26. PRAŽSKÉ HEMATOLOGICKÉ DNY

Hematologie 2026

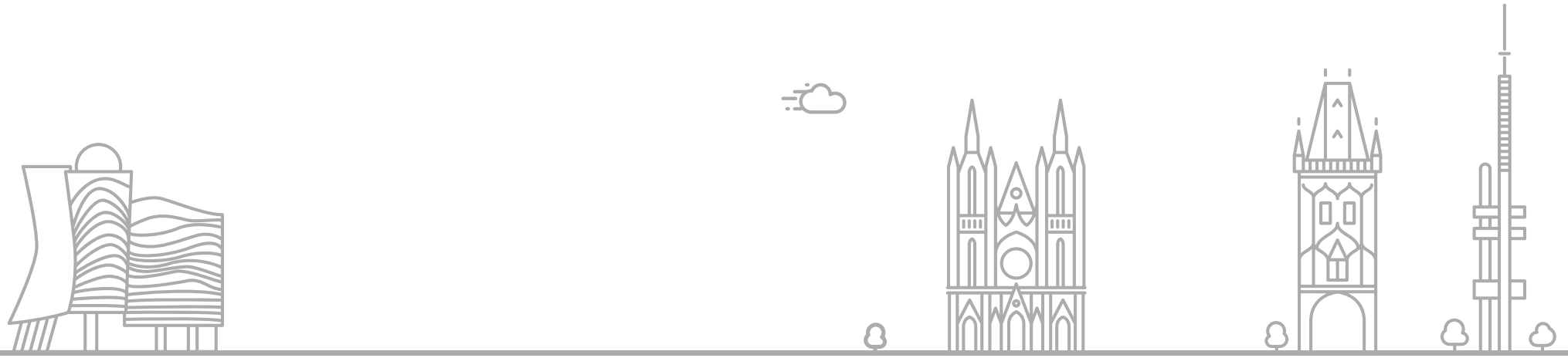
KAM NÁS POSOUVÁ TO NEJLEPŠÍ Z ČESKÉ A SVĚTOVÉ HEMATOLOGIE

21.–23. 1. 2026

Clarion Congress Hotel Prague
(Freyova 33, Praha 9 – Vysočany)

Odborná veřejnost

PROGRAM KONFERENCE A SBORNÍK ABSTRAKTŮ



OBSAH

ÚVODNÍ SLOVO

Vážené kolegyně, vážení kolegové!

Vítáme Vás na výročním setkání Hematologie 2026, který představuje to nejlepší z české hematologie, uvádí novinky z oboru a přináší i obsáhlou edukaci. Již v době psaní tohoto úvodu je zřejmé že se bude jednat o zatím nejúspěšnější ročník, alespoň z pohledu účasti, kdy bylo submitováno 107 abstraktů a měsíc před zahájením setkání bylo přihlášeno více než 600 účastníků.

Za zásadní považujeme kromě ústních sdělení posterovou sekci. Obé ukazuje rozsah a úroveň české hematologie, zároveň umožňuje setkání a diskuse lékařů a vědců z různých oblastí. Kromě přednášek zahraničních hostů, včetně Konstanze Döhner, prezidentky EHA v rámci již tradičního společného bloku EHA a ČHS, s nimi (a nejen s nimi) můžeme diskutovat v rámci Meet the Expert workshopů. Edukační program i diskuse pak zahrnují taková témata jako je dopad umělé inteligence na naši práci, otevřené otázky paliativní péče a řadu dalších. Opět se podařilo připravit velmi atraktivní ošetřovatelský program.

V neposlední řadě jsme moc rádi, že Hematologie 2026 vytvořila prostor pro velký počet odborných setkání nad tématy, která jsou důležitá jak pro odbornou péči a výzkum, tak pro podporovatele z řad farmaceutických firem a zároveň i interakci v rámci akademických pracovních skupin.

Přejeme nám všem, abychom si letošní Hematologii 2026 užili, ocenili výsledky české hematologie, načerpali poznatky a inspiraci a vzájemně se povzbudili na další cestě.

Marek Trněný
Předseda Programového výboru

Jan Trka
Předseda Vědeckého výboru

POŘADATEL

SPOLEK ČESKÝCH LÉKAŘŮ V PRAZE ČLS JEP

pod záštitou:
České hematologické společnosti ČLS JEP

České společnosti pro trombózu a hemostázu ČLS JEP

ve spolupráci s:

I. interní klinikou - klinikou hematologie VFN a 1. lékařské fakulty UK v Praze - *Marek Trněný*

Ústavem hematologie a krevní transfuze *- Petr Cetkovský*

Klinikou dětské hematologie a onkologie FN Motol a 2. lékařské fakulty UK v Praze - *Lucie Šrámková*

Hematologickou klinikou FN Královské Vinohrady a 3. lékařské fakulty UK v Praze - *Tomáš Kozák*

VÝBORY

PROGRAMOVÝ VÝBOR

→ předseda

Marek **Trněný**
1.LF UK a VFN Praha

→ členové

Anna **Jonášová**
1.LF UK a VFN Praha

Kateřina **Machová Poláková**
ÚHKT Praha

Julia **Starková**
2.LF UK a FN Motol Praha

Tomáš **Stopka**
1.LF UK a VFN Praha

Cyril **Šálek**
ÚHKT Praha

Jan **Zuna**
2.LF UK a FN Motol Praha

VĚDECKÝ VÝBOR

→ předseda

Jan **Trka**
2.LF UK a FN Motol Praha

→ členové

Meritxell **Alberich-Jorda**
UMG AV ČR Praha

Jan **Blatný**
LF MU a FN Brno

Nikola **Čuřík**
ÚHKT Praha

Vladimír **Divoký**
UP Olomouc

Michael **Doubek**
LF MU a FN Brno

Petr **Dulíček**
LF UK a FN Hradec Králové

Eva **Froňková**
2.LF UK a FN Motol Praha

Jaromír **Gumulec**
FN Ostrava

Tomáš **Jelínek**
LF OU FN Ostrava

Markéta **Kalinová**
3.LF UK a FNKV Praha

Magdalena **Klánová**
1.LF UK a VFN Praha

Pavel **Klener**
1.LF UK a VFN Praha

Ester **Mejstříková**
2. LF UK a FN Motol Praha

Marek **Mráz**
LF MU Brno

Jan **Novák**
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Adam **Obr**
ÚHKT Praha

Jakub **Radocha**
LF UK a FN Hradec Králové

Jan **Starý**
2.LF UK a FN Motol Praha

Michal **Šimíček**
LF OA a FN Ostrava

Martin **Špaček**
1.LF UK a VFN Praha

Jan **Vydra**
ÚHKT

Daniela **Žáčková**
LF MU a FN Brno

→ garanti ošetřovatelského programu

Darja **Hrabánková**
1.LF UK a VFN Praha

Lenka **Turková**
3.LF UK a FNKV Praha

Lucie **Vylitová**
ÚHKT Praha

Jitka **Wintnerová**
2.LF UK a FN Motol Praha

HODNOTITELÉ ABSTRAKTŮ

Meritxell Alberich-Jorda – UMG AV ČR Praha
Jan Blatný – LF MU a FN Brno
Nikola Čuřík – ÚHKT Praha
Vladimír Divoký – UP Olomouc
Michael Doubek – LF MU a FN Brno
Eva Froňková – 2.LF UK a FN Motol Praha
Tomáš Jelínek – LF OU FN Ostrava
Anna Jonášová – 1.LF UK a VFN Praha
Markéta Kalinová – 3.LF UK a FNKV Praha
Pavel Klener – 1.LF UK a VFN Praha
Kateřina Machová Poláková – ÚHKT Praha
Ester Mejstříková – 2. LF UK a FN Motol Praha
Marek Mráz – LF MU Brno
Jan Novák – 3.LF UK a FNKV Praha
Adam Obr – ÚHKT Praha
Jakub Radocha – LF UK a FN Hradec Králové
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Cyril Šálek – ÚHKT Praha
Martin Špaček – 1.LF UK a VFN Praha
Lucie Šrámková – 2.LF UK a FN Motol Praha
Jan Trka – 2.LF UK a FN Motol Praha
Marek Trněný – 1.LF UK a VFN Praha
Jan Vydra – ÚHKT Praha
Jan Zuna – 2.LF UK a FN Motol Praha
Daniela Žáčková - LF MU a FN Brno

Vědecký výbor konference děkuje všem, kteří se podíleli na hodnocení abstraktů.

ORGANIZAČNÍ ZAJIŠTĚNÍ KONFERENCE

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MEDAILONKY VYZVANÝCH ŘEČNÍKŮ

Heřmanského přednáška:
GENOMICS AND TREATMENT IN WALDENSTROM'S MACROGLOBULINEMIA

Steven TREON, M.D., Ph.D.

Steve Treon is the Director of the Bing Center for Waldenstrom's Macroglobulinemia (WM) at the Dana Farber Cancer Institute (DFCI), a Professor of Medicine at Harvard Medical School, and Chair of the WM Clinical Trials Group.

His laboratory first identified highly recurring activating mutations in MYD88 and CXCR4 using whole genome sequencing. Dr. Treon's laboratory also identified that Bruton's tyrosine kinase (BTK) was a downstream target of mutated MYD88, and enabled a clinical trial with the BTK inhibitor ibrutinib that resulted in the first-ever approval of a drug by the U.S. FDA and the European Medicines Agency for WM. Dr. Treon also made major contributions to the investigation and advancement of many novel agents used to treat WM including monoclonal antibodies, nucleoside analogues, bendamustine, proteasome inhibitors, and BTK inhibitors.

Dr. Treon has delivered numerous scientific and clinical lectures worldwide, and his scientific work has earned "Best of ASH" designations. He has published extensively on topics in WM and myeloma with over 280 peer-reviewed original reports, authoritative reviews, editorials, and chapters included in high-impact journals and textbooks. He has been the principal organizer of the International Workshops on WM since 2002. Dr. Treon is an active member of the Workshop Committee for the International Myeloma Society (IMS), and was the Chair of the 17th International Myeloma Workshop. His scientific and clinical research contributions in WM have been acknowledged by many awards; Robert A. Kyle Award, the J.G. Waldenstrom Lifetime Achievement Award, the L. Strauss Leukemia Foundation Outstanding Cancer Investigator Award, designation as "America's Top Doctor" by U.S. News and World Report, the prestigious "One Hundred Award" from the Massachusetts General Hospital for Outstanding Cancer Research, the Bruce Waterfall Memorial Award from Weill Cornell Medical School, the Medical Oncology Discoverer Award for seminal work in Medical Oncology by the DFCI.

Neuwirtova přednáška:
NOVEL CAR T/NK CELL THERAPIES

Naoki HOSEN, M.D., Ph.D.

Professor, Department of Hematology and Oncology, Graduate School of Medicine, Osaka University, Japan; Professor, Division of Cellular Immunotherapy, Immunology Frontier Research Center (IFReC), Osaka University.

Career and Professional Experience: Has held various clinical and academic positions within Osaka University, including postdoctoral and research fellowships. Board-certified hematologist with clinical experience in treating hematological malignancies. Extensive research involvement in cellular immunotherapy, especially related to hematologic cancers like multiple myeloma.

Research Interests: Hematologic Oncology – Focus on blood cancers, particularly multiple myeloma. Cancer Immunotherapy – Development of novel CAR-T cell therapies and monoclonal antibodies. Tumor-specific Antigens – Discovery of unique surface markers for targeted therapies (e.g., integrin β7 in its activated form). Translational Medicine – Bridging lab research with clinical applications.

Key Research Contributions: Identified activated integrin β7 as a tumor-specific target for CAR-T therapy in multiple myeloma. Developed monoclonal antibodies and CAR constructs targeting novel antigens specific to malignant cells. Published numerous articles in high-impact journals in the fields of hematology, oncology, and immunology.

CSH/EHA joint symposium

čtvrtek 22. 1. 2026 od 14:45 hodin v hlavních sálech Zenit + Nadir

Prof. Dr. Konstanze Döhner

Prof. Konstanze Döhner is the president of the European Hematology Association. She is a senior physician at the Department of Internal Medicine III at Ulm University Hospital and heads the Section of Myeloid Neoplasia located there.
Konstanz Döhner studied Medicine at the University Medical School in Heidelberg, Germany and received her M.D. degree in 1992. From the beginning she worked in the field of hematology with a special focus on myeloid malignancies, in particular acute myeloid leukemia (AML). From 1995 to 1997 she spent a postdoctoral fellowship at the German Cancer Research Center (Professor Dr. Peter Lichter) in Heidelberg, Germany, and the Hospital for Sick Children, Department of Genetics (Dr. S.W. Scherer) Toronto, Canada. Her main scientific interest was/is the molecular characterization of AML/myeloid malignancies and the translation of her findings into clinical studies. Very early she became a Board member of the German-Austrian AML Study Group (AMLSG) and here she established one of the leading reference laboratories for molecular diagnostics in AML. The identification of molecular markers within a very short time window (48hrs) was a major basis for the development of risk-adapted treatment regimens as performed within the AMLSG. In 2000, Konstanze Döhner moved to the Department of Hematology/Oncology at the University Hospital Ulm (Germany) where she is working as an Assistant Professor until today. She became head of the laboratory for Cytogenetic and Molecular Diagnostics in myeloid leukemias and is now leading the section of Myeloid neoplasms in the department. In 2003 Konstanze Döhner received her board certification as Hematologist and Oncologist and in 2005 she finished her "Habilitation" and became Professor. Konstanze Döhner is involved in a large number of clinical AML studies. She is member of the European LeukemiaNet MRD working party. In 2018, she was elected as a Board Member of the European Hematology Association (EHA) where she became Chair of the EHA Education Committee and Chair of the EHA Specialized Working Group AML. In 2021, she was elected as a member of the EHA Executive Board and in 2023 she became President Elect followed by her presidency that has started in June 2025 for a 2 years term. Beside AML she also has clinical and scientific interest in myeloproliferative neoplasms. Here together with the University Hospital of Aachen she was one of the founders of the German Study Group on Myeloproliferative Neoplasms (GSG-MPN). One centerpiece of the GSG-MPN Study group is the MPN BioRegistry study for BCR::ABL1 negative MPN. Finally, Konstanze Döhner is involved in a large number of national and international scientific cooperations, which is also reflected by numerous highly ranked publications.

Uri Ilan, M.D.

Dr. Ilan is the AI Lead at the Máxima Innovation Center, Princess Máxima Center for Pediatric Oncology.
He studied medicine at the Hebrew University of Jerusalem. Medical Training: Completed pediatric residency at Hadassah Medical Center (Jerusalem), followed by a fellowship in pediatric hematology and oncology in Jerusalem and later at the Birmingham Children's Hospital, UK. Current Position: AI Lead, *Princess Máxima Center for Pediatric Oncology* (Utrecht, the Netherlands). He leads efforts to integrate artificial intelligence tools into pediatric oncology for clinical decision support and research.
Research Focus: Development of AI-driven platforms to interpret complex clinical and genomic data in pediatric cancer. Creator and scientific lead of the "Capricorn" AI platform, developed in collaboration with Google Research, which transforms structured patient data into targeted scientific literature searches. Development of an AI assistant for multidisciplinary tumor boards, designed to improve decision-making and global access to expert care in childhood cancer.
Major Projects: Contributor to the International Leukemia Targeted Board (iLTB), a multinational initiative for precision treatment of relapsed and refractory pediatric leukemia, integrating molecular profiling, immunophenotyping, and drug sensitivity testing.

Assoc. Prof. Tomas Jelinek, MD, PhD

Associate Professor Tomáš Jelínek is a leading Czech expert in the field of hematologic malignancies, particularly multiple myeloma.
He graduated from medical school, earned his MUDr. and Ph.D. degrees, and currently serves as Deputy Head for Science and Research at the Department of Hematooncology, Faculty of Medicine, University of Ostrava / University Hospital Ostrava. His clinical and research work focuses mainly on multiple myeloma – its diagnosis, treatment, monitoring, and prognosis. He has contributed to studies examining the significance of circulating tumor plasma cells in myeloma patients and the biology of extramedullary myeloma (disease spreading outside the bone marrow).

Research and publications

- Author and co-author of numerous scientific papers on novel treatment algorithms for myeloma, immunotherapy, minimal residual disease (MRD), and PET/CT imaging standardization.
- In 2025, he received the European Hematology Association (EHA) Physician Scientist Research Grant, recognizing his international impact and scientific excellence.

Blok I

středa 21. 1. 2026 od 11:00 hod. v hlavních sálech Zenit + Nadir

Co bychom měli vědět o léčbě hemofilie v roce 2026?

doc. MUDr. Jan Blatný, Ph.D.

Pracuje na Oddělení dětské hematologie a biochemie Dětské nemocnice FN Brno, kde odpovídá za Komplexní hemofilické centrum certifikované jak ČNHP (www.cnhp.cz) tak EUHANET (www.euhanet.org) a Centrum pro trombózu a hemostázu. Je rovněž docentem pediatrie a členem Oborové rady Pediatrie na Lékařské fakultě Masarykovy university.
Mezi jeho odborné klinické a výzkumné zájmy vždy patřila hematologie se zaměřením především (ale nejen) na trombózu a hemostázu, včetně jejich vrozených a získaných poruch a život ohrožujících krvácení u dětí i dospělých.
Je prezidentem EAHAD, koordinátorem ČNHP a členem řady dalších národních i mezinárodních odborných společností, institucí a pracovních skupin.
Od listopadu 2020 do dubna 2021 byl ministrem zdravotnictví České republiky.
Je autorem či spoluautorem více, než 100 publikací.

Riziko venózního tromboembolizmu u nemocných s lymfomem

prof. MUDr. Petr Dulíček, Ph.D.

Promoval na LF UK v Hradci Králové v r. 1988. Po promoci nastoupil jako sekundární lékař na OLDN FN, od r. 1990 pak na Oddělení klinické hematologie ve FN v HK. Zde pracuje dosud, t.č. na IV. Interní hematologické klinice. V roce 1999 obhájil kandidátskou disertační práci, v roce 2004 habilitační a v r. 2023 byl jmenován profesorem vnitřního lékařství. V letech 2002-2012 byl členem revizní komise ČHS při ČLS JEP, od r. 2015 je místopředsedou ČSTH. V roce 1997 strávil 2 měsíce jako visiting clinician na Mayo Clinic v Rochesteru.

Vrodené erytrocytózy: pretrvávajúca diagnostická a klinická výzva v ére molekulárnej medicíny

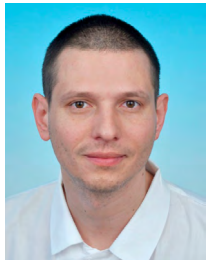
doc. Mgr. Monika Horváthová, Ph.D.

Je přednostkou Ústavu biologie Lékařské fakulty Univerzity Palackého (LF UP) v Olomouci. Vystudovala genetiku na Univerzitě Komenského v Bratislavě a doktorandské studium dokončila na LF UP v Olomouci. Je zástupkyní pro poruchy červené krevní řady olomouckého centra v evropské síti ERN-EuroBloodNet. Ve své vědecké práci se věnuje molekulární patofyziologii vrozených anémií a erytrocytóz a propojení procesu erytropoézy s metabolismem železa.

Blok II

středa 21. 1. 2026 od 13:15 hod. v hlavních sálech Zenit + Nadir

Nežádoucí účinky CAR T-cell terapie a bispec. protilátek



MUDr. František Folber, Ph.D.

Absolvoval Lékařskou fakultu Masarykovy univerzity v roce 2006 a od té doby pracuje na Interní hematologické a onkologické klinice Fakultní Nemocnice Brno, kde je nyní vedoucím programu transplantací a buněčné terapie a vedoucím lékařem transplantační jednotky. Je členem Transplantační sekce České hematologické společnosti, místopředsedou České společnosti pro genovou a buněčnou terapii a spoluautorem národních doporučení pro transplantace krvetvorných buněk a terapii CAR-T lymfocyty.

Transfuze jako součást paliativní péče v hematologii?

doc. MUDr. Anna Jonášová Ph.D.

Vedoucí skupiny pro Myelodysplastický syndrom (MDS), Akutní myeloidní leukemii a dalších Myeloproliferativních onemocnění na I. interní klinice Všeobecné fakultní nemocnice a I. Lékařské fakulty V Praze.

doc. MUDr. MgA. Kateřina Rusinová, Ph.D.

Přednostka kliniky paliativní medicíny VFN Praha

Vrozené mutace a myeloidní nádory

Ing. Lucie Láníková, Ph.D.

Působí na Ústavu molekulární genetiky AV ČR v Praze od roku 2014, kde se věnuje studiu vrozených mutací u hematologických onemocnění se zaměřením na regulaci JAK/STAT a HIF signálních drah. Doktorské studium absolvovala na Lékařské fakultě Univerzity Palackého v Olomouci, poté pracovala jako postdoktorandka v laboratoři prof. Prchala na University of Utah v Salt Lake City, kde se zaměřila na molekulární mechanismy erytropoézy a genetické predispozice k myeloproliferativním onemocněním. Dlouhodobě spolupracuje s prof. Prchalem i s klinickými pracovišti na translaci molekulárních poznatků do diagnostické praxe a je autorkou či spoluautorkou řady odborných publikací v oblasti experimentální hematologie.

Blok III

čtvrtek 22. 1. 2026 od 11:00 hod. v hlavních sálech Zenit + Nadir

Potransplantační lymfoproliferace

prof. MUDr. Jan Novák, Ph.D.

Hematologii se věnuje od roku 2007 a krom zahraničních stáží je věrný FNKV Praha. Zkoumá atypické T-lymfocyty a věnuje se léčbě akutních leukémií, B-NHL a CLL.

MUDr. Markéta Racková, Ph.D.

Lékařka na Klinice dětské hematologie a onkologie 2.LF UK a FN Motol, výzkumná pracovnice v laboratořích CLIP. Zabývá se léčbou a výzkumem dětských non-Hodgkinských lymfomů (NHL), zejména potransplantačními lymfoproliferativními onemocněními (PTLD). Aktivně působí ve skupině i-BFM a v European Intergroup for Childhood Non-Hodgkin Lymphoma (EICNHL), kde je členkou komise pro vzácné formy NHL. V rámci obou skupin mimo jiné vede mezinárodní projekt zaměřený na studium patogeneze PTLD a koordinuje mezinárodní Česko-rakouský registr PTLD.

Alogenní transplantace – myelodysplastický syndrom

MUDr. Markéta Šťastná Marková

Absolvovala 1. lékařskou fakultu Univerzity Karlovy. Poté pracovala na III. Interní klinice a Oddělení klinické hematologie I. Interní kliniky Všeobecné fakultní nemocnice v Praze. Atestovala v oboru vnitřní lékařství a hematologie a krevní transfúze. Aktuálně pracuje na Ústavu hematologie a krevní transfúze, jako klinický lékař, kde se věnuje především problematice alogenních transplantací hemopoetických buněk, intenzivní péči v hematologii a především transplantační léčbě myelodysplastického syndromu. Svoji kvalifikaci si rozšířila na odborných stážích ve Velké Británii – Manchester, Christie Hospital a University of Minnesota, USA.

Akutní lymfoblastová leukémie

doc. MUDr. Mgr. Cyril Šálek, Ph.D.

Specializuje se na léčbu a výzkum akutních leukémií dospělých. Podílí se tvorbě léčebných standardů vydávaných Českou hematologickou společností. Je autorem několika akademických klinických studií, jež českým pacientům umožňují časný přístup k moderní cílené terapii. Zastává pozici místopředsedy České leukemické skupiny – pro život (CELL), aktivně působí v Evropské pracovní skupině pro akutní lymfoblastovou leukémii dospělých (EWALL).

Blok VI

pátek 23. 1. 2026 od 12:00 hod. v hlavních sálech Zenit + Nadir

Novinky v diagnostice a léčbě DLBCL- postASH

prof. MUDr. Pavel Klener, Ph.D.

- I. Interní klinika- hematologie, VFN a 1. LF UK a Ústav patologické fyziologie 1. LF UK
- klinický hematolog, VŠ pedagog a vědec se zaměřením na problematiku nehodgkinských lymfomů
- autor >110 publikací v impaktovaných časopisech
- řešitel / spoluřešitel 17 grantových projektů AZV a GAČR (2 x cena MZČR za nejlepší projekt)
- školitel PhD studentů (9 x absolventi, 5 x vedeni)

Hodgkinův lymfom 2025: Klíčová sdělení

MUDr. Alice Sýkorová, Ph.D.

Působí na IV. interní hematologické klinice FN Hradec Králové, kde vede ambulantní provoz a klinickou Hodgkinovu skupinu. Specializuje se na diagnostiku a léčbu maligních lymfomů. Je členkou Kooperativní lymfomové skupiny a České skupiny pro studium Hodgkinova lymfomu. Problematice lymfomů se věnuje také publikačně na národní i mezinárodní úrovni a pravidelně přednáší na odborných konferencích.

Praktický přístup k léčbě CLL: současné možnosti a jejich optimální využití

doc. MUDr. Martin Šimkovič, Ph.D.

Hematolog a docent vnitřního lékařství na IV. interní hematologické klinice FN Hradec Králové, kde vede program CLL. Je absolventem Univerzity Karlovy a je atestován v interním lékařství i hematologii. Aktivně se podílí na mezinárodních klinických studiích zaměřených na nové cílené terapie CLL a B-buněčných malignit. Je autorem či spoluautorem více než 130 publikací a pravidelným účastníkem kongresů ASH, EHA a IWCLL. Působí ve výboru České skupiny pro CLL (ČSCLL), podílí se na národních doporučeních.

SCHÉMA ODBORNÉHO PROGRAMU

STŘEDA 21. 1. 2026

8:00 | 9:00 | 10:00 | 11:00 | 12:00 | 13:00 | 14:00 | 15:00 | 16:00 | 17:00 | 18:00 | 19:00 | 20:00

sály ZENIT/ NADIR

- 8:00 – 18:00 REGISTRACE
- Uvítací občerstvení
- 11:00 – 12:45 **Blok I** Edukační program Přednášková sdělení
- Přestávka na kávu
- 13:15 – 15:00 **Blok II** Edukační program Přednášková sdělení
- Přestávka na kávu
- 15:30 – 17:30 **SLAVNOSTNÍ ZAHÁJENÍ** Heřmanského přednáška Neuwirtova přednáška
- 17:45 – 19:00 Posterová sekce - prezentace (sál Meridian)
- 19:00 Diskuse u posterů a večeře (sál Meridian + foyer)

sál AQUARIUS

- 8:00 – 18:00 REGISTRACE
- 9:00 – 10:45 Statistický workshop

ČTVRTEK 22. 1. 2026

8:00 | 9:00 | 10:00 | 11:00 | 12:00 | 13:00 | 14:00 | 15:00 | 16:00 | 17:00 | 18:00 | 19:00 | 20:00

sály ZENIT/ NADIR

- 8:00 – 19:00 REGISTRACE
- 8:30 – 9:15 CSL Behring
- Přestávka na kávu
- 11:00 – 12:45 **Blok III** Edukační program Přednášková sdělení
- 13:30 – 14:30 Novartis
- 14:45 – 16:15 **Blok IV** EHA / CSH JOINT SYMPOSIUM (blok v AJ)
- 16:45- 18:15 **Blok V** Best of papers / best of abstracts
- 18:30 – 19:15 Bristol-Myers Squibb

sály TYCHO/ KEPLER

- 8:00 – 19:00 REGISTRACE
- 8:30 – 9:15 Gilead
- 9:30 – 10:30 Meet the expert workshop 1 (blok v AJ)
- 11:00 – 12:30 **Ošetřovatelský program I**
- 12:35 – 13:15 Vzdělávací seminář pro sestry
- 14:00 – 15:30 **Ošetřovatelský program II** workshop
- 16:00 – 17:30 Pacientský seminář nejen pro pacienty
- 18:30 – 19:15 Eli Lilly
- 19:30 Večeře

sály LEO/ VIRGO

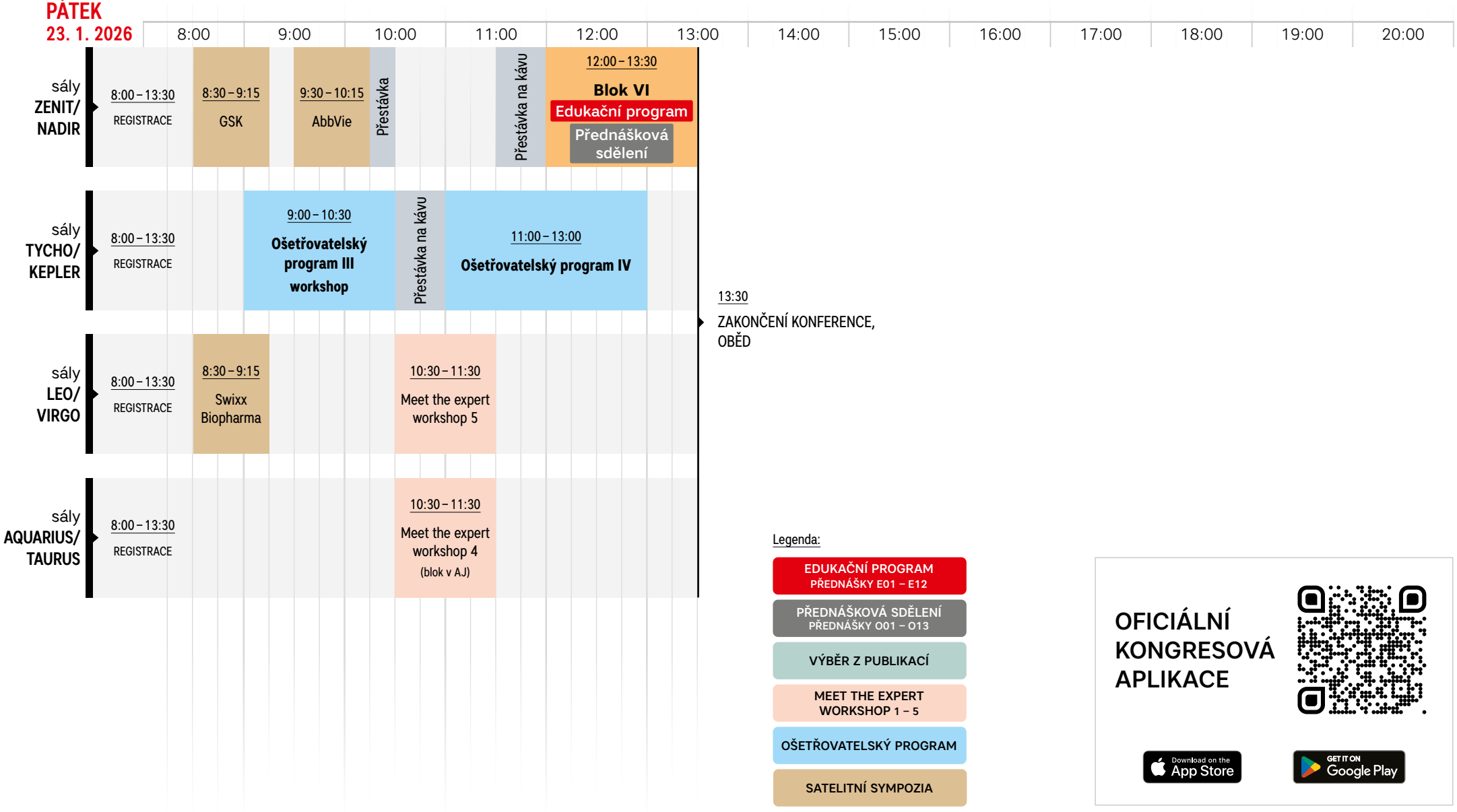
- 8:00 – 19:00 REGISTRACE
- 9:30 – 10:30 Meet the expert workshop 2
- Oběd
- 13:45 – 14:30 Johnson &Johnson
- Přestávka na kávu

sály AQUARIUS/ TAURUS

- 8:00 – 19:00 REGISTRACE
- 9:30 – 10:30 Meet the expert workshop 3 (blok v AJ)

SCHÉMA ODBORNÉHO PROGRAMU

PÁTEK 23. 1. 2026



Legenda:

- EDUKAČNÍ PROGRAM – PŘEDNÁŠKY E01 – E12
- PŘEDNÁŠKOVÁ SDĚLENÍ – PŘEDNÁŠKY O01 – O13
- VÝBĚR Z PUBLIKACÍ
- MEET THE EXPERT WORKSHOP 1 – 5
- OŠETŘOVATELSKÝ PROGRAM
- SATELITNÍ SYMPOZIA

OFICIÁLNÍ KONGRESOVÁ APLIKACE

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Schůze výborů a jednání na základě osobního pozvání:

středa 21. 1. 2026

8:30 – 10:00
Schůze výboru ČHS ČLS JEP (salónek Stella, 3. patro)

10:00 – 10:45
Zasedání redakční rady časopisu Transfuze a Hematologie *Dnes* (salónek Stella, 3. patro)

19:00 – 19:45
Zasedání pracovní skupiny pro ALL (CELL) (salónek Stella, 3. patro)

19:00 – 21:00
Zasedání CELL/MIND (salónek Aquarius, 3. patro)

19:00 – 20:00
Zasedání registru pacientů ČSTH (salónek Taurus, 3. patro)

19:00 - 20:00
Schůze výboru Hodgkinův lymfom (salónek Zodiac, 2. patro**)**

čtvrtek 22. 1. 2026

7:30 – 8:15
Zasedání HEMATOLOGIE-online.cz (salónek Zodiac, 2. patro)

12:30 – 13:30
Schůze Transplantační sekce ČHS ČLS JEP (salónek Stella, 3. patro)

Firemní zasedání na základě osobního pozvání:

čtvrtek 22. 1. 2026

12:35 – 13:35
Recordati Rare Diseases (salónek Aquarius, 3.patro)

19:30 – 21:00
GlaxoSmithKline, s.r.o. (salónek Taurus, 3.patro)

19:30 – 21:00
Johnson&Johnson (salónek Stella, 3.patro)

19:30 – 21:30
AbbVie s.r.o. (salónek Zodiac, 2.patro)

pátek 23. 1. 2026

7:15 – 8:15
Bristol-Myers Squibb spol. s r.o. (salónek Stella, 3. patro)

7:30 – 8:15
Novartis s.r.o. (salónek Zodiac, 2. patro)

PODROBNÝ PROGRAM KONFERENCE

středa 21. 1. 2026

středa 21. 1. 2026

→ SÁL AQUARIUS

9:00 – 10:45

Statistický workshop:
analýza přežití pro kliniky a začínající vědce
Vydra J., Pecherková P. (Praha)

→ SÁLY ZENIT + NADIR

11:00 - 12:45

Odborný program – Blok I

Předsedající: Gumulec J. (Ostrava), Starý J. (Praha)

11:00 – 11:20

E01 **Co bychom měli vědět o léčbě hemofilie v roce 2026?**
Blatný J. (Brno)

11:20 – 11:40

E02 **Riziko venózního tromboembolizmu u nemocných s lymfomem**
Dulíček P. (Hradec Králové)

11:40 – 11:55

O01 ASH 2025
Multiple Reciprocal Interactions within B-cell Receptor and AKT Signaling Pathways Regulate Response to EZH2 Inhibition in Germinal Center-derived Diffuse Large B-cell Lymphoma
Havránek O. (Praha) a spol.

11:55 – 12:10

O02 EHA 2025
Repression of miR-29 via MYC Leads to Increased CD40 Signaling in Transformed Follicular Lymphoma
Mráz M. (Brno) a spol.

12:10 – 12:25

O03 ESID EHA SIOPE FOCUSED SYMPOSIUM
Clonal dynamics of T cells and treatment outcomes in Pediatric Lymphocytic variant Eosinophilic syndrome
Mejstříková E. (Praha) a spol.

12:25 – 12:45

E03 **Vrodené erytrocytózy: pretrvávajúca diagnostická a klinická výzva v ére molekulárnej medicíny**
Horváthová M. (Olomouc) a spol.

12:45 – 13:15 **Přestávka na kávu**

13:15 - 15:00

Odborný program – Blok II

Předsedající: Machová K. (Praha), Žáčková D. (Brno)

13:15 – 13:35

E04 **Vrozené mutace a myeloidní nádory**
Láníková L. (Praha)

13:35 – 13:50

O04 EHA 2025
JAK2 R1063H Germline variant in AML: association with oncogenic IDH 1/2 drivers resulting in cooperative leukemogenesis and therapeutic implications
Hlušičková Kaprálová K. (Olomouc) a spol.

středa 21. 1. 2026

13:50 – 14:05

O05 **EHA 2025**

CHRONIC MYELOID LEUKEMIA (CML) treatment discontinuation after a two-step dose reduction. The first results of the phase 2 study HALF.

Mayer J. (Brno) a spol.

14:05 – 14:20

O06 **ASH 2025**

DNA-based MRD monitoring enhances risk stratification during TKI dose reduction in CML: Evidence from the clinical trial

Machová K. (Praha) a spol.

14:20 – 14:40

E05 Transfuze jako součást paliativní péče v hematologii?

Jonášová A., Rusinová K. (Praha)

14:40 – 15:00

E06 Nežádoucí účinky CAR T-cell terapie a bispec. protilátek

Folber F. (Brno)

15:00 – 15:30 **Přestávka na kávu**

15:30 – 17:30

SLAVNOSTNÍ ZAHÁJENÍ KONFERENCE

Předsedající: Cetkovský P. (Praha), Hrabánková D. (Praha), Kozák T. (Praha), Šrámková L. (Praha), Trka J. (Praha), Trněný M. (Praha)

15:30 – 16:30

Zahájení konference

15:30-16:00 Zahájení
16:00-16:30 Předání cen ČHS ČLS JEP za nejlepší publikace v roce 2024 a jejich prezentace: Tomáš Jelínek (Ostrava), Marek Mráz (Brno), Markéta Kubričanová Žaliová (Praha)

16:30 – 17:00

HEŘMANSKÉHO PŘEDNÁŠKA

Treon S. (Boston, MA; USA):

GENOMICS AND TREATMENT IN WALDENSTROM'S MACROGLOBULINEMIA

17:00 – 17:30

NEUWIRTOVA PŘEDNÁŠKA

Hosen N. (Osaka; Japan):

NOVEL CAR T/NK CELL THERAPIES

17:45 - 19:00

Posterová sekce (sál Meridian)

od 19:00

Diskuze u posterů, večeře (sál Meridian + foyer)

POSTEROVÁ SEKCE

Moderovaná diskuse:

středa 21. 1. 2026 od 17:45 hodin (sál Meridian)

AKUTNÍ LYMFOBLASTICKÁ LEUKÉMIE (Postery P01 – P11)

Diskusi řídí: Starý J. (Praha)

P01

L-asparaginase alters redox homeostasis in acute lymphoblastic leukemia EHA 2025

Kolárik M. (Praha) a spol.

P02

Monocytic Switching in DUX4-Positive BCP-ALL: Challenges in MRD Assessment and Prognostic Impact

Palavandishvili N. (Praha) a spol.

P03

Surface protein profiling of childhood B-cell precursor acute lymphoblastic leukemia

Palavandishvili N. (Praha) a spol.

P04

High metabolic activity in leukemic cells is associated with more aggressive phenotype and reduced treatment efficacy EHA 2025

Starková J. (Praha) a spol.

P05

Investigating Prednisone-Induced Signaling Pathway Changes in Pediatric T-ALL EHA 2025

Kužílková D. (Praha) a spol.

P06

Implementation of Extended Molecular Diagnostics in the Clinical Management of Adult ALL Patients ESH 2025

Polívková V . (Praha) a spol.

P07

Clinical value of advanced genomic testing in adult Philadelphia-negative B-ALL: Results from the CZ national leukemia study group (CELL) ASH 2025

Křížková J. (Praha) a spol.

P08

ACUTE TOXICITIES DURING INDUCTION AND FIRST CONSOLIDATION TREATMENT OF YOUNG ADULTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA: COMPARISON OF ALL CELL 2023 JUNIOR AND GMALL 07/2003 PROTOCOLS – A SINGLE CENTER EXPERIENCE CLEAR 2025

Láznička A. (Praha) a spol.

P09

Analýza genomových aberací u dospělých pacientů s akutní lymfoblastickou leukemií pomocí sekvenování dlouhým čtením

Dvořáčková B. (Brno) a spol.

P10

Brexucabtagene autoleucel v léčbě akutní lymfoblastové leukemie – pilotní analýza CELL a srovnání s daty mezinárodních souborů

Hrabovský Š. (Brno) a spol.

P11

CD19 Chimeric Antigen Receptor-T Cells with Enhanced Tscm Immunophenotype produced by piggyBac transposon system for Therapy of Refractory B-cell Malignancies- Preliminary Results of Phase I Clinical Trial EHA 2025

Štach M. (Praha) a spol.

AKUTNÍ MYELOIDNÍ LEUKÉMIE I. (Postery P12 – P19)

Diskusi řídí: Šálek C. (Praha)

P12

FLT3 INHIBITORS MIDOSTAURIN AND GILTERITINIB ENHANCE CYTARABINE ACCUMULATION BY AFFECTING OCTN1 AND ENT1 TRANSPORTERS IN AML CELLS EHA 2025

Dudičová S. (Hradec Králové) a spol.

P13

IDENTIFICATION OF CELLULAR MECHANISMS THAT CAN LEAD TO DRUG RESISTANCE TO FLT3 INHIBITOR GILTERITINIB IN AML EHA 2025

Čečková M. (Hradec Králové) a spol.

P14

Integrative analysis of the interplay between peripheral immune landscape and leukemic blast adaptation in acute myeloid leukemia ASH 2025

Cerovská E. (Praha) a spol.

P15

Evaluation of novel structures as potential FLT3 inhibitors in AML EHA 2025

Rataj J. (Hradec Králové) a spol.

P16

Analysis of AML cells in vitro: Sensitivity to CK1 inhibition vs. genetic background

13th International Conference Analytical Cytometry

Plešingerová H. (Brno) a spol.

P17

PD-L1 expression on leukemia cells is dynamic and correlates with glycolytic rate ESH 2025

Kořánová T. (Praha) a spol.

P18

Neocentromeres in complex acute leukemia karyotypes as an indicator of chromosomal instability and disease progression

15th European Cytogenomics Conference

Mendlíková I. (Praha) a spol.

P19

Optické mapování genomu akutních myeloidních leukémií (AML): nové pohledy na strukturní varianty a jejich klinický význam

XXXV. Izakovičov memoriál 2025

Jarošová M. (Brno) a spol.

AKUTNÍ MYELOIDNÍ LEUKÉMIE II. (Postery P20 – P27)

Diskusi řídí: Šrámková L. (Praha)

P20

SQSTM1/p62 Inhibition Impairs Mitophagy and Cellular Respiration in AML Cells

Acute Myeloid Leukemia „Molecular and Translational": Advances in Biology and Treatment

Kuželová K. (Praha) a spol.

P21

Common features of stress response to drugs targeting the nucleus and mitochondria ESHAML2025

Wolfová K. (Praha) a spol.

P22

Redox Dependency and Antioxidant Vulnerability in MLL-Rearranged Leukemia

Nemes D. (Praha) a spol.

P23

APL-Like Acute Myeloid Leukemia With KMT2A-PTD and MYC Amplification in an Elderly Patient: A Case Report

Beránková M. (Hradec Králové) a spol.

P24

Perfusion model of microvascular endothelium activation by AML-derived secreted factors

Röselová P. (Praha) a spol.

P25

CD123-specific CAR-T cells produced by Linear DNA-based piggyBac transposon system – initiation of phase I clinical trial in patients with AML EHA 2025

Štach M. (Praha) a spol.

P26

DETERMINANTS OF OUTCOMES AFTER ALLOGENEIC TRANSPLANTATION (ALLOHCT) IN PATIENTS ≥70 YEARS OF AGE – SINGLE CENTRE ANALYSIS EBMT 2025

Jindra P. (Plzeň) a spol.

P27

The Transplant Program Quality Council meetings as the effective tool for the QMS introduction and maintenance and the implementation of new requirements EBMT 2025

Dobrovolná M. (Praha) a spol.

CHRONICKÁ MYELOIDNÍ LEUKÉMIE (Postery P28 – P34)

Diskusi řídí: Čuřík N. (Praha)

P28

Ponatinib-based Combination Therapies Including Asciminib and Venetoclax Were Effective on Patient-derived Xenograft Models of Chronic Myeloid Leukemia in Blast Phase ASH 2025

Čuřík N. (Praha) a spol.

P29

Allele-dependent activity of promoters of genes SLC22A4 and SLC22A5 encoding imatinib transporters in chronic myeloid leukemia blast cells ASH 2025

Burda P. (Praha) a spol.

P30

Residual normal hematopoietic stem cells predict early hematological toxicity in chronic myeloid leukemia EHA 2025

Kašša S. (Brno) a spol.

P31

Evaluating reverse transcriptase efficiency in CML: Consequences for deep molecular response classification and treatment-free remission decisions ASH 2025

Machová K. (Praha) a spol.

P32

Functional analysis of a novel germline JAK2 variant associated with thrombocytosis

Dvořáček L. (Praha) a spol.

P33

Functional Characterization of a Novel Germline JAK2 R989fs Mutation ASH 2025

Zimolová V. (Praha) a spol.

P34

Designing and testing a CML Quality of Life Tracker, a digital solution to facilitate effective and consistent communication between patients with CML and healthcare providers regarding the impact on their daily life

ESH-iCMLf 27th Annual John Goldman Conference on Chronic Myeloid Leukemia: Biology and Therapy

Žáčková D. (Brno) a spol.

LYMFOMY I (Postery P35 – P45)

Diskusi řídí: Papajík T. (Olomouc)

P35

Epcoritamab with rituximab + lenalidomide (R2) and epcoritamab maintenance deliver deep and durable remissions in previously untreated (1L) follicular lymphoma (FL): 3-year outcomes from EPCORE NHL-2 arms 6 and 7 ASH 2025

Belada D. (Hradec Králové) a spol.

P36

Fixed-duration epcoritamab + R-CHOP in patients with newly diagnosed DLBCL and high IPI scores (3–5) led to sustained remissions and disease-free survival beyond 3 years: results from the EPCORE NHL-2 trial ASH 2025

Belada D. (Hradec Králové) a spol.

P37

Epcoritamab + R-mini-CHOP results in 2-year remissions and high MRD negativity rates in elderly patients with newly diagnosed DLBCL: results from the EPCORE NHL-2 trial ASH 2025

Belada D. (Hradec Králové) a spol.

P38

Fixed-duration epcoritamab monotherapy induces high response and MRD-negativity rates in elderly patients with newly diagnosed large B-cell lymphoma (LBCL) and comorbidities: results from EPCORE DLBCL-3 ASH 2025

Belada D. (Hradec Králové) a spol.

P39

Epcoritamab + GemOx achieves durable >2-year remissions in relapsed/refractory (R/R) 2L+ diffuse large B-cell lymphoma (DLBCL): long-term data reinforce clinical potential of the regimen across a diverse patient population ASH 2025

Belada D. (Hradec Králové) a spol.

P40

Age-stratified prognostic performance of patient- vs disease-related IPI factors in large B-cell lymphoma ASH 2025

Vodička P. (Praha) a spol.

P41

Treatment patterns in patients aged 80+ years with LBCL: Propensity score analysis reveals comparable outcomes between R-CHOP and R-miniCHOP ASH 2025

Vodička P. (Praha) a spol.

P42

Predictive performance of the LEO comorbidity index in patients aged 80+ years with large B-cell lymphoma ASH 2025

Vodička P. (Praha) a spol.

P43

Improved survival with third-line CAR-T therapy vs alternative treatment in DLBCL: A propensity score–matching analysis ASH 2025

Vodička P. (Praha) a spol.

P44

Prognostic impact of diagnosis-to-treatment interval in follicular lymphoma patients treated with immunochemotherapy: Evidence from an international cohort with independent validation ASH 2025

Vodička P. (Praha) a spol.

P45

FLIPI24 as a prognostic model in marginal zone lymphoma: Results from an international multi-cohort analysis ASH 2025

Vodička P. (Praha) a spol.

LYMFOMY II (Postery P46 – P56)

Diskusi řídí: Drgoňa L. (Bratislava; SK)

P46

First disclosure of epcoritamab + R-ICE in patients with relapsed/refractory diffuse large B-cell lymphoma (R/R DLBCL) eligible for autologous stem cell transplantation (ASCT): EPCORE NHL-2 EHA 2025; SOHO 2025

Trněný M. (Praha) a spol.

P47

Diagnosticky opomíjené defekty negativně ovlivňující prognózu a přežití pacientů s difúzním velkobuněčným B-lymfomem

Marečková A. (Brno) a spol.

P48

Pre-treatment circulating tumor DNA predicts survival of diffuse large B-cell lymphoma patients and allows genetic and biological evaluation ASH 2025

Hamová I. (Praha, Vestec) a spol.

P49

Frontline Regimens and Consolidation in PMBCL: A Pooled Real-World Analysis from the Mayo Clinic and CLSG

Procházka V. (Olomouc) a spol.

P50

Four cycles of R-CHOP show the same effectivity as six cycles in real-world DLBCL patients with localized, non-bulky disease independently of other unfavourable risk factors EHA 2025

Maláriková D. (Praha) a spol.

P51

Cell Cycle Dependent and Independent Regulation of AKT Activity in Germinal Center B-cell-like Diffuse Large B-cell lymphoma – a Dual Role of Cyclin Dependent Kinase 2 ASH 2025

Rajmonová A. (Praha) a spol.

P52

Phosphoinositide dependent protein kinase 1 is a key metabolic regulator and potential therapeutic target in lymphoma ICML 2025

Heřman V. (Praha) a spol.

P53

Targeted Gene Expression Profiling for Rapid ABC/GCB Stratification of DLBCL Patients: Evaluating the Prognostic and Predictive Impact of COO Subtyping ISLH 2025

Navrkalová V. (Brno) a spol.

P54

The Efficacy and Toxicity of CNS Prophylaxis in Diffuse Large B-cell Lymphoma (CLSG-CNS-01): A Randomized, Multicenter, Prospective Phase 3 Trial ICML 2025

Mociková H. (Praha) a spol.

P55

Comparable PFS in isolated parenchymal CNS vs systemic relapse of DLBCL: Results from a large real-world cohort ASH 2025

Masár M. (Praha) a spol.

P56

Impact of Primary CNS Prophylaxis in High CNS-IPI DLBCL: Analysis from the Czech National Lymphoma Registry ASH 2025

Hricko S. (Brno) a spol.

CLL / LYMFOMY III (Postery P57 – P64)

Diskusi řídí: Špaček M. (Praha)

P57

Vitamin D and Other Micronutrients Influence Prognosis of Patients with Non-Hodgkin lymphoma (NHL). ASH 2025

Hájek M. (Brno) a spol.

P58

Epcoritamab combinations demonstrate promising efficacy in patients (pts) with Richter Transformation (RT): first results from arms 2B (epcor + lenalidomide [LEN]) and 2C (epcor + R-CHOP) of the phase 1b/2 EPCORE CLL-1 trial ASH 2025

Šimkovič M. (Hradec Králové) a spol.

P59

Clonal shifts of TP53 mutations in patients treated with BTK and BCL2 inhibitors: analysis of accompanying genomic changes iwCLL2025

Malčíková J. (Brno) a spol.

P60

Casein kinase 1δ/ε inhibition mediates chronic lymphocytic leukemia suppression by a combination of cell cycle block and decreased response to microenvironmental stimuli MILESTONE International Conference CK1

Mikulová A. (Brno) a spol.

P61

Measurable Residual Disease in Chronic Lymphocytic Leukemia (CLL) in Clinical Practice: A Single-Center Report Over the Years 2018–2024 IWCLL2025

Mihályová J. (Ostrava) a spol.

P62

Clonality assessment in multiclonal chronic lymphocytic leukemia using Sanger and next-generation sequencing approaches iwCLL - 2025

Plevová K. (Brno) a spol.

P63

Validation of Novel Prognostic Models in Symptomatic Waldenström's Macroglobulinemia Based on Real-World Data ASH 2025

Kaščák M. (Ostrava)

P64

Leukémie z velkých granulárních lymfocytů, zkušenosti pracoviště

Turcsanyi P. (Olomouc) a spol.

MYELOMY (Postery P65 – P73)

Diskusi řídí: Radocha J. (Hradec Králové)

P65

Circulating tumor cells for the staging of multiple myeloma: a European pooled analysis of 2446 newly diagnosed patients EHA 2025

Žihala D. (Ostrava) a spol.

P66

High-Dimensional Profiling Reveals Predominant Depletion of NK Cell Effector Phenotypes in Relapsed/Refractory Multiple Myeloma Patients Treated with anti-CD38 Therapy ASH 2025

Venglář O. (Ostrava) a spol.

P67

Biology of Circulating Tumor Cells (CTCs) in Multiple Myeloma (MM): Comparative Transcriptomic Profiling Reveals Egression-Associated Changes Related to CTC Burden ASH 2025

Bílek D. (Ostrava) a spol.

P68

Selective depletion of B-cell lineage subsets during treatment with anti-BCMA vs anti-GPRC5D bispecific antibodies (bsAbs) underlies different risk of infections in patients with multiple myeloma (MM) EHA 2025

Žihala D. (Ostrava) a spol.

P69

Genomic landscape of para-skeletal and extramedullary plasmacytomas in multiple myeloma patients EHA 2025

Štork M. (Brno) a spol.

P70

A Multicenter Phase 2 Study Designed to Optimize the Schedule of Belantamab Mafodotin Plus Bortezomib and Dexamethasone in Relapsed Refractory Multiple Myeloma EHA 2025

Popková T. (Ostrava) a spol.

P71

De-escalated Teclistamab Dosing in Relapsed/Refractory Multiple Myeloma: Czech Myeloma Group Real-World Evidence Study EHA 2025

Menšíková K. (Brno) a spol.

P72

CHARACTERIZATION OF OPHTHALMIC EXAMINATION FINDINGS (OEFS) AND IMPACT ON READING AND DRIVING IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA (RRMM) TREATED WITH BELANTAMAB MAFODOTIN (BELAMAF) EHA 2025

Popková T. (Ostrava) a spol.

P73

Evolution of Myeloma Bone Disease Over Time: Comparison of Selected Biomarkers in MGUS, SMM and active MM ASH 2025

Minařík J. (Olomouc) a spol.

VZÁCNÁ KREVNÍ ONEMOCNĚNÍ (Postery P74–P79)

Diskusi řídí: Mejstříková E. (Praha)

P74

Prime editing-mediated correction of FANCA mutation in primary cells from Fanconi anemia patients Genome Engineering: CRISPR Frontiers

Peterková L. (Praha) a spol.

P75

Hepatitis-Associated vs Idiopathic Paediatric Aplastic Anaemia: Clonal Architecture and Disease Trajectory EWOG MDS/SAA 2025

Dulla D. (Praha) a spol.

P76

Fostamatinib přináší nové možnosti kombinační léčby pacientů s imunitními cytopeniemi

Červinek L. (Brno) a spol.

P77

Metabolic consequences of distinct SF3B1 mutations in myelodysplastic neoplasms 4th Prague Symposium on Cancer Metabolism

Dostálová Merkerová M. (Praha) a spol.

P78

Altered T Cell Bioenergetics in Hypoplastic Myelodysplastic Neoplasms and Idiopathic Aplastic Anemia The 18th International Congress on MDS

Pinc Z. (Praha) a spol.

P79

Etiologie a léčba splanchnické trombózy ve východních Čechách

Kriegler T. (Chrudim) a spol.

EXPERIMENTÁLNÍ A TRANSLAČNÍ HEMATOLOGIE (Postery P80–P84)

Diskusi řídí: Škvárová Kramarzová K. (Praha)

P80

Chronic inflammation drives HSC aging, clonal expansion, and altered infection response in mice EHA-SWG 2025

Grusanovic S. (Praha) a spol.

P81

Polatuzumab Vedotin Induced Activation of B-cell Receptor Signaling Creates a Synthetic Lethality with BTK and AKT Inhibition in Mantle Cell Lymphoma ASH 2025

Seňavová J. (Praha) a spol.

P82

C/EBPδ preserves hematopoietic stem homeostasis and regulates myeloid commitment in a context-dependent manner EHA Recon 2025

Ribeiro Bas I. (Praha) a spol.

P83

Novel EuroFlow Quality Assessment on B-cell Precursor Acute Lymphoblastic Leukemia Minimal Residual Disease. Program development and Four Rounds of Implementation EHA 2025

Reiterová M. (Praha) a spol.

P84

Chimerism analysis after allogeneic hematopoietic stem cell transplantation: clinical relevance and methodology 38th European Immunogenetics & Histocompatibility Conference

Stefflová L. (Praha) a spol.

čtvrtek 22. 1. 2026

8:30 - 9:15 Satelitní sympozia

CSL Behring s.r.o. (sály Zenit/Nadir)

Gilead Sciences s.r.o. (sály Tycho/Kepler)

9:30 – 10:30

Meet the expert workshopy 1 – 3

→ SÁLY TYCHO + KEPLER *(BLOK V AJ)*

1 How I treat Waldenstrom macroglobulinemia

Steven Treon (Boston; USA) moderuje: Marek Trněný (Praha)

→ SÁLY LEO + VIRGO

2 „Výživové mýty pod lupou: Praktický průvodce populárními otázkami“

Adam Obr (Praha) & David Erban (Praha)

→ SÁLY AQUARIUS + TAURUS *(BLOK V AJ)*

3 „AI in health - where are we going?“

Uri Ilan (Utrecht; NL) moderuje: Jan Zuna (Praha)

10:30 – 11:00 **Přestávka na kávu**

→ SÁLY ZENIT + NADIR

11:00 – 12:45

Odborný program – Blok III

Předsedající: Jindra P. (Plzeň), Šrámková L. (Praha)

11:00 – 11:20

E07 **Novinky v biologii a léčbě akutní lymfoblastové leukémie 2025**
Šálek C. (Praha)

11:20 – 11:35

O07 EHA 2025
Elevated circulating tumor cell (CTC) numbers associate with a highly proliferative (PR) and genomically complex phenotype in newly diagnosed multiple myeloma (NDMM) patients
Ševčíková T. (Ostrava) a spol.

11:35 – 11:50

O08 ASH 2025
Longitudinal immune monitoring in patients treated with CD19 and BCMA CAR-T cells using standardized 8 color flow cytometry panel reveals substantial differences among products kinetics and persistence
Venglář O. (Ostrava) a spol.

11:50 – 12:05

O09 ASH 2025
Immune and DNA-damage responses in the intestinal stroma of patients presenting with lower gastro-intestinal symptoms after allogeneic stem cell transplantation
Kuba A. (Olomouc)a spol.

čtvrtek 22. 1. 2026

12:05 – 12:25

E08 Alogenní transplantace – myelodysplastický syndrom

Marková M. (Praha)

12:25 – 12:45

E09 Potransplantační lymfoproliferace

Novák J., Racková M. (Praha)

12:45 – 13:45 **Oběd (hotelová restaurace Veduta, 2. patro)**

13:30 - 14:30 Satelitní sympozia

Novartis s.r.o. (sály Zenit/Nadir, 13:30-14:30)

Johnson&Johnson (sály Leo/Virgo, 13:45-14:30)

eha

Česká hematologická společnost ČLS JEP

→ **SÁLY ZENIT + NADIR**

14:45 – 16:15

Odborný program – Blok IV
EHA / CSH JOINT SYMPOSIUM *(BLOK V AJ)*

Předsedající: Döhner K. (Ulm; Germany); Žák P. (Hradec Králové)

14:45 – 15:15

Perspectives on Current Diagnosis and Follow-up Strategies in Adult AML Patients

Döhner K. (Ulm; Germany)

15:15 – 15:45

AI for oncologists, when data thinks

Ilan U. (Utrecht; NL)

15:45 – 16:15

Multiple myeloma in 2026 and Beyond

Jelínek T. (Ostrava)

16:15 – 16:45 **Přestávka na kávu**

čtvrtek 22. 1. 2026

16:45 – 18:15

Odborný program – Blok V
Best of papers / best of abstracts

Předsedající: Cetkovský P. (Praha), Zuna J. (Praha)

Nejlepší publikace českých autorů v roce 2025

Uvede: Špaček M. (Praha)

BP01 **Detection of clinically relevant variants in the TP53 gene below 10% allelic frequency: A multicenter study by ERIC, the European Research Initiative on CLL**
Pavlová Š. (Brno)

Uvede: Šálek C. (Praha)

BP02 **NGS-MRD negativity in post-HSCT ALL spares unnecessary therapeutic interventions triggered by borderline qPCR results without an increase in relapse risk**
Šeferna K. (Praha)

Oceněná sdělení mladých hematologů

Uvede: Procházka V. (Olomouc)

O10 ASH 2025
Optimizing risk stratification in patients aged 80+ years with large B-cell lymphoma: Comparison of prognostic indices in three pooled international cohorts
Vodička P. (Praha) a spol.

Uvede: Stopka T. (Praha)

O11 ASH 2025
Anthracyclines induce epigenetic changes in ALDH1A that promote chemoresistance in AML, revealing a potential treatment strategy co-targeting ALDH1A inhibition with hypomethylating agents
Shaikh M. (Praha) a spol.

18:30 - 19:15 Satelitní sympozia

Bristol-Myers Squibb spol. s r.o. (sály Zenit/Nadir)
Eli Lilly ČR, s.r.o. (sály Tycho/Kepler)

19:30 **Večeře (hotelová restaurace Veduta, 2. patro)**

pátek 23. 1. 2026

pátek 23. 1. 2026

8:30 - 9:15 Satelitní sympozia

GlaxoSmithKline, s.r.o. (sály Zenit/Nadir)

Swixx Biopharma s.r.o. (sály Leo/Virgo)

9:30 - 10:15 Satelitní sympozium

AbbVie s.r.o. (sály Zenit/Nadir)

10:30 – 11:30

Meet the expert workshopy 4 – 5

→ **SÁLY AQUARIUS + TAURUS** *(BLOK V AJ)*

4 MRD in AML

Konstanze Döhner (Ulm; Germany) moderuje: Jan Trka (Praha)

→ **SÁLY LEO + VIRGO**

5 Konzumpční koagulopatie v klinické praxi

Peter Salaj (Praha) & Jan Bláha (Praha)

11:30 – 12:00 **Přestávka na kávu**

→ **SÁLY ZENIT + NADIR**

12:00 – 13:30

Odborný program – Blok VI

Předsedající: Kozák T. (Praha), Papajík T. (Olomouc)

12:00 – 12:20

E10 Novinky v diagnostice a léčbě DLBCL- postASH

Klener P. (Praha)

12:20 – 12:40

E11 Praktický přístup k léčbě CLL: současné možnosti a jejich optimální využití

Šimkovič M. (Hradec Králové)

pátek 23. 1. 2026

12:40 – 12:55

O12 ASH 2025

Bulky disease and/or very high LDH define a high-risk LBCL subgroup within IPI 1–2, but only in younger patients

Vodička P. (Praha) a spol.

12:55 – 13:10

O13 EHA 2025

Tafasitamab (tafa) plus Lenalidomide (len) and Rituximab (R) for Patients With Relapsed or Refractory Follicular Lymphoma (R/R FL): Results from the Phase 3 inMIND Study

Trněný M. (Praha) a spol.

13:10 – 13:30

E12 **Hodgkinův lymfom 2025: Klíčová sdělení**

Sýkorová A. (Hradec Králové)

13:30 **ZAKONČENÍ KONFERENCE**

Trka J. (Praha), Trněný M. (Praha)

Oběd (hotelová restaurace Veduta, 2. patro)

SAVE THE DATE!

Srdečně Vás zveme
na 27. pražské hematologické dny – HEMATOLOGIE 2027
Termín: 27. – 29. 1. 2027

OŠETŘOVATELSKÝ PROGRAM

čtvrtek 22. 1. 2026

→ SÁLY TYCHO + KEPLER

11:00 - 12:30

Ošetřovatelský program I. - "Infekce pod kontrolou"
edukační program

Předsedající: Vylitová L. (Praha), Wintnerová J. (Praha)

11:00 – 11:20

ES01 **Standardní a cílená protiepidemická opatření na pracovištích Kliniky dětské hematologie a onkologie 2. LF UK a FN Motol z pohledu Oddělení nemocniční hygieny a epidemiologie**
Hrončeková J. (Praha)

11:20 – 11:40

ES02 **Sepse u dětských hematoonkologických pacientů – závažná komplikace léčby**
Čermáková J. (Praha)

11:40 – 12:00

ES03 **Hygienické režimy a zkušenosti s jejich aplikací v ÚHKT**
Labská K. (Praha)

12:00 – 12:20

ES04 **Jak chránit planetu a neohrozit pacienta?**
Hráčková H. (Praha)

Přestávka

12:30 - 13:10

Vzdělávací seminář: Jak zjednodušit práci sestry a život hematoonkologickým pacientům se sekundární imunodeficiencí
Milota T.: Sekundární imunodeficience – základy diagnostiky a léčby v hematoonkologii (15 min.)
Henebergová D.: Cesta k lepší spolupráci pacienta – imunoglobulinová léčba krok za krokem (20 min.)

12:30 – 14:00 **Oběd (restaurace Veduta, 2. patro)**

14:00 – 15:30

Ošetřovatelský program II. – Workshop Psychologie

„Jak se dělá duševní zdraví: co si lze z psychoterapie odnést jako univerzální poznání pro duševní zdraví"
Miroslav Světlák
(Ústav lékařské psychologie a psychosomatiky Masarykovy univerzity v Brně)

pátek 23. 1. 2026

→ SÁLY TYCHO + KEPLER

9:00 – 10:30

Ošetřovatelský program III. – Workshop - Komunikační dovednosti s mediačním nástrojem

Petra Absolonová (Brno)

10:30 – 11:00 **Přestávka na kávu**

11:00 – 13:00

Ošetřovatelský program IV – Varia

Předsedající: Hrabánková D. (Praha), Turková L. (Praha)

S01 **„Jídlo jako lék“**
Růžičková L. (Praha)

S02 **Uzdrav mysl - Mindfullnes**
Malyszková K. (Ostrava)

S03 **„Aromaterapie k duševní harmonii“**
Vondrová M. (Praha)

S04 **Jak dopadl projekt „Prevence syndromu vyhoření“ na Klinice dětské hematologie a onkologie?**
Wintnerová J. (Praha)

S05 **Fytoterapie a koření v hematoonkologii – vliv na účinnost léčby**
Vedrová J., Rozsívalová P. (Praha)

S06 **Od stigmat k standardům - konopí v moderní ošetřovatelské péči**
Koňařík M. (Ostrava)

S07 **Bertíkovo uzdravení jako nástroj pro komunikaci závažné diagnózy u dětí nízkého věku s onkologickým onemocněním**
Keslová P. (Praha)

13:00 ZAKONČENÍ KONFERENCE, OBĚD

čtvrtek 22. 1. 2026

16:00 - 17:30

Organizuje: Pacientská organizace Lymfom Help

→ SÁLY TYCHO + KEPLER

1. pacientský seminář nejen pro pacienty - Onkopsychologie
“Síla jemných emocí při zvládání nemoci aneb možnosti snížení prožitků NEJISTOTY při onkologické léčbě, ale i po ní.”
Martin Pospíchal (Praha)

SAVE THE DATE!

Srdečně Vás zveme
na 27. pražské hematologické dny – HEMATOLOGIE 2027
Termín: 27. – 29. 1. 2027

SATELITNÍ SYMPOZIA

ČTVRTEK 22. 1. 2026

CSL Behring

8:30-9:15 SÁLY ZENIT / NADIR

HEMATOONKOLOGIE V POHYBU: OD DAT K UZDRAVENÍ, OD DOPORUČENÍ K NÁVRATU DO ŽIVOTA

Předsedající a moderátor:
prof. MUDr. Marek Trněný, CSc.

CESTA K UZDRAVENÍ: ROSTOUCÍ PŘEŽÍVÁNÍ A PRACOVNÍ REINTEGRACE HEMATOONKOLOGICKÝCH PACIENTŮ
prof. RNDr. Ladislav Dušek, Ph.D.

OD GUIDELINES K PRAXI: JAK IMUNOGLOBULINOVÁ LÉČBA MĚNÍ PÉČI O HEMATOONKOLOGICKÉ PACIENTY
prof. MUDr. Roman Hájek, CSc.

DLOUHODOBÉ KOMPLIKACE MODERNÍ HEMATOONKOLOGICKÉ LÉČBY: LZE JIM PŘEDCHÁZET?
prof. MUDr. Michael Doubek, Ph.D.

ČTVRTEK 22. 1. 2026

GILEAD
Creating Possible

8:30-9:15 SÁLY TYCHO / KEPLER

CAR T NOVÁ CZ/SK LÉČEBNÁ DOPORUČENÍ PRO ŘEŠENÍ NEŽÁDOUCÍCH ÚČINKŮ
CAR T 6letá RWD V ČR A KAZUISTIKY

NOVÁ CZ/SK LÉČEBNÁ DOPORUČENÍ PRO ŘEŠENÍ NEŽÁDOUCÍCH ÚČINKŮ A 6letá RWD V ČR
MUDr. František Folber, Ph.D.

Diskuze

2 KAZUISTIKY U KOMPLIKOVANÝCH PACIENTŮ
MUDr. Robert Pytlík, Ph.D.

Diskuze

ČTVRTEK 22. 1. 2026

NOVARTIS

13:30-14:30 SÁLY ZENIT / NADIR

"ŽÍT, NEJEN PŘEŽÍVAT"

Moderátoři:
MUDr. Petra Bělohlávková, Ph.D.
prof. MUDr. Jaroslav Čermák, CSc.

KDYŽ SE ÚČINNOST POTKÁ S BEZPEČNOSTÍ A ZLEPŠENÍM KVALITY ŽIVOTA: NOVÁ ÉRA INICIÁLNÍ LÉČBY CML
doc. MUDr. Daniela Žáčková, Ph.D.

ZROZENÍ PERORÁLNÍ MONOTERAPIE PNH
prim. MUDr. Jaromír Gumulec

MPN, KDYŽ SYMPTOMY PŘESTANOU BÝT TÉMA
prof. MUDr. Michael Doubek, Ph.D.

Diskuse

ČTVRTEK 22. 1. 2026

Johnson&Johnson

13:45-14:30 SÁLY LEO / VIRGO

HEMATOLOGICKÝ VÍCEBOJ

Předsedající:
prof. MUDr. Marek Trněný, CSc.
(I. interní klinika – klinika hematologie VFN Praha)

1.disciplína: **PRVNÍ LINIE TERAPIE CLL**
MUDr. Martin Špaček, Ph.D.
(I.interní klinika – klinika hematologie VFN Praha)

2.disciplína: **PRVNÍ LINIE TERAPIE MM**
prof. MUDr. Luděk Pour, Ph.D.
(Interní hematologická a onkologická klinika, FN Brno)

3.disciplína: **ČTVRTÁ A DALŠÍ LINIE TERAPIE MM**
MUDr. Jan Straub
(I.interní klinika – klinika hematologie VFN Praha)

ČTVRTEK 22. 1. 2026

Bristol Myers Squibb™

18:30-19:15 SÁLY ZENIT / NADIR

DATA ÚČINNOSTI A BEZPEČNOSTI BREYANZI V LÉČBĚ LYMFOMU

Předsedající:
prof. MUDr. Marek Trněný, CSc.

POTVRZUJÍ DATA Z REÁLNÉ PRAXE DLOUHODOBOU ÚČINNOST LISOCELU U DLBCL?
doc. MUDr. David Belada, Ph.D.

NEŽÁDOUCÍ ÚČINKY CAR-T: EXISTUJÍ NĚJAKÉ PREDIKTIVNÍ FAKTORY?
MUDr. František Folber, Ph.D.

ZKUŠENOSTI S CAR-T TERAPIÍ BREYANZI VE VFN, MOŽNOST LÉČBY PRO FL
MUDr. Kamila Polgárová, Ph.D.

ČTVRTEK 22. 1. 2026

Lilly
A MEDICINE COMPANY

18:30-19:15 SÁLY TYCHO / KEPLER

INHIBITORY BRUTONOVY TYROZINKINÁZY U RELABOVANÉ/REFRAKTERNÍ CHRONICKÉ LYMFOCYTÁRNÍ LEUKEMIE (R/R CLL) A RELABOVANÉHO/REFRAKTERNÍHO LYMFOMU Z BUNĚK PLÁŠTĚ (R/R MCL) – AKTUÁLNÍ MOŽNOSTI LÉČBY

Předsedající:
prof. MUDr. Tomáš Papajík, CSc.
(FN Olomouc)

BTKi V ALGORITMU LÉČBY R/R CLL V ČESKÉ REPUBLICE, AKTUÁLNÍ MOŽNOSTI LÉČBY
MUDr. Martin Špaček, Ph.D.
(VFN Praha)

BTKi V ALGORITMU LÉČBY R/R MCL V ČESKÉ REPUBLICE, AKTUÁLNÍ MOŽNOSTI LÉČBY
prof. MUDr. Pavel Klener, Ph.D.
(VFN Praha)

Diskuse, shrnutí a závěr
prof. MUDr. Tomáš Papajík, CSc.
(FN Olomouc)

PÁTEK 23. 1. 2026

GSK

8:30-9:15 SÁLY ZENIT / NADIR

OD TEORIE K PRAXI: ZAMĚŘENO NA MYELOFIBRÓZU

Předsedající:
doc. MUDr. Anna Jonášová, Ph.D.
(I. interní klinika – klinika hematologie - VFN v Praze)

JAK2 INHIBITORY U CYTOPENICKÝCH PACIENTŮ
prof. MUDr. Michael Doubek, Ph.D.
(Interní hematologická a onkologická klinika - FN Brno)

CO MŮŽEME OČEKÁVAT OD LÉČBY MOMELOTINIBEM?
MUDr. Petra Bělohlávková, Ph.D.
(IV.interní hematologická klinika – FN Hradec Králové)

ÚSPĚŠNÁ A VELMI DOBŘE TOLEROVANÁ LÉČBA POSTPOLYCYTEMICKÉ MYELOFIBRÓZY MOMELOTINIBEM (OMJJARA®) VE 3. LINII LÉČBY INHIBITOREM JANUSOVÝCH KINÁZ
MUDr. Olga Černá
(Interní hematologická klinika - FN Královské Vinohrady)

PÁTEK 23. 1. 2026

Swixx BioPharma
Modern Medicines for All

8:30-9:15 SÁLY LEO / VIRGO

INTERAKTIVNĚ SE ZANUBRUTINIBEM: 4× JINAK V KLINICKÉ PRAXI

DRUHÁ LINIE LÉČBY CLL A VÝZVY VOLBY TERAPIE
MUDr. Martin Špaček, Ph.D.
(VFN Praha)

WALDESTRÖMOVA MAKROGLOBULINÉMIE SE VZÁCNOU NEUROLOGICKOU KOMPLIKACÍ
MUDr. Adriana Heindorfer
(Krajská nemocnice Liberec)

REFRAKTERNÍ LYMFOM MARGINÁLNÍ ZÓNY A ROZHODNUTÍ, KTERÁ MĚNÍ PRŮBĚH
MUDr. Robert Pytlík, Ph.D.
(ÚHKT Praha)

NA POMEZÍ DVOU B LYMFOPROLIFERACÍ
MUDr. Juraj Ďuraš
(FN Ostrava)

PÁTEK 23. 1. 2026

abbvie

9:30-10:15 SÁLY ZENIT / NADIR

VENETOKLAX JAKO ZÁKLAD KOMBINOVANÉ TERAPIE

Předsedající:
prof. MUDr. Michael Doubek, Ph.D.
(Interní hematologická a onkologická klinika FN Brno)

KAM SMĚŘUJE LÉČBA CLL?
MUDr. Martin Špaček, Ph.D.
(I. interní hematologická klinika 1. LF UK a VFN v Praze)

CO PŘINÁŠÍ LÉKAŘŮM A PACIENTŮM ČASOVĚ OMEZENÁ LÉČBA – video

INTEGRACE PACIENTSKÝCH VÝSLEDKŮ DO KLINICKÉHO ROZHODOVÁNÍ: MOŽNOSTI A VÝZVY
MUDr. Dominika Écsiová
(IV. interní hematologická klinika FN Hradec Králové)

Diskuzní panel k tématu aktuálních možností a zkušeností s cílenou léčbou:
prof. MUDr. Michael Doubek, Ph.D.
MUDr. Dominika Écsiová
prof. MUDr. Tomáš Papajík, CSc.
MUDr. Martin Šimkovič, Ph.D.
MUDr. Martin Špaček, Ph.D.

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SBORNÍK ABSTRAKTŮ

SBORNÍK ABSTRAKTŮ

EDUKAČNÍ PROGRAM

(ABSTRAKTY E01 – E12)

SBORNÍK ABSTRAKTŮ

HEŘMANSKÉHO PŘEDNÁŠKA

GENOMICS AND TREATMENT IN WALDENSTROM'S MACROGLOBULINEMIA

Steven P. Treon
Bing Center for Waldenstrom's Macroglobulinemia, Dana Farber Cancer Institute; and Harvard Medical School, Boston MA USA.

INTRODUCTION

Waldenström macroglobulinemia (WM) is a B-cell lymphoid neoplasm resulting from the accumulation of a clonal population of lymphocytes, lymphoplasmacytic cells, and plasma cells, which secrete a monoclonal IgM. WM corresponds to lymphoplasmacytic lymphoma (LPL) as defined in the International Consensus Classification of Mature Lymphoid Neoplasms, and the World Health Organization classification systems.[1,2] Most cases of LPL are WM; less than 5 percent of cases are IgA-secreting, IgG-secreting, or non-secreting LPL.[2] The key mutations in WM include MYD88, CXCR4 and TP53. Up to half of WM patients have loss of the long arm (q) of chromosome 6. Acquired BTK mutations are common in those patients who progress on BTK inhibitors. The importance of these mutations to the pathogenesis and management of WM is discussed below.

MYD88 MUTATIONS

Mutated MYD88 ($MYD88^{Mut}$) is found in 95-97% of WM patients, nearly all of which are of the L265P variant.[3-8] AS-PCR is preferable for $MYD88^{L265P}$ detection since next-generation sequencing (NGS) may miss $MYD88^{L265P}$ in 35% of WM patients, particularly in those with a bone marrow (BM) disease burden of <10%.[9] The signaling cascades triggered by $MYD88^{Mut}$ are dependent on Hematopoetic Cell Kinase (HCK), BTK and IL-1 receptor-associated kinases (IRAKs).[10-16] HCK activates BTK-dependent NFKB and ERK1/2 signaling, whilst IRAK1 and IRAK4 molecules trigger NFKB independent of BTK. Up to 5% of WM patients are MYD88 wild-type ($MYD88^{WT}$). Many of these patients carry NFKB-activating mutations distal to BTK signaling.[17] $MYD88^{WT}$ patients have a higher risk for disease transformation and show shorter overall survival (OS).[18,19] $MYD88^{Mut}$ status can also differentially impact outcomes with BTK inhibitors (discussed below). Current workshop guidelines recommend the determination of $MYD88^{Mut}$ status as part of the diagnostic workup by AS-PCR for the $MYD88^{L265P}$ variant. If negative, NGS may be used to identify any non-$MYD88^{L265P}$ variants.[20]

CXCR4 MUTATIONS

$CXCR4^{Mut}$ are found in 30-40% of WM patients.[4,21] Over 40 CXCR4 nonsense and frameshift variants in the C-terminal domain have been identified in WM patients.[4,7,24-27] Nonsense ($CXCR4^{Mut/NS}$) variants such as $CXCR4^{S338X}$ cause truncation of the C-terminal regulatory domain while frameshift ($CXCR4^{Mut/FS}$) variants result from insertions or deletions.[4,21-23] In response to CXCL12, $CXCR4^{Mut}$ triggers BTK, AKT, and ERK signaling, which promotes BM chemotaxis and ibrutinib-resistance.[24-26] CXCR4 antagonists such as plerixafor or ulocuplumab can sensitize $CXCR4^{Mut}$-expressing WM cells to ibrutinib.[24-26]

$CXCR4^{Mut/NS}$ WM patients present with higher BM disease burden and serum IgM (sIgM) levels and are more likely to have symptomatic hyperviscosity. $CXCR4^{Mut}$ patients, particularly those with nonsense variants, may also have a shorter time to initial treatment versus those with either $CXCR4^{WT}$ or $CXCR4^{Mut/FS}$.[7,22,23] One study also showed shorter OS in patients with $CXCR4^{Mut/NS}$ versus $CXCR4^{WT}$ or $CXCR4^{Mut/FS}$.[27] $CXCR4^{Mut/NS}$ may also adversely impact treatment outcomes with BTK inhibitors versus $CXCR4^{WT}$ or $CXCR4^{Mut/FS}$.[28] The importance of $CXCR4^{Mut}$ subtype on ibrutinib outcomes was evaluated in 180 symptomatic WM patients receiving ibrutinib. $CXCR4^{Mut/NS}$ was associated with lower major response rate (MRR) and shorter PFS versus those with $CXCR4^{Mut/FS}$ or $CXCR4^{WT}$.[28] NGS may miss two-thirds of patients with $CXCR4^{Mut}$, particularly those with lower BM disease burden and clonality.[29] Newer NGS platforms may improve detection.[27] Current workshop guidelines recommend that $CXCR4^{Mut}$ status be considered part of the diagnostic workup by NGS, particularly in patients being considered for BTK inhibitor therapy.[20]

TP53 ALTERATIONS

Alterations in TP53 ($TP53^{Alt}$) in bone marrow CD19-selected WM cells occur in 5-15% of treatment-naïve WM patients, including $TP53^{Mut}$ and 17p deletions.[7,30,31] $TP53^{Alt}$ WM patients showed shorter OS and/or PFS with BTK inhibitors.[30-32.] The incidence of $TP53^{Alt}$ was reported to be higher (25-30%) in both treatment naïve and previously treated WM patients using next generation sequencing (NGS) in unselected bone marrow mononuclear cells. Most of the previously treated patients in that study received alkylators (85%) and/or nucleoside analogues (22%).[32] However, the high incidence of $TP53^{Alt}$ disease raised the possibility of co-incidental clonal hematopoeisis (CHiP) clones which are frequently found in WM patients, and impact time to first therapy.[33] To further clarify the incidence of landscape of TP53 alterations in previously treated WM patients, we performed NGS and observed an $TP53^{Alt}$ incidence rate of 12%. Importantly, previously treated WM patients exposed to alkylators and/or nucleoside analogues showed a higher incidence of TP53 alterations compared to non-chemotherapy treated patients.[34] Notably, previously treated WM patients with double $TP53^{Alt}$ hits had an inferior overall survival that was more prevalent among patients treated with both alkylators and nucleoside analogues, as well as those re-treated with chemotherapy.[34] Current workshop guidelines recommend evaluating for $TP53^{Alt}$ at diagnosis and relapse using NGS as their presence may guide treatment considerations.[20] As these studies have been qualitative, no cutoffs for $TP53^{Alt}$ have been established.

DELETIONS IN CHROMOSOME 6q

Deletions in 6q (del6q) are present in up to half of WM patients at diagnosis and are almost always are heterozygous. 6q is of particular interest since important regulators of BTK (IBTK), MYD88/NFKB (TNFAIP3, HIVEP2, TRAF3IP2, IRAK1BP1), and regulators of apoptosis (FOXO3, BCLAF1, PERP) are located at this locus.[35] Serial whole exome sequencing identified homozygous deletions in 6q in WM patients progressing on ibrutinib, including evolution from heterozygous to homozygous loss of 6q at the time of progression.[36]

BTK MUTATIONS

BTK^{Cys481} is the binding site for covalent BTK inhibitors (cBTK-i), including ibrutinib, zanubrutinib, acalabrutinib, orelabrutinib and tirabrutinib. BTK^{Cys481} variants are the most common mutations associated with acquired ibrutinib-resistance in WM patients.[37] Multiple clones bearing different BTK^{Cys481} mutations can occur within individual WM patients who progress on ibrutinib.[37] WM cells expressing the $BTK^{Cys481Ser}$ mutation show ibrutinib-resistance and re-activation of BTK-PLCγ2-ERK1/2 signaling.[16] Use of ERK1/2 inhibitors triggers apoptosis in $BTK^{Cys481Ser}$ expressing cells, and re-sensitization to ibrutinib.[16] Moreover, ERK1/2 re-activation is accompanied by IL-6 and IL-10 release which protects co-cultured wild-type BTK^{Cys481} WM cells from ibrutinib, demonstrating a paracrine means for propagating cBTK-i resistance.[16]

GENOMICS AND TREATMENT APPROACH IN WM

The recommendations presented below considered recent consensus panel guidance.[38] For symptomatic treatment-naïve patients, chemoimmunotherapy with bendamustine and rituximab (Benda-R), Dexamethasone, Rituximab, and Cyclophosphamide (DRC), as well as cBTK-i can be considered. For chemoimmunotherapy, Benda-R may offer an advantage over DRC since the former may offer deeper responses and longer PFS.[39,40] For $MYD88^{Mut}$ only patients, using a cBTK-i may be appropriate to minimize the risk for acquired $TP53^{Alt}$. As all cBTK-i exhibit similar activity in $MYD88^{Mut}$ only patients, the choice should consider accessibility and adverse event profile, including risk for atrial fibrillation in patients at risk.[38] For $CXCR4^{Mut}$ patients requiring a rapid response, Benda-R or zanubrutinib are active options.[41,42,43,44] Rituximab should be held in any rituximab-containing regimens, and plasmapheresis should be performed in those with symptomatic hyperviscosity. Rituximab should also be held in patients without symptomatic hyperviscosity and chemotherapy offered alone until the serum IgM levels are <4,000 mg/dL to avoid triggering a hyperviscosity crisis.[38] The median time to a major response was 2.8 months in $CXCR4^{Mut}$ WM patients receiving zanubrutinib in the ASPEN

study, comparable to Benda-R.[41] Benda-R may be preferable in patients with bulky disease or symptomatic light chain amyloidosis.[38,44]

Zanubrutinib can also be considered in CXCR4^{Mut} patients who do not need rapid disease control since a shorter time to major response, deeper responses, and longer PFS were observed versus ibrutinib.[41] For MYD88^{WT} patients, zanubrutinib is favored for symptomatic, treatment-naïve patients since high response levels and long-term disease control can be achieved.[41] Benda-R and proteasome-inhibitor (PI) -based therapy are reasonable alternatives in CXCR4^{Mut} or MYD88^{WT} patients.[38] TP53^{Alt} status can be considered in positioning BTK inhibitors. Zanubrutinib is preferable for TP53^{Alt} WM patients given the ASPEN study findings showing higher levels of activity and long-term disease control versus ibrutinib.[32,41]

The recommendations for previously treated, symptomatic WM patients that are noted below considered recent consensus panel guidance.[43] The consensus panel noted that biological age, co-morbidities and fitness, nature of relapse, patient preferences, hematopoietic reserve, and MYD88, CXCR4, and TP53 mutation status should be considered in treatment selection. For MYD88^{Mut} only patients who are refractory or in first relapse following initial chemoimmunotherapy, cBTK-i can be considered. As all cBTK-i exhibit similar response activity in MYD88^{Mut} only patients, the choice of agent should consider accessibility, disease morbidity, and adverse event profile in WM (summarized in Ref. 44). For MYD88^{Mut}CXCR4^{Mut} patients who are refractory or in first relapse after initial chemoimmunotherapy, zanubrutinib may be preferable.[44] In MYD88^{WT} WM patients, zanubrutinib is preferable after initial chemoimmunotherapy.[32,41] Zanubrutinib is also preferred for TP53^{Alt} WM patients, as noted above.[32,41]

Benda-R is an option regardless of genomic subtype for WM patients who are refractory to initial cBTK-i therapy.[44] However, in younger patients, the use of alkylators should be mitigated given the potential for acquiring TP53^{Alt}.[34] As discussed above, rituximab should be held in any rituximab-containing regimens, and plasmapheresis should be performed in those with symptomatic hyperviscosity. Rituximab should also be held in patients without symptomatic hyperviscosity and chemotherapy offered alone until the serum IgM levels are <4,000 mg/dL to avoid triggering a hyperviscosity crisis.[38,43] For those progressing after initial cBTK-i response, options include Benda-R, PI-based therapy, venetoclax, or pirtobrutinib. Alkylator exposure should be avoided, particularly in patients <70 years or with TP53^{Alt}. Venetoclax may be preferable for these patients since it is highly active in WM patients previously exposed to cBTK-i or with CXCR4^{Mut} disease.[45,46] The activity of venetoclax in MYD88^{WT} or TP53^{Alt} WM patients remains to be clarified. Pirtobrutinib is an option post-cBTK-i therapy, though its activity in MYD88^{WT} or MYD88^{Mut}CXCR4^{Mut} patients is not known.[47,48] Benda-R or PI-based regimens can also be considered for those progressing on a cBTK-i as these regimens appear active across all genomic subtypes.[42,44,49] Additional options in second or later relapse include re-use of chemotherapy if a response lasted for >3 years, alternative chemoimmunotherapy, nucleoside analogues, or everolimus.[38] Clinical trials should also be prioritized in patients with relapsed disease.

Benda-R is more suitable for WM patients with bulky extramedullary disease since data on BTK inhibitors in patients with bulky disease is limited. For WM patients with symptomatic light chain amyloidosis, consensus recommendations favor Benda-R or PI-based therapy followed by consolidation with high-dose chemotherapy and autologous stem cell transplant in suitable WM patients.[50] Covalent BTK-inhibitors are highly active and show durable responses in WM patients with CNS disease (Bing Neel Syndrome).[51-53]

EMERGING TREATMENT OPTIONS

Newer agents being developed for WM include the BTK degrader BGB-16673 and the BCL2 inhibitor sonrotoclax. In 27 heavily pre-treated WM patients (median prior therapies of 3), the overall and major response rates to single-agent BGB-16673 were 82% and 74% and were not impacted by

MYD88, CXCR4 or TP53 mutation status.[54] Responses were observed in patients carrying BTK mutations associated with acquired resistance to covalent (BTK^{Cys481}) and non-covalent (BTK^{Leu528}) BTK inhibitors. Treatment was well tolerated, and no episodes of atrial fibrillation were observed. The efficacy of single-agent sonrotoclax has also been evaluated in a Phase 1 study in 19 previously treated WM patients. In this Phase 1 study, patients received 80, 160, and 320 mg daily. The overall and major response rates were 79% and 58%.[55]

Combination studies are also underway with BTK and BCL2 inhibitors in WM. Zanubrutinib in combination with ixazomib and dexamethasone (ZID) is being investigated in a study in China (NCT04463953) and has shown high levels of response activity and good tolerance in symptomatic, treatment-naïve patients.[56] The overall, major, and VGPR/CR remission rates were 100%, 96%, and 46%, respectively. The median time to response was 2 months. Patients with mutations in *CXCR4* had similar VGPR/CR rates. The combination of Benda-R with acalabrutinib is being investigated in a multicenter (BRAWM Study) as first line therapy in WM.[57] Patients received one year of acalabrutinib along with 6 cycles of Benda-R. In a preliminary report, the major response rate was 100%, with 42% of patients achieving a VGPR/CR. Patients without CXCR4 mutations showed better VGPR/CR rates at cycle 12. The multicenter ZEBRA study has recently been initiated, and it will combine 15 months of zanubrutinib with four cycles of Benda-R (NCT06561347). The combination of pirtobrutinib with venetoclax is also being investigated in previously treated, symptomatic patients (NCT05734495).[58] Patients receive 2 years of treatment in this study. The MRR was 87%, though responses were impacted by CXCR4 mutation status.[58] Combination studies with zanubrutinib and sonrotoclax are also contemplated in WM. The combination of acalabrutinib plus rituximab is also under investigation in patients with demyelinating neuropathy and concurrent IgM monoclonal gammopathy (NCT05065554). In Germany, the CZAR-1 study is investigating the efficacy and safety of carfilzomib in combination with ibrutinib versus ibrutinib alone in treatment-naïve and previously treated WM (NCT04263480). A second German study is also investigating the combination of Benda-R and ibrutinib (NCT03620903). Immunotherapies targeting WM are also advancing. A clinical trial with the antibody drug conjugate locastuximab tesirine that targets CD19 is enrolling WM patients with symptomatic, previously treated WM (NCT05190705). Notably, loncastuximab was highly active in previously treated $TP53^{Alt}$ WM patients in this study.[59] A study with the CD3/CD20 bispecific antibody epcoritamab has also been initiated in symptomatic previously treated WM (NCT06510491).

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NEUWIRTOVA PŘEDNÁŠKA

NOVEL TARGETS FOR CAR-T CELLS FOR HEMATOLOGICAL CANCERS

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Cancer-specific cell-surface antigens are ideal targets for CAR-T cell therapy but are likely to have previously been identified in transcriptome or proteome analyses. We screened >10,000 anti-MM mAb clones and identified MMG49 and R8H283 as MM-specific mAbs. MMG49 specifically recognizes the active conformer of integrin β_7. Elevated expression and constitutive activation of integrin β_7 conferred high MMG49 reactivity on MM cells, whereas MMG49 binding was scarcely detectable in other cell types including normal integrin β_7^+ lymphocytes. T cells transduced with MMG49-derived chimeric antigen receptor (CAR) exerted anti-MM effects without damaging normal hematopoietic cells, and is now being tested in a clinical trial (***Nat Med*** 23, 1436–1443, 2017).

SBORNÍK ABSTRAKTŮ

R8H283 specifically recognized CD98hc. R8H283 did not react with monomers of CD98hc; instead, it bound CD98hc in heterodimers with a CD98 light chain (CD98lc). Although CD98 heterodimers were also present on normal leukocytes, R8H283 did not react with them. The glycoforms of CD98hc present on normal leukocytes were distinct from those present on MM cells, which may explain the lack of R8H283 reactivity to normal leukocytes (***Sci Transl Med*** 14, eaax7706, 2022).

Acute myeloid leukemia (AML)-specific target antigens are difficult to identify. We identified KG2032 as a monoclonal antibody specifically bound to AML cells in about half of patients, but not to normal leukocytes other than B lymphocytes. KG2032 reacted with a subset of HLA-DRB1 molecules, specifically those in which the 86th amino acid was not aspartic acid. KG2032 reacted minimally with nonhematopoietic tissues. These results indicate that KG2032 reactivity is highly specific for AML cells in patients who carry KG2032-reactive HLA-DRB1 alleles and who received allo-HCT from a donor carrying KG2032-nonreactive HLA-DRB1 alleles. KG2032-derived CAR T or natural killer cells showed significant anti-leukemic activity, suggesting that they may cure patients with AML who are incurable with allo-HCT (***Nat Cancer*** 6**,** 595–611, 2025).

Our findings showed that a cancer-specific targets, which cannot be identified by transcriptome or proteome analyses, can be found by extensive screening of primary human tumor samples.

E01
CO BYCHOM MĚLI VĚDĚT O LÉČBĚ HEMOFILIE V ROCE 2026

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Hemofilie je vzácná dědičná porucha srážení krve, která postihuje především muže. Dle hladiny chybějícího faktoru VIII (Hemofilie A) či IX (Hemofilie B) ji dělíme na lehkou, střední a těžkou formu. Především u těžké formy dochází ke spontánnímu krvácení zejména do kloubů a měkkých tkání, což bez léčby vede k vývoji cílového kloubu a později k rozvoji hemofilické artropatie jež významně omezuje kvalitu života. V době, kdy nebyla účinná léčba dostupná se málokterý jedinec s těžkou formou nemoci dožil konce druhé dekády svého života. Proto je u těchto osob nutná profylaktická léčba jejíž cílem je nejen zabránit vzniku cílového kloubu, ale jakémukoli závažnému krvácení. Až do počátku tohoto století s sebou taková léčba nesla zátěž ve smyslu pravidelné a časté i.v. aplikace chybějícího faktoru a možného rizika vzniku inhibitoru (protilátek proti léčebně podávanému faktoru).

Velký rozvoj terapeutických možností na poli hemofilie v posledních deseti letech výrazně zlepšil kvalitu života hemofiliků a umožnil léčbu šitou na míru. Sdělení se věnuje tomu, co bychom o nových léčebných postupech měli vědět na prahu roku 2026.

Léčba faktorová
Původní substituční léčba (podávání chybějícího faktoru) doznala zásadních změn. Tou hlavní je fakt, že lék se nepodává již v intervalu dní, ale týdnů. Z tohoto pohledu nejpokročilejší v oblasti hemofilie A je molekula s názvem efanesoctocog alfa v Evropě komerčně dostupná pod názvem Altuvoct.

Jedná se o koncentrát FVIII s ultra prodlouženým poločasem díky jeho nezávislosti na endogenním vWF. To se projevuje udržením hladiny FVIII nad 40%

po dobu 4 dní a také tím, že hladina před další dávkou neklesne pod 15% (u adolescentů a dospělých)[1]. Recentně byly prezentované předběžné výsledky dlouhodobé studie XTEND-ed[2], které potvrzují účinnost a bezpečnost dokázanou v pilotních studiích. Pacienti při podávání 50 IU/kg 1x týdně dosáhli velmi nízkého ABR (<1) a procento léčených bez spontánního krváceni bylo 91,7% u adolescentů a dospělých, resp. 97,7% u dětí. Léčba je dobře tolerovaná, bez neočekávaných nežádoucích účinků.

Retrospektivní analýza RWD (real-world-data) ze 4 center v USA[3] ukazuje účinnost Altuvoctu u 39 pacientů, u nichž došlo k výraznému snížení ABR oproti původní profylaxi SHL/EHL ale i emicizumabem a to na 0,8 pro všechna krváceni a 0,2 pro spontánní (průměry).

U hemofilie B jsou pak k disposici již delší dobu dvě léčiva s komerčním názvem Alprolix (eftrenonacog alfa) a Idelvion (albutrepenonacog alfa), které díky významně prodlouženému poločasu lze podávat v intervalu až dvou týdnů při dosažení velmi dobré a spolehlivé ochrany před krvácením jak během profylaxe, tak při chirurgických výkonech[4,5].

Léčba pomocí FVIII napodobujících léčiv

Zásadní přelom v léčbě hemofilie nastal v roce 2017 kdy do klinické praxe v Evropě (i v ČR) byl zaveden „emicizumab“, komerčním názvem Hemlibra. Výsledky studií Haven 1-7[6-12] prokázaly jeho účinnost a bezpečnost pro osoby s hemofilií A s inhibitorem i bez něj ve všech věkových kategoriích. O tom, že i v naší zemi jsou velmi bohaté zkušenosti s tímto preparátem hovoří např. publikace shrnující celonárodní data týkající se léčba emicizumabem[13]. Na Evropské úrovni pak například práce skupiny PedNet[14], v USA pak recentně práce Genentech[15]. Z těchto dat plyne, že výrazně nadpoloviční většina osob (zejména dětí) léčených emicizumabem je bez krvácení, které by vyžadovalo léčbu, a hlavně u osob s inhibitorem dochází i k významnému snížení počtu všech krvácení ve srovnání s předchozí léčbou. Lék je účinný i u tzv. PUPs (před tím neléčených pacientu), jimž ho lze podávat již krátce po narození bez potřeby zavádět opakovaný, či permanentní žilní vstup. Není však zatím vyřešena otázka, zda zahájení léčby pomocí emicizumabu změní pravděpodobnost vzniku inhibitoru, nebo ji jen odsune do pozdějšího věku, protože i když je lék velmi účinný, občas ke krvácení dojít může a pak je léčba faktorem FVIII nutná[16]. Probíhají i klinické studie, které mapují účinnost a bezpečnost emicizumabu u jiných chorob se sníženou hladinou FVIII, např u typu 3 vWD[17], nebo u získané hemofilie[18].

Emicizumab však brzy nebude jedinou FVIII napodobující (mimikující) látkou. V pokročilé fázi klinického zkoušení jsou další molekuly. Např. NXT007[19], léčivo s vyšším potenciálem generovat trombin a tím i s lepším a trvalejším efektem a delším poločasem. Uvedená práce popisující zatím dostupné výsledky studie fáze 3 potvrzuje jeho bezpečnost a vysokou efektivitu (více než 80% pacientů ve studii bylo zcela bez krvácení – ABR 0).

Další molekulou v této skupině léčiv je denecimig, nazývaný Mim8, v probíhající studii fáze 3[20]. Procento osob s nulovým krvácením se v citované práci pohybuje kolem 70% a nebyly zaznamenány žádné znepokojivé bezpečnostní signály (např trombózy). Lék muže být podáván v až měsíčním intervalu.

Rebalanční léčba

Prvními i v EU registrovanými zástupci tzv. „rebalanční“ léčby jsou inhibitory TFPI. Snížením aktivity TFPI pomocí protilátek (a tím pádem zvýšení trombogenního potenciálu pacientovy plazmy) lze do jisté míry kompenzovat tendenci ke krvácení danou vrozenou hemofilií. Zástupci TFPI inhibitorů jsou concizumab (Alhemo) a marstacimab (Hympavzi). I tyto léky lze podávat podkožně a v principu jsou účinné u hemofilie A i B bez ohledu na přítomnost inhibitoru. Marstacimab v klinickém hodnocení fáze 3[21] prokázal snížení ABR u osob s hemofilií a inhibitorem k počtu blížícímu se 0 (medián). V téměř 20% případů vznikly při léčbě marstacimabem protilátky, které ale nebyly neutralizující a neovlivnily efekt léku. Data o použití concizumabu u osob s hemofilií bez inhibitoru pak ukazují[22], že použití tohoto léčiva je non-inferiorní ke standardní profylaxi. Lék je třeba aplikovat podkožně denně.

Dalším z „rebalančních" léčiv je fitusiran (malá interferenční RNA snižující expresi antitrombinu v jaterní buňce). Tento lék zatím v EU registrován není, ale dostupná data a jejich metaanalýza[23] ukazuje, že pro pacienty s hemofilií a inhibitorem tato léčba snižuje počet krvácení v porovnání s on-demand léčbou nebo profylaxi s by-pass léčivy. Podobný trend, ale bez statistické významnosti, je zaznamenán i u osob s hemofilií bez inhibitoru. Je však třeba počítat s vyšším rizikem tromboembolických příhod a s možnou elevací jaterních enzymů v souvislosti s léčbou fitusiranem.

Genová léčba

Pro genovou léčbu jsou nyní v EU registrovány dva preparáty valoctocogene roxaparvovec (Roctavian) pro hemofilii A, etranacogene dezaparvovec (Hemgenix) pro hemofilii B. Zdá se, že slibnější je GT pro léčbu hemofilie B. Data spojená s ukončením studie HOPE-B[24] týkající se léku Hemgenix u 50 léčených prokazují po 5 letech od podání GT významné a trvalé snížení počtu krvácení (ABR 1,52) a hladina FIX se po celou dobu pohybovala nad 36% bez významného kolísání. Spotřeba léčebně podávaného FIX (např. při případném krvácení) klesla o 96% oproti předchozí léčbě (profylaxe faktorem). Pouze 1 pacient se musel k faktorové léčbě vrátit. Elevace jaterních testů byla přítomna u cca 20% osob a vyžadovala steroidní léčbu kratší než 3 měsíce u většiny z nich. Pozdní onkogenicita ani hepatotoxicita s léčbou v dané kohortě spojena nebyla. Všichni pacienti budou dále sledování (plán je 15 let).

Výsledky pětileté léčby Roctavian (valoctocogene roxaparvovec) byly prezentovány na ISTH 2025. Aktivita FVIII měřená chromogenně byla v pátém roce léčby víceméně stabilní a srovnatelná s rokem 4 (medián hladiny FVIII 6,2% vs. 6,5%). 48.2% léčených bylo bez krvácení po celých 5 let léčby. Nicméně 25 ze 139 léčených se v průběhu této doby vrátilo na profylaxi faktorem[25]. Při hodnocení 16 pacientů z Brazílie nebylo zaznamenáno zhoršení HJHS score během 5 let léčby, což je nepřímý důkaz prevence rozvoje, resp. zhoršení hemofilické artropatie u takto léčených osob s hemofilií A[26].

Znepokojující je však fakt (komunikace v rámci relevantního SSC ISTH), že dle výsledku šetření v dubnu 2025 bylo celosvětově léčených jen 19 pacientů mimo klinické studie, a to jen ve třech zemích. Hlavním problémem se zdá být zatím nedořešený režim úhrady genové léčby hemofilie A s ohledem na nepředvídatelnou expresi genu a délku trvání efektu této léčby.

Závěr

V posledních 10 letech došlo k zásadním změnám v možnostech léčby hemofilie. Významně se zvýšila efektivita léčby a kvalita života osob s hemofilií. Nepohodlí spojené s léčbou téměř pominulo, nebo bylo výrazně redukováno. Mnoho otázek však stále zůstává nezodpovězených a řada z nich teprve vyvstane. Je nicméně uspokojivé, že další vývoj v této oblasti pokračuje. Je třeba jej bedlivě sledovat.

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E02
RIZIKO VENÓZNÍHO TROMBOEMBOLIZMU U NEMOCNÝCH S LYMFOMEM

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Úvod: Zvýšená frekvence výskytu venózního tromboembolizmu (VTE) je u nemocných s nádory dobře známa (1,2). Asi 10-12 % idiopatických trombóz je l. příznakem nádorového onemocnění, které se pak manifestuje do jednoho roku. Také výskyt VTE během léčby je jasně defininován. VTE výrazně zvyšuje riziko nemocnosti a úmrtnosti (3). Sklon k trombóze se u jednotlivých nádorů silně liší, proto není jednoduché vypracovat doporučené postupy stran profylaxe a terapie VTE v onkologii. To stejné platí pro hematologická nádorová onemocnění (HM).

Lymfomy a riziko VTE: Ve americké studii 400 000 veteránů v letech 2006-2021 s diagnostikovanou malignitou byl výskyt HM v 10 % (n=40 010 p.). Kumulativní incidence VTE (v 12 měsících) byla největší u ALL (18,6 %), u agresivních NHL 11 % a m. Hodgin 9,5 % (4). V retrospektivní studii zahrnující 16755 p. s NHL byla akutní VTE spojena se 70 % zvýšením rizika úmrtí během 6 měsíců (HR 1.7, 95 % CI: 1.5–1.9) po vztažení k věku, komorbiditám, stadiu lymfomu a jeho histopatologii (5). Lymfomy patří HM s vysokým rizikem VTE (6,7,8,9)**.** Rizikové faktory (RF) nejsou totožné jako onkologii. V literárním průřezu posledních 20 let bylo vybráno 25 publikací, kde je riziko demonstrováno jako největší během prvních 2 měsíců po dg. lymfomu, pak toto riziko klesá (6). Rizika VTE u lymfomů můžeme rozdělit na 2 velké skupiny:

1/ asociované s lymfomem

2/ obecné RF

Ad,1

Histologie lymfomu
Ve skupině B-NHL, DLBCL je VTE nejčastější a je větší než u folikulárního lymfomu, či u jiného indolentního lymfomu (10). Během 1. roku od dg. DLBCL se VTE vyskytuje u 10-12%, u indolentních lymfomů 1,5-4% (11). Periferní T cell lymfomy patří také do skupiny s vysokým rizikem VTE (8). Naopak incidence VTE je u nemocných s Hodgkinovým lymfomem dle GHSG 3.3% (12).

Místo postižení. Riziko VTE je zvýšené u CNS lymfomů. V sérii 42 p. s primárním mozkovým lymfomem se VTE vyskytla v 59,5% (13). V jiných pracech je toto číslo menší (27,2%) (14).

Postižení mediastina a "bulky disease". Ve skupině 42 p. s primárním mediastinálním lymfomem, mělo 15 p. (35,7%) VTE (15). Postižení mediastina je spojeno s 8x větším rizikem VTE, extranodální postižení pak s 2,3 větším rizikem.

Stádium onemocnění. Větší tumorózní masa je spojena s větším rizikem VTE (8), ale ne všechny studie s tím souhlasí (10).

Laboratorní znaky. Na lymfomy obecně není vhodné Khoranův skórovací systém jako v onkologii (16). Jinak hodnocené markery byly LDH, velikost trombocytů aj., ale vždy s nejednotnými závěry.

Mezinárodní prognostické indexy (IPI). U agresivních lymfomů se používají více než 25 let prediktivní modely stran prognózy (17). Vycházejí z hodnocení věku, stadia onemocnění, "performance status", LDH a extranodální postižení.

Čas od diagnózy. Riziko VTE, jak již bylo uvedeno je největší během prvních měsíců po stanovení diagnózy a pak klesá, což souvisí s masou nádoru a zahájením chemoterapie. Zhou a spol popsal, že 64% (51/80) pacientů dostalo VTE během prvních 3 cyklů chemoterapie (18).

Typ terapie. Přidání dalšího léka do léčebného schematu není jednoduché zhodnotit, protože se jedná většinou o agresivnější formy onemocnění u polymorbidnějších pacientů. Nemocní s pokročilým stadium Hodginova lymfomu léčení schématem BEACOPP (místo ABVD), mají větší riziko VTE (19). Take podání BEACOPP každých 14 dnů místo 21 je rizikovější stran VTE (12) Přidání doxorubicinu do režimu CVP zvyšuje riziko VTE u jedinců sh B cell lymfomy (DLBCL and FL) (19).

Zavedení centrálního žilního katetru. Zavedený centrální žilní katetr (CVC) u nemocných s lymfomem zvyšuje riziko VTE (11). V jedné studii z Číny byla incidence trombózy v žilách horní končetiny u PICCu 7.1% (40/565) u nemocných s lymfomem, bez rozdílu dle histologie (20). Byla však vyšší než nehematologických malignit.

2/ Obecné rizikové faktory VTE
Mezi obecné rizikové factory řadíme věk nemocného, osobní anamnézu VTE, přítomnost vrozeného trombofilního stavu (zjištěného např. v rámci vyšetření 1. pokrevních příbuzných), BMI, komorbidity a také stupeň mobility. U některých typů lymfomu je větší záchyt přítomnosti protilátky typu lupus anticoagulant (LA) (21), u některých je snížená fibrinolýza (22).

Profylaxe a léčba VTE u nemocných s lymfomem
Primární profylaxe VTE je u nemocných s lymfomem individuální, po zhodnocení všech výše uvedených rizikových faktorů. Pokud je nemocný převážně na nemocničním lůžku, měl by být zajištěn LMWH. V případě terapie VTE postupuje stejně jako u solidních nádorů (23,24). Také v tzv. "modré knize" české onkologické společnosti jsou doporučení jasně formulována, lze z nich vyjít i u nemocných s lymfomem. Není mnoho doporučení, která by se specializovala jenom na lymfom. Stručně řečeno, LMWH v terapeutické dávce zůstává lékem volby akutní VTE, pokud je clearance kreatininu ≥30 mL/min (25). Tuto dávku dáváme většinou jeden měsíc, pak dávku 75% terapeutické. Po 6 měsících lze léčbu ukončit, pokračování je v případě přetrvávající aktivity základní choroby nebo obecně po individuálním zhodnocení poměru přínosů

a rizika, snášenlivosti a preferencí pacienta. Takto postupujeme i u asymptomatických, náhodně zjištěných trombóz Dk či plicních embolií, protože tyto jsou častým nálezem při zobrazovacích vyšetření (CT, UZ aj.). Častým nálezem je trombocytopenie během terapie. Pro léčbu symptomatického trombózy související s katetry se doporučuje antikoagulační terapie minimálně 3 měsíce, pokud je CVC zavedena (24). Odborný panel podporovaný pracovní skupinou Gruppo Italiano Malattie Ematologiche dell'Adulto pro trombózu a hemostázu vytvořil formální konsenzus ohledně mezních hodnot krevních destiček pro bezpečnou léčbu LMWH u pacientů s hematologickými nádory a trombocytopenií (25). Dávku LMWH upravujeme při počtu krevních destiček < 50 × 10^9/L. (26). V klinické praxi je třeba posoudit rizikové faktory krvácení a VTE u jednotlivého pacienta, protože postižení určitých orgánů může potenciálně zvýšit riziko krvácení (např. lokalizace lymfomu v gastrointestinálním nebo centrálním nervovém systému**).** Používání přímých perorálních antikoagulans (DOAC) se stává bezpečnou a účinnou alternativou k LMWH v onkologii, takže ji lze využít i při VTE u lymfomů, zejména v případě dlouhodobé antikoagulační terapie.

Závěry. Lymfomy patří mezi neoplazie s vysokým rizikem VTE. Agresivní lymfomy mají přibližně 10–15% výskyt VTE v prvním roce. Toto riziko je ještě vyšší, pokud je nemoc lokalizována v CNS nebo způsobuje mediastinální masu. Riziko je nejvyšší na začátku od diagnózy až po první cykly chemoterapie. Intenzivní aktivita onemocnění, nehybnost, umístění CVC a podávání chemoterapie antracykliny – to vše přispívá k riziku VTE již na začátku. Hodnotící skóre VTE vyvinuté pro pacienty se solidními nádory, jako je Khorana skóre, nepředpovídají riziko VTE u pacientů s lymfomem. V nepřítomnosti ověřeného rizikového skóre nelze poskytnout žádné doporučení podložené důkazy pro profylaxi VTE u ambulantních pacientů podstupujících protinádorovou léčbu. Aktuální strategií je individuální hodnocení poměru rizika a přínosů s ohledem na výše uvedené rizikové factory, proto je potřeba prospektivních studií k doporučení primární profylaxe pro nemocné s lymfomem. Je totiž velkou škodou pro nemocného, když je vyléčen ze závažného onemocnění, nebo je v dlouhodobé remisi, ale jeho kvalita života je pak značně limitována posttrombotickým syndromem.

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SBORNÍK ABSTRAKTŮ

E03
VRODENÉ ERYTROCYTÓZY: PRETRVÁVAJÚCA DIAGNOSTICKÁ A KLINICKÁ VÝZVA V ÉRE MOLEKULÁRNEJ MEDICÍNY

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Erytrocytóza je stav charakterizovaný zvýšenou koncentráciou erytrocytov v krvnom riečisku. Absolútna erytrocytóza je charakterizovaná zvýšeným objemom erytrocytovej hmoty, čo sa pre diagnostické účely často interpretuje ako zvýšenie hladiny hemoglobínu (Hb) a hematokritu (Hct) nad hornú hranicu normy, s prihliadnutím na vek, pohlavie a nadmorskú výšku dlhodobého pobytu. Absolútnu erytrocytózu je potrebné odlíšiť od relatívnej erytrocytózy, ktorá je spôsobená znížením objemu plazmy v dôsledku straty telesných tekutín alebo ako súčasť Gaisböckovho syndrómu.

V rámci diferenciálnej diagnostiky absolútnej erytrocytózy je prvým krokom vylúčenie polycythemia vera (PV), získaného myeloproliferatívneho ochorenia charakterizovaného nadmernou proliferáciou všetkých krvotvorných línií a mutáciami v JAK2 kináze, najčastejšie V617F. Diagnostickým markerom je aj nízka alebo subnormálna hladina erytropoetínu (EPO). PV patrí medzi primárne erytrocytózy vyznačujúce sa hyperproliferáciou erytroidných progenitorov.

Absolútna získaná erytrocytóza môže byť aj sekundárna, vznikajúca v dôsledku chronickej hypoxie centrálneho alebo periférneho pôvodu, napríklad pri pľúcnych ochoreniach, vrodených srdcových vadách s pravo-ľavým skratom, pobyte vo vysokých nadmorských výškach alebo fajčení. Ďalšie príčiny zahŕňajú nádory produkujúce EPO, exogénne faktory, ako sú lieky stimulujúce erytropoézu (EPO, ESA, SGLT2 či VEGF inhibítory), androgény alebo stav po transplantácii obličiek či TEMPI syndróm. Hladiny EPO sú pri sekundárnych formách obvykle zvýšené. V posledných rokoch sa pozornosť venuje erytrocytóze asociovanej s liečbou inhibítormi SGLT2, používanými u pacientov s diabetom, zlyhávaním srdca a chronickým ochorením obličiek. Mierny vzostup Hb a Hct nastáva v dôsledku normalizácie tubulointersticiálnej funkcie, zvýšenej produkcie endogénneho EPO a celkovej úpravy renálneho a metabolického prostredia. Štúdie nepreukázali zvýšené trombotické riziko, a preto si SGLT2-indukovaná erytrocytóza spravidla nevyžaduje hematologickú intervenciu.

Samotné vrodené erytrocytózy predstavujú veľmi zriedkavú a zároveň heterogénnu skupinu geneticky podmienených stavov, často spojených s pozitívnou rodinnou anamnézou a dlhotrvajúcou izolovanou erytrocytózou. Príčinami môžu byť mutácie v bunkových signálnych dráhach regulujúcich samotné erytroidné progenitory (gény *EPOR, JAK2*), alebo v signálnych dráhach regulujúcich hypoxickú (HIF) signalizáciu a produkciu EPO (gény *HIF2A*, *VHL*, *PHD2, EPO*), či afinititu hemoglobínu ku kyslíku (gény *HBA1*, *HBA2*, *HBB* a *BPGM*). Hladiny EPO sú variabilné, od nízkych pri primárnej familiárnej a kongenitálnej erytrocytóze (PFCP) asociovanej s mutáciami v EPOR až po normálne či zvýšené u ďalších typov. Ukazuje sa, že niektoré mutácie vo VHL či HIF2A pritom nespôsobujú len zvýšenú produkciu EPO, ale aj priamu hyperproliferáciu erytroidných progenitorov, ktorá prispieva k výslednému fenotypu.

Erytrocytózy spojené s mutáciami v samotnom *EPO* géne sú pomerne nedávno ustanovenou podskupinou familiárnych erytrocytóz. Príčinné mutácie zvyšujú expresiu EPO, prípadne vedú k produkcii hyperaktívneho *„hepatic-like“* EPO s atypickou glykozyláciou.

Ďalšími génmi identifikovanými u tzv. idiopatických, „inak nešpecifikovaných“, erytrocytóz bez odhalenej príčiny sú *PIEZO1*, kódujúci mechanosenzitívny iónový kanál, s patogénnymi variantmi spojenými so zvýšeným Hb

a známkami hemolýzy; *SLC30A10*, transportér mangánu, pri mutáciách spojený s hypermanganéziou, erytrocytózou, dystóniou, cirhózou a nedostatkom železa; *ACO1*, bifunkčný protein regulujúci mimo iné transláciu HIF2A; a *HFE*, podieľajúci sa na regulácii systémovej homeostázy železa. Presná kauzalita *ACO1* a *HFE* s erytrocytózou zatiaľ nie je úplne objasnená.

Naša analýza českej kohorty pacientov s vrodenou erytrocytózou ukázala, že 67 % z nich nieslo zárodočné mutácie v EPOR alebo HIF signálnej dráhe. V deviatich prípadoch boli príčinou erytrocytózy patogénne varianty nachádzajúce sa v nekódujúcich sekvenciách génov *EPO* alebo *VHL*. U časti pacientov boli nájdené varianty nejasného významu v *EPOR* alebo *JAK2* géne, ktoré sa chovajú ako alely s nízkou penetranciou a rizikom rozvoja myeloidných neoplázií.

Liečba vrodených a idiopatických erytrocytóz je do značnej miery individualizovaná. Cytoredukcia nie je indikovaná. Flebotómie sa využívajú len pri symptómoch hyperviskozity alebo zvýšenom riziku trombózy, pričom u hypoxických alebo HIF/VHL-mutantných foriem môžu byť kontraindikované. Optimalizácia kardiovaskulárnych rizikových faktorov a nízke dávky aspirínu sú často súčasťou manažmentu. Nedávna štúdia ukázala, že kombinácia flebotómií a nízkych dávok aspirínu vykazuje u PFCP možný prínos z hľadiska prevencie cievnych príhod. V prípade získaných sekundárnych erytrocytóz je liečba cielená na korekciu primárnej príčiny, napríklad oxygenoterapia alebo chirurgická resekcia EPO-produkujúcich nádorov.

Nové terapeutické prístupy pre PV, ako rusfertide alebo sapablursen, predstavujú liečivá ovplyvňujúce metabolizmus železa a jeho dostupnosť pre erytropoézu prostredníctvom modulácie hepcidinu. Ich testovanie v klinických štúdiách ukázalo zníženie potreby venepunkcií, lepšiu kontrolu Hct a zlepšenie vybraných symptómov. Hoci údaje u neklonálnych foriem erytrocytóz chýbajú, tieto výsledky naznačujú potenciál cielenej modulácie osi hepcidin–železo aj v tejto oblasti. Rovnako tak je možné uvažovať o využití HIF-1/2 inhibítorov v prípade erytrocytóz s mutáciami v HIF signálnej dráhe. Potenciál JAK2 inhibície ruxolitinibom bol testovaný u Čuvašskej polycytémie spojenej s vrodenou VHL mutáciou R200W.

Pokroky v molekulárnej diagnostike, najmä exomové a celogenómové sekvenovanie, umožňujú odhaľovať nové patogénne varianty a vysvetľovať prípady doteraz považované za idiopatické, čo má významný dopad na klasifikáciu, prognózu a budúci vývoj cielenej terapie pre pacientov s vrodenými erytrocytózami.

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E04
VROZENÉ MUTACE A MYELOIDNÍ NÁDORY

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Myeloidní nádory představují heterogenní skupinu hematologických malignit, jejichž vznik a průběh je výsledkem komplexní interakce somatických genetických alterací, epigenetických změn a vrozené genetické predispozice. Tradičně byly myeloproliferativní neoplazie (MPN), myelodysplastický syndrom (MDS) a akutní myeloidní leukemie (AML) vnímány především jako onemocnění vznikající na podkladě získaných mutací v hematopoetických kmenových buňkách. V posledních letech se však stále více ukazuje, že významnou roli v iniciaci onemocnění, formování fenotypu, riziku komplikací i pravděpodobnosti leukemické transformace hrají zárodečné (germinální) genetické varianty.

V páté revizi klasifikace WHO[1] jsou myeloidní neoplazie asociované se zárodečnou predispozicí nově uznány jako samostatná kategorie, což podtrhuje jejich klinický význam a potřebu systematického vyhledávání těchto stavů v běžné hematologické praxi. Klinická relevance germinálních mutací spočívá nejen ve zvýšeném riziku rozvoje myeloidního nádoru, ale také v ovlivnění věku manifestace, typu onemocnění, spektra přidružených komplikací (zejména trombózy), odpovědi na léčbu a zásadních implikacích pro rodinné příslušníky pacienta.

Zárodečné predispozice k myeloidním nádorům zahrnují široké spektrum genetických změn s rozdílnou penetrancí a klinickým dopadem. Na jedné straně stojí vysoce penetrantní, vzácné mutace v genech jako *RUNX1*, *GATA2*, *DDX41* nebo *ANKRD26,* které často vedou k familiárnímu výskytu MDS nebo AML, mnohdy již v mladším věku. Na straně druhé se nacházejí časté, nízce penetrantní alely, které samy o sobě nezpůsobují onemocnění, ale vytvářejí „fertilní půdu“ pro vznik somatických mutací a klonální hematopoézy. Typickým příkladem této druhé skupiny je haplotyp *JAK2* 46/1, který významně zvyšuje pravděpodobnost vzniku somatické mutace *JAK2*-V617F a rozvoje MPN.

Z klinického hlediska jsou myeloproliferativní neoplazie modelovým příkladem onemocnění, u nichž se prolíná vliv zárodečných a somatických genetických faktorů. Aktivující mutace v signální dráze JAK-STAT, zejména *JAK2*-V617F, mutace v exonu 12 genu *JAK2*, a dále mutace v *MPL* a *CALR*, jsou hlavními somatickými „driver“ událostmi. Stále více důkazů však ukazuje, že fenotyp

onemocnění, riziko trombózy a pravděpodobnost progrese do myelofibrózy či AML jsou výrazně modulovány přítomností zárodečných variant v *JAK2* a dalších genech regulujících cytokininovou signalizaci.

Zvláštní pozornost si v tomto kontextu zaslouží zárodečné mutace v genu *JAK2*. Ty lze rozdělit do několika funkčních kategorií: (i) extrémně vzácné gain-of-function mutace způsobující hereditární erytrocytózu nebo trombocytózu, které fenotypově napodobují MPN, (ii) nízce penetrantní varianty a haplotypy predisponující ke vzniku *de novo* MPN a (iii) germinální mutace, které samy o sobě nemusí vyvolat plně rozvinuté onemocnění, ale významně ovlivňují biologii hematopoetických kmenových buněk a chování následně vzniklého maligního klonu.

Naše práce se zaměřuje právě na tuto třetí skupinu a na detailní charakterizaci klinického a biologického dopadu zárodečné mutace *JAK2*-R1063H.[2] Pomocí unikátního myšího modelu nesoucího germinální mutaci *Jak2*-R1063H[3] jsme prokázali, že tato mutace zásadním způsobem ovlivňuje hematopoézu již na úrovni hematopoetických kmenových buněk. Dochází k jejich funkčnímu vyčerpání, předčasnému stárnutí a posunu diferenciace směrem k myeloidní a megakaryocytární linii, což se klinicky manifestuje zvýšenou trombopoézou a produkcí destiček se zvýšeným trombogenním potenciálem. Mutace *Jak2*-R1063H vede ke vzniku trombotických komplikací nezávisle na hladinách trombopoetinu a v nepřítomnosti klasických rizikových faktorů. Zvýšené hladiny D-dimerů a známky aktivované koagulace naznačují, že vrozené změny v signalizaci JAK-STAT mohou přímo přispívat k protrombotickému stavu.

Dalším klíčovým zjištěním našeho výzkumu je kooperace germinální mutace *JAK2*-R1063H se somatickou mutací *JAK2*-V617F.[4] Mutace *JAK2*-R1063H patří mezi tři nejčastější vrozené varianty u MPN[5] a je spojena se zvýšeným rizikem trombotických komplikací a leukemické transformace.[3] Experimentální modely ukazují, že přítomnost germinální varianty zesiluje signalizaci JAK-STAT, podporuje klonální expanzi a urychluje progresi onemocnění. V modelu leukemické transformace indukované onkogenem MLL-AF9 bylo prokázáno, že *Jak2*-R1063H významně zvyšuje agresivitu onemocnění a zkracuje přežití[3], což poskytuje mechanistické vysvětlení klinických pozorování zvýšeného rizika AML u části pacientů s touto variantou (nepublikované výsledky).[6]

Z pohledu klinické praxe tato data podtrhují význam testování germinální genetické predispozice u pacientů s myeloidními nádory bez ohledu na věk v době diagnózy. Zejména u nemocných s atypickým průběhem MPN, časným výskytem trombotických komplikací, rodinnou anamnézou hematologických onemocnění nebo neobvyklou progresí choroby lze germinální genetické vyšetření považovat za vhodnou součást diagnostického algoritmu. Zárodečné mutace tak představují důležitý, dosud často opomíjený faktor podílející se na patogenezi myeloidních nádorů. Zárodečné varianty v genu *JAK2* nejen predisponují ke vzniku MPN, ale mohou rovněž ovlivňovat výsledný klinický fenotyp, včetně rizika trombotických komplikací a leukemické transformace. Začlenění germinální genetiky do klinického hodnocení pacienta tak otevírá nové možnosti prevence, časné intervence a cílené terapie.

[1] *WHO Classif. Tumours Editor. Board. 2024. Haematolymphoid Tumours, Part A, Vol. 11: WHO Classification of Tumours. Lyon, France: Int. Agency Res. Cancer. 5th ed. https://publications.iarc.who.int/637.*

[2] *Kapralova K, et al. Blood. 2016 Sep 8;128(10):1418-23.*

[3] *Zimolova V, et al. Leukemia. 2025;39(11):2745-2757.*

[4] *Mambet C, et al. Blood. 2018;132(25):2695-2699.*

[5] *Benton CB, et al. Cancer. 2019 Jun 1;125(11):1855-1866.*

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E05
TRANSFUZE JAKO SOUČÁST PALIATIVNÍ PÉČE V HEMATOLOGII?

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Paliativní péče v hematologii představuje specifickou oblast, která se v mnoha ohledech odlišuje od paliativní péče v solidní onkologii. Hematologická maligní onemocnění, jako jsou myelodysplastické syndromy (MDS), akutní myeloidní leukemie (AML) a myeloproliferativní neoplazie (MPN), se často vyznačují chronickým či subakutním průběhem s významnou interindividuální variabilitou prognózy. I v pokročilých fázích onemocnění může docházet k prolongovanému přežití, a to zejména díky intenzivní podpůrné léčbě. V tomto kontextu nabývá paliativní přístup zásadního významu nikoliv jako péče terminální, ale jako integrální součást dlouhodobého managementu nemocných s vysokou symptomovou zátěží.

Dominantním klinickým problémem u pacientů s MDS, AML a MPN jsou cytopenie, především anémie, která představuje hlavní determinantu funkčního stavu pacienta. Chronická anémie se klinicky manifestuje výraznou únavou, slabostí, sníženou tolerancí fyzické zátěže, námahovou i klidovou dušností, tachykardií, kognitivním zpomalením a celkovým poklesem výkonnostního stavu (ECOG/WHO PS). Tyto symptomy mají přímý dopad na soběstačnost nemocných, jejich schopnost setrvat v domácím prostředí a na celkovou kvalitu života (quality of life, QoL). Z paliativního i hematologického hlediska je proto transfuzní podpora základním nástrojem symptomatické léčby, jejímž cílem není korekce laboratorních parametrů, ale klinická úleva a zachování funkční kapacity pacienta.

Transfuzní závislost je typickým rysem pokročilých MDS a části pacientů s MPN, stejně jako u nemocných s refrakterní nebo relabující AML, kteří nejsou kandidáty intenzivní protinádorové léčby. V paliativním kontextu však někdy dochází k mylné interpretaci transfuzní terapie jako nepřiměřeně extenzivní či neindikované léčby.

Takový pohled je v současné době překonaný, protože cílem paliativní medicíny je maximalizace kvality života v souladu s hodnotami a přáními pacienta a transfuzní podpora právě tyto cíle může dobře naplňovat. U symptomatické anémie má transfuzní léčba jasný klinický přínos – vede ke snížení únavy, dušnosti a závratí, zlepšuje fyzickou výkonnost, umožňuje pacientovi zůstat mobilní a soběstačný a často oddaluje nutnost institucionalizace.

V tomto kontextu je velmi podstatné, že v českém prostředí již vznikla a jsou k dispozici oficiální odborná doporučení pro podávání transfuzní podpory v domácím prostředí v paliativní péči, připravená ve spolupráci České společnosti paliativní medicíny a Společnosti pro transfuzní lékařství ČLS JEP (1). Existence těchto doporučení představuje důležitý krok směrem k legitimizaci domácích transfuzí jako odborně promyšlené, bezpečné a eticky ukotvené součásti paliativního managementu vybraných pacientů s hematologickými malignitami, zejména s MDS a u starších nemocných s AML. Doporučení jasně vymezují indikační a vylučovací kritéria, kladou důraz na symptomatický přínos, informovaný souhlas a předem domluvenou intenzitu péče při komplikacích a potvrzují, že hlavní bariéry rozvoje domácích transfuzí nejsou primárně legislativní, ale organizační, etické a kapacitní. Současně tato doporučení akcentují, že transfuzní podpora má být v paliativním kontextu vedena výhradně podle klinického efektu a kvality života pacienta a měla by být ukončena ve chvíli, kdy opakované transfuze již nepřinášejí prokazatelnou symptomovou úlevu, případně když jejich celková zátěž a rizika – pro pacienta, pečující i systém – začnou převyšovat očekávaný benefit, což vyžaduje otevřenou, opakovanou a dobře dokumentovanou komunikaci s pacientem a jeho blízkými.

Současně je důležité, že se téma domácích transfuzí začíná vynořovat i v českém odborném písemnictví v podobě kazuistických sdělení (2), která ukazují, že při pečlivém výběru nemocných a dodržení standardizovaného postupu

lze transfuzní přípravky podat v domácím prostředí bezpečně a s jasným klinickým přínosem (typicky úleva od únavy a dušnosti, zlepšení funkční kapacity, někdy i získání času v přijatelné kvalitě života). Tato sdělení zároveň pojmenovávají limity postupu: popisují časovou a personální náročnost pro terénní tým, potřebu jasné logistiky (transport, identifikace, monitoring a řešení reakcí), riziko, že výkon „vyblokuje" kapacitu pro jiné pacienty, a v neposlední řadě bariéry implementace v ČR – od tradičního vnímání transfuze jako výkonu „patřícího do nemocnice", přes omezené kompetence některých nelékařských profesí v terénu až po nevyjasněné či neadekvátně nastavené úhradové mechanismy, které zatím brání širšímu rozšíření této praxe.

Z hematologického pohledu je nutné zdůraznit, že adekvátní podpůrná péče, včetně transfuzí erytrocytů a v indikovaných případech trombocytů, má potenciál ovlivnit nejen kvalitu života, ale i celkové přežití (overall survival, OS). Zlepšení výkonnostního stavu a redukce symptomové zátěže snižují riziko komplikací, jako jsou pády, infekce, malnutrice či dekompenzace kardiopulmonálních onemocnění, které jsou častou příčinou morbidity a mortality u starších hematologických pacientů. Prodloužení přežití v paliativním kontextu přitom nelze chápat izolovaně, ale jako prodloužení času, který může nemocný prožít v přijatelném funkčním stavu a v prostředí, které preferuje.

Specifikem hematologických diagnóz je také významná psychosociální dimenze onemocnění. Pro pacienty i jejich rodiny je dlouhodobá transfuzní závislost a opakovaný kontakt se zdravotnickým systémem značnou zátěží. Časté hospitalizace nebo ambulantní návštěvy mohou paradoxně snižovat přínos samotné léčby a vést k vyčerpání pacienta i pečujících. Z pohledu paliativní péče je proto klíčové hledat cesty ke snížení této zátěže, aniž by byla omezena účinná symptomatická léčba. Jednou z perspektivních možností je rozvoj modelů domácí nebo komunitní transfuzní péče, které by umožnily podávání transfuzí v domácím prostředí či v rámci specializované paliativní ambulance. Přestože tento přístup vyžaduje jasně definovaná indikační kritéria, logistické zajištění a mezioborovou spolupráci, je plně v souladu s cíli paliativní medicíny a preferencemi většiny pacientů.

Zásadním předpokladem úspěšné integrace paliativní péče do hematologické praxe je překonání předsudků na obou stranách. Hematologové mohou mít obavu, že zapojení paliativního týmu povede k oslabení naděje pacienta nebo k tlaku na ukončení aktivní léčby. Paliativní specialisté naopak mohou vnímat pokračující transfuzní a podpůrnou léčbu jako neadekvátní „přeléčování". Klíčem k řešení těchto rozporů je otevřená komunikace, jasné vymezení rolí a přijetí paralelního modelu péče, v němž paliativní tým poskytuje expertní podporu v oblasti symptom managementu, hodnotové anamnézy a práce s rodinou, zatímco hematolog nadále řídí specifickou léčbu onemocnění.

Závěrem lze konstatovat, že paliativní péče v hematologii musí být chápána jako aktivní, dynamický a dlouhodobý proces, který zahrnuje cílenou léčbu symptomů, zejména anémie a dalších cytopenií, a respektuje individuální trajektorii onemocnění a hodnoty a preference pacienta. Odepírání transfuzní podpory u symptomatických pacientů s MDS, AML či MPN nelze v současné době považovat za odůvodněný přístup, ale naopak za selhání základního principu péče zaměřené na kvalitu života. Zdůrazňování přínosu transfuzní léčby, možnosti jejího podávání v domácím prostředí a významu paralelní paliativní podpory v průběhu celé trajektorie onemocnění může zásadně přispět ke zlepšení péče o tuto specifickou a zranitelnou skupinu pacientů.

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E06
NEŽÁDOUCÍ ÚČINKY CAR-T TERAPIE A BISPECIFICKÝCH PROTILÁTEK

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Úvod

Autologní T lymfocyty s chimerickým antigenním receptorem (CAR-T) a bispecifické protilátky (bsAb) se staly již běžnou součástí léčby některých hematologických malignit. Mezi nejčastější indikace patří relabované/refrakterní non-hodgkinské B lymfomy, akutní B lymfoblastická leukemie a mnohočetný myelom.

Nežádoucí účinky můžeme rozdělit na akutní (do dne 30 od podání), opožděné (do dne 100) a pozdní (po dni 100). Léčba CAR-T je prováděna jednorázově a nese s sebou více závažnějších nežádoucích účinků. V případě bsAb, vzhledem k opakovanému podávání, se jejich akutní a opožděná toxicita často překrývají.

Akutní toxicita

Syndrom z uvolnění cytokinů (CRS) zastupuje vůbec nejčastější akutní komplikaci. Následkem aktivace imunitních buněk a systémového šíření cytokinů dochází k rozvoji syndromu systémové zánětlivé odpovědi, který se projevuje febriliemi, hypotenzí a případně i hyposaturací. K léčbě patří antipyretika, antibiotika, katecholaminy, tocilizumab, dexametazon.

Vzácnější **syndrom aktivovaných makrofágů při CRS (CRS/MAS)** a **syndrom hemofagocytární lymfohistiocytózy (IEC-HS)** se projevuje těžkou refrakterní pancytopenií, hypofibrinogenemií s koagulopatií, extrémní elevací ferritinu, zvýšením jaterních testů a triglyceridů a nálezem hemofagocytózy v kostní dřeni. K léčbě užíváme kortikoidy, anakinru, ruxolitinib, etoposid.

Syndrom neurotoxicity (ICANS) představuje druhou nejčastější akutní komplikaci. Projevuje se různými formami encefalopatie, dále obvykle expresivní afázií, poruchou orientace v čase a prostoru, zmateností a neklidem, případně třesem a poruchami jemné motoriky. Závažnější formy zahrnují křeče, periferní parézy, těžké poruchy vědomí až koma a mozkový edém. Kromě symptomatické léčby se užívají kortikoidy, anakinra, siltuximab, intratékální chemoterapie.

Neurotoxicita spojená se zánětlivou reakcí v místě nádoru (TIAN) se může objevit v případě primární nebo sekundární infiltrace mozku primárním onemocněním. Projevuje se lokálním otokem nebo elektrofyziologickými abnormalitami v místě postižení. Léčba vyžaduje antiedematózní kortikoidy a manitol, případně antiepileptika.

Syndrom závratí a ataxie (IEC-DACS) je specifikem CAR-T přípravků proti antigenu GPRC5D. Tato mozečková toxicita se projevuje závratěmi a poruchou koordinace pohybů a může být závažná až imobilizující. Léčba zahrnuje kortikoidy a imunoglobuliny.

Kožní, nehtová a rohovková toxicita je typická při imunoterapii proti antigenu GPRC5D. Projevuje se vyrážkou, svěděním, změnami až odlučováním nehtů a zarudnutím očí. Léčba je obvykle lokální, případně se uplatňují systémové kortikoidy.

Hematologickou toxicitu (ICAHT) způsobuje nejprve chemoterapie, poté systémová zánětlivá odpověď a později také nedostatečná hematopoetická rezerva kostní dřeně, projevuje se neutropenií a trombocytopenií. K léčbě kromě transfuzí užíváme granulocytární růstové faktory, trombopoetinová mimetika, imunoglobuliny, kortikoidy, ruxolitinib.

Kardiovaskulární toxicita může vést k rozvoji nebo akcentaci srdečního selhání, městnání a arytmií. U pacientů se závažnějšími formami CRS nebo akutními kardiálními komplikacemi je doporučeno sledování, byť patologie přetrvává jen zřídka.

Plicní komplikace se nejvíce vyskytují u pacientů s CRS vyššího stupně a projevují se hypoxií, plicním edémem, pleurálními výpotky a plicní embolií. Pleurální výpotky, které jsou přítomny na počátku léčby, často vyžadují

terapeutickou intervenci a spíše přetrvávají. Naproti tomu nově vzniklé pleurální výpotky obvykle drenáž nevyžadují a spíše odezní.

Opožděná toxicita

Hematologická toxicita může přetrvávat i po prvním měsíci a přecházet do chronicity. Měli bychom vyloučit jiné možné sekundární příčiny (infekce, relaps, myelodysplastický syndrom, hemofagocytární syndrom). V léčbě lze kromě výše uvedeného zvážit také podporu autologními periferními krvetvornými kmenovými buňkami, mezenchymální stromální buňky, případně alogenní transplantaci krvetvorných buněk.

Pohybová a neurokognitivní neurologická toxicita (MNT) patří mezi méně časté neurologické nežádoucí účinky u CAR-T přípravků proti antigenu BCMA. Projevuje se parkinsonismem, poruchami kognitivních funkcí a změnami osobnosti. Dalším možným příznakem jsou parézy periferních nervů, nejčastěji n. facialis. Léčba je poměrně svízelná a zahrnuje kortikoidy, imunoglobuliny, plazmaferézu nebo cytostatika.

Imunitně podmíněná enterokolitida (IEC-EC) je vzácnou komplikací CAR-T terapie proti antigenu BCMA. Projevuje se silnými a četnými průjmy. V léčbě se kromě podpůrných opatření používají kortikoidy, infliximab nebo vedolizumab.

Infekční komplikace odrážejí komplex neutropenie, poruchy T lymfocytární imunity, aplázie B lymfocytů, hypogamaglobulinemie a imunosupresivní medikace. Krátkodobá neutropenie je spojena s rizikem systémových bakteriálních a kvasinkových infekcí, delší pak s infekcemi mykotickými. Defekt lymfocytární a protilátkové imunity zvyšuje výskyt virových infekcí a pneumocystové pneumonie. V léčbě se uplatňují antibiotika, antivirotika, antimykotika a substituce imunoglobulinů, důležitá je i adekvátní antimikrobiální profylaxe a vakcinace.

Pozdní toxicita

Zvýšené riziko **infekčních komplikací** může přetrvávat i mnoho měsíců. Problematické jsou zejména respirační a jiné virové infekce a pneumocystová pneumonie.

Jako **sekundární malignity (SPM)** jsou označována maligní onemocnění jiných buněk, tkání nebo orgánových systémů, která se objeví až s delším odstupem. Vzhledem k výraznému předléčení pacientů chemoterapií, radioterapií nebo i transplantací však nelze všechny tyto malignity svádět pouze na nežádoucí vliv imunoterapie. Nejčastěji jsou nacházeny malignity hematologické (myelodysplastický syndrom, akutní myeloidní leukemie), kožní nádory a neoplázie respiračního a gastrointestinálního traktu. Malignity vycházející z T nebo CAR-T lymfocytů jsou velmi vzácné.

Závěr

K dispozici jsou **doporučené postupy** pro screening, diagnostiku, klasifikaci a léčbu nežádoucích účinků terapie CAR-T lymfocyty a bispecifickými protilátkami, které jsou si velmi podobné, ale v některých detailech se liší.

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EO7
NOVINKY V BIOLOGII A LÉČBĚ AKUTNÍ LYMFOBLASTOVÉ LEUKÉMIE 2025

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Na poli akutní lymfoblastové leukémie jsme svědky odklonu od intenzivní chemoterapie směrem k precizně řízené terapii založené na cílené léčbě, imunoterapii a buněčné terapii. Nejvýraznější pokrok byl dosažen u B-ALL, zatímco u T-ALL se objevují první průlomová data zejména díky genovým editacím CAR-T.

Ph-negativní B-ALL: integrace imunoterapie do časných linií

U Ph-negativní B-ALL byla definitivně potvrzena zásadní role blinatumomabu v časných fázích léčby. Randomizovaná studie fáze III ECOG-ACRIN E1910 prokázala, že přidání blinatumomabu do konsolidace vedlo k významnému zlepšení celkového přežití (OS 92 % vs. 67 % při samotné chemoterapii; HR 0,20; 95% CI 0,08–0,54; p = 0,002) a snížení rizika relapsu. Přínos byl

patrný ve všech věkových i rizikových skupinách a úzce koreloval s dosažením MRD negativity.[1]

Příslib většího komfortu pro pacienty i lékaře představují data o subkutánním blinatumomabu. Ve studii fáze I/II u dospělých s relabující nebo refrakterní Ph-negativní ALL bylo dosaženo CR/CRh/CRi u 89–92 % pacientů a MRN negativity přibližně u 90 %. Odhadované 12měsíční OS dosahovalo 63–70 %, při přijatelné toxicitě (CRS ≥3 u 17–23 %, neurologická toxicita převážně reverzibilní).[2]

U relabujících pacientů zůstává klíčovým terapeutickým nástrojem inotuzumab ozogamicin. Retrospektivní analýzy ukázaly, že kumulativní dávka ≤3,3 mg/m² je spojena s podobnou účinností, nižší myelotoxicitou a nižší nerelapsovou mortalitou po následné alo-HSCT než dávka >3,3 mg/m². Riziko VOD bylo v obou skupinách srovnatelné.[3]

Význam dlouhodobé kontroly onemocnění dokumentuje aktualizovaný follow-up studie CALGB 10403, která hodnotila intenzivní pediatrický režim u adolescentů a mladých dospělých. Desetileté OS dosáhlo 56 % a EFS 44 %, přičemž časné dosažení negativity MRN bylo nejsilnějším prediktorem dlouhodobého přežití.[4]

Ph-pozitivní ALL: bezchemoterapeutické přístupy jako nový standard

Nejzásadnější změna léčebného paradigmatu se týká Ph-pozitivní ALL. Randomizovaná studie fáze III GIMEMA ALL2820 jednoznačně prokázala větší účinnost bezchemoterapeutického režimu kombinujícího ponatinib s blinatumomabem oproti imatinibu s chemoterapií. Kompletní remise bylo dosaženo v 94,4 % vs. 79,4 % (p = 0,001), MRD negativita v den +133 v 70,2 % vs. 52,7 % (p = 0,009) a 18měsíční EFS bylo 89,9 % vs. 76,8 % (p = 0,011). Přínos byl zachován i u pacientů s genotypem *IKZF1*plus.[5]

Podpůrná data přinesla také post-hoc analýza studie PhALLCON, která ukázala rychlejší dosažení hluboké molekulární odpovědi při léčbě ponatinibem oproti imatinibu, což se odrazilo v nižší potřebě alogenní transplantace v první remisi.[6]

Další významný posun představují nové TKI. Olverembatinib v kombinaci s redukovanou chemoterapií dosáhl CR/CRi u >94 % pacientů a MRN negativity u 56–66 %, včetně nemocných s mutací T315I. OS a EFS ve 2 letech přesahovaly 90 %.[7] Flumatinib vedl k CR/CRi u 98,6 % pacientů, s dvouletým OS kolem 79 %.[8]

Relabující a refrakterní B-ALL: CAR-T a biologické determinanty odpovědi

Na poli relabující a refrakterní B-ALL byla potvrzena zásadní role CAR-T terapie. Obecabtagen autoleucel (obe-cel) ve studii FELIX dosáhl mediánu EFS 11,9 měsíce a mediánu OS 17,1 měsíce, přičemž 38–40 % pacientů zůstávalo v dlouhodobé remisi bez nutnosti následné transplantace.[9]

Analýzy prezentované na ASH 2025 ukázaly, že kvalita CAR-T produktu (vyšší podíl naivních/paměťových T buněk, nižší exhausce) a nižší nádorová nálož před lymfodeplecí jsou klíčovými prediktory dlouhodobého přežití.[10]

Zvláštní pozornost byla věnována časnému použití CAR-T u MRN-pozitivních pacientů v první kompletní remisi, kde bylo dosaženo 100% negativity MRN při minimální toxicitě, bez ICANS.[11]

T-ALL: genově editované univerzální CAR-T

Byla prezentována první klinická data přinášející naději na zavedení účinné a bezpečné buněčné terapie u T-ALL. Při výrobě genově editovaných univerzálních CD7 CAR-T buněk (BE-CAR7) se využívá vícenásobné genové editace k odstranění CD7 a TCR/CD52 z efektorových buněk, čímž se eliminuje fratricida a umožní cílení CD7+ leukemických klonů. Ve studii bylo dosaženo kompletní remise u všech léčených pacientů s relabující nebo refrakterní T-ALL, s MRN negativitou přibližně u 80 % a možností úspěšného přemostění k alogenní transplantaci u většiny nemocných.[12]

Data z kongresů EHA a ASH 2025 ukazují, že léčba ALL vstoupila do éry precizní, biologicky a imunologicky řízené terapie, v níž imunoterapie a buněčné terapie postupně nahrazují konvenční chemoterapii. Tyto změny zásadně redefinují standardy péče i dlouhodobou prognózu dospělých pacientů s ALL.

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E08
ALOGENNÍ TRANSPLANTACE U MDS

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Myelodysplastický syndrom (MDS) představuje vysoce heterogenní skupinu onemocnění s výraznou variabilitou klinického průběhu – od mírných cytopenií s relativně dlouhou délkou dožití až po velmi agresivní chorobu transformující se do vysoce rizikové akutní myeloidní leukemie (AML). Alogenní transplantace krvetvorných kmenových buněk (allo-HSCT) zůstává jedinou kurativní léčebnou možností. Rozhodování o indikaci a načasování transplantace je komplexní a závisí na rizikové kategorii dle klinických a biologických parametrů, věku a fyzické zdatnosti pacienta, komorbiditách a transplantačních faktorech. V praxi se opíráme o škály IPSS (1997) a IPSS-R (2012), na nichž stojí většina dostupných transplantačních studií, přičemž novější IPSS-M (2022) integruje 31 somatických mutací a zvyšuje přesnost odhadu OS a rizika transformace do AML; a zároveň tato stratifikace pomáhá I odhadnout úspěšnost ve smyslu přežití po provedené transplantaci.

Kromě rizika relapsu po transplantaci přináší zásadní rizika neúspěchu i samotný výkon, ke kterému je možné indikovat pacienty způsobilé touto léčbou projít.

Vysoce rizikové MDS a načasování transplantace: U „fit“ pacientů s vyšším rizikem (IPSS intermediate-2/high; IPSS-R high/very high, případně vyšší kategorie IPSS-M) je časné provedení allo-HSCT po nalezení vhodného dárce upřednostněným přístupem, protože v této skupině opakovaně prokázalo benefit ve srovnání s konzervativními strategiemi, včetně léčby hypometylačními látkami.

Z molekulárního pohledu přinesla důležité zjištění analýza, kde se přínos allo-HSCT projevil i u nemocných s TP53 mutacemi, zvláště pokud neměli pacienti další genetická rizika. U těchto pacientů s TP53 mutacemi a komplexními změnami v karyotypu je nutné indikaci k transplantaci bedlivě zvážit.

Nízkorizikové MDS: V nízkém/intermediate-1 riziku dle IPSS-R vykazují pacienti po allo-HSCT sice nižší riziko relapsu, avšak výrazně vyšší NRM .U onemocnění s nižším rizikem, je tedy odklad výkonu s výhodou, ale je nutné zvážit allo-HSCT u nemocných se život ohrožující neutropenií či trombocytopenií, u transfúzně závislé anémie refrakterní na ESA/luspatercept, po selhání lenalidomidu u del(5q), nebo při vysokorizikových mutacích (např. TP53) a v samém začátku akcelerace.

Léčba před transplantací (cytoredukce, hypometylačními látkami -HMA, či intenzivní chemoterapií - ICT). Randomizovaná srovnání optimálního postupu před allo-HSCT neexistují. U nemocných bez nadbytku blastů se obvykle postupuje přímo k transplantaci. Mezinárodní expertní panel doporučuje zvážit cytoredukci při >10 % blastů, a to jednak ke snížení nádorové masy, jednak k získání času pro dohledání dárce. Retrospektivní analýzy ukazují rozporné výsledky: některé naznačují lepší přežití u nemocných s clearance blastů v čase transplantace, jiné přínos nepotvrzují. Není jasné, zda delší OS odráží

nižší nádorovou zátěž, nebo méně agresivní biologii u responderů. Navíc hrozí toxicity a progrese bránící plánované transplantaci .

Volba dárce a dostupnost. Spektrum dárců se rozšířilo a většina pacientů dnes má dostupného dárce díky využití mismatchovaných a haploidentických příbuzenských dárců. Přesto platí, že pokud je rychle dostupný HLA-shodný sourozenec (MSD) či HLA-shodný nepříbuzný dárce (MUD), zůstávají upřednostňováni. V reálné praxi rozhoduje časová naléhavost, riziko progrese, logistika (rychlost typizace a aktivace dárců) a zkušenost centra s konkrétními postupy.

Přípravné režimy (conditioning): myeloablativní MAC vs s redukovanou intenzitou RIC. Volba intenzity kondicionování je kompromisem mezi antileukemickým účinkem a toxicitou/NRM. Myeloablativní režimy (MAC) poskytují silnější eradikaci choroby, ale jsou zatíženy vyšším NRM; volí se proto u mladších, fit nemocných s agresivnějším onemocněním. Režimy se sníženou intenzitou (RIC) mají nižší časnou toxicitu a spíše spoléhají na graft-versus-MDS efekt; u starších a pacientů s komorbiditami představují standardní přístup. Volba zohledňuje věk, HCT-CI, aktuální stav MDS, typ dárce a institucionální zkušenost.

Prevence relapsu po allo-HSCT a monitorace. Možnosti profylaktického či preemptivního ovlivnění relapsu u MDS jsou omezené. V praxi se využívají dárcovské lymfocytární infuze (DLI) a hypometylační látky (HMA) v preemptivní indikaci, při známkách hrozícího relapsu dle MRD nebo při klesajícím chimerismu. V jednotlivých případech I profylakticky u very high risk onemocnění. Nalezení vhodných pacientů k udržovací profylaktické léčbě po transplantaci je stale předmětem studií. Balancování mezi antileukemickým účinkem a indukcí GvHD je klíčové, zejména u DLI.

Závěr:
Allo-HSCT je jedinou kurativní léčbou MDS napříč rizikovými kategoriemi, avšak s významným rizikem NRM, infekčních komplikací a GvHD. U vysoce rizikových a „fit“ nemocných je časná transplantace po nalezení vhodného dárce racionální strategií s prokázaným přínosem v přežití. U nízkorizikových nemocných je transplantace vyhrazena pro selektované případy se závažnými, na konzervativní léčbu refrakterními cytopeniemi nebo s vysokým rizikem.

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E09
POTRANSPLANTAČNÍ LYMFOPROLIFERACE

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Potransplantační lymfoproliferativní onemocnění (PTLD) představují široké spektrum lymfoidních proliferací v důsledku externě navozené imunosuprese po transplantaci solidního orgánu nebo kostní dřeně. Zahrnují jak indolentní proliferace, polyklonální polymorfní onemocnění, tak agresivní maligní lymfomy, které velmi připomínají stejné typy lymfomů v imunokompetentní populaci a významně přispívají k morbiditě a mortalitě transplantovaných pacientů.

Vzhledem k heterogenitě těchto onemocnění není ani jejich patogeneze jednotná a kompletně objasněná.

U velké většiny PTLD hraje zásadní roli virus Epsteina-Barrové (EBV), kterým je promořeno 90–95 % světové populace. Během primoinfekce virus infikuje naivní B-lymfocyty a následně dochází k jejich polyklonální expanzi a expresi vysoce imunogenních virových proteinů (LMP, EBNA). U imunokompetentních jedinců tyto antigeny spouštějí cytotoxickou T-buněčnou odpověď, která eliminuje většinu infikovaných B-buněk. Malá část z nich však expresi virových antigenů potlačí a uniká imunitnímu dohledu. Tyto latentně infikované B-buňky přetrvávají v organismu celoživotně a při oslabení T-buněčné imunity, jako je tomu právě u transplantovaných pacientů během imunosuprese, mohou začít pod vlivem EBV znovu proliferovat. Nadměrná proliferace buněk usnadní vznik sekundárních mutací a následnou transformaci do PTLD [1].

Většina z těchto EBV+ onemocnění se rozvine do 1 roku od transplantace (tzv. časné PTLD) a jedná se o zralé B-buněčné CD20+ proliferace. Tyto proliferace jsou časté hlavně u dětských pacientů, u kterých tvoří až 90% všech PTLD. Je to dáno hlavně tím, že většina dětí je při transplantaci EBV negativní a riziko vzniku PTLD při primoinfekci je díky absenci EBV-specifických T-lymfocytů vyšší než při reaktivaci. U dospělých pacientů tvoří tyto proliferace přibližně 50% všech PTLD.

Histologicky se jedná zejména o PTLD typu časných lézí, polymorfní PTLD a PTLD difuzní velkobuněčný lymfom (PTLD-DLBCL). V těchto podtypech PTLD se EBV vyskytuje téměř výhradně ve fázi latence II a III, tedy je transkripčně aktivní. Výsledky genomických studií ukazují, že EBV+ PTLD mají nižší procento změn počtu kopií (copy number alterations, CNA) a nižší mutační nálož než histologicky stejné proliferace u imunokompetentních jedinců [2]. Na základě toho se dá předpokládat, že vliv EBV je na vznik a udržení proliferace těchto buněk zásadní.

Méně prozkoumanou skupinou PTLD jsou i vzhledem k jejich raritě pozdní PTLD (více než 1 rok od transplantace), EBV-negativní PTLD nebo jiné než B-buněčné PTLD (proliferace T-lymfocytů nebo NK-buněk) [3], [4].

EBV-negativní a pozdní PTLD vznikají mechanismy, které jsou méně přímo řízeny virovou onkogenezí a jedná se spíše o kombinaci dlouhodobé imunitní dysregulace, chronické antigenní stimulace a věkem nebo terapií navozené genomové instability. Chronická imunosuprese postupně narušuje imunitní dohled, což umožňuje expanzi B-buněčných klonů se získanými somatickými mutacemi. Pokud u některých hraje roli infekce, jedná se o zejména časné fáze onkogeneze. Patogeneze těchto forem se tak více podobá skutečné maligní transformaci a fenotypově i molekulárně se přibližují de novo maligním lymfomům. V souladu s touto hypotézou jsou např. současné znalosti o patogenezi PTLD Burkittova lymfomu (PTLD-BL). Tento typ proliferace se téměř výhradně prezentuje jako pozdní PTLD a EBV se téměř u všech vyskytuje ve fázi latence I. To naznačuje, že EBV hraje zásadní roli hlavně v rané fázi vývoje onemocnění, než dojde ke vzniku MYC translokace, která se stane hlavním driverem onkogeneze a virus transkripčně umlčí [2].

Vzácné podtypy PTLD jako např. T-buněčná lymfocytární leukémie z velkých granulárních buněk (T-cell large granular lymphocytic leukemia, T-LGL) dále

ilustrují heterogenitu PTLD [5]. Proliferace typu LGL mohou spontánně regredovat, ale v některých případech i progredovat do agresivních forem.

PTLD jsou ve srovnání s ostatními malignitami vzácná onemocnění. Jejich léčba se opírá o několik málo prospektivních studií, naprostá většina dat pochází z retrospektivních analýz malých skupin pacientů. Níže uvádíme seznam terapeutických možností. Vzhledem ke klíčové roli EBV infekce a imunitní dysregulace v patogenezi PTLD, by byla ideální, resp. kauzální léčbou PTLD eliminace EBV a obnovení funkce T lymfocytů.

1. Eliminace EBV. EBV je gama herpesvirus, který ke své replikaci nevyužívá virovou, ale hostitelskou DNA-polymerázu. Proto jsou standardní antivirotika u EBV neúčinná. K eliminaci EBV lze využít buněčné terapie, např. ex vivo expandované EBV-specifické T lymfocyty nebo EBV-specifické CAR-T. V současnosti se používají v léčbě PTLD po alo-SCT, v managementu PTLD po transplantaci solidních orgánů (SOT) zůstávají experimentální metodou [6].
2. Obnovení kompetence T lymfocytů/redukce imunosuprese (RIS). Jedná se o logický krok vycházející z patofyziologie nemoci. Rozsah intervence je dán typem transplantovaného orgánu, dobou od transplantace a ev. historií rejekčních epizod. Obecná doporučení obsahují redukci dávky calcineurinových inhibitorů na cca 50% a ukončení léčby antimetabolity. Redukce imunosuprese může být účinná jako „monoterapie“ u nedestruktivních (hyperplastických) lézí. U monomorfních PTLD je redukce imunosuprese sice základní strategií, velmi zřídka však samostatnou. Jediná prospektivní studie redukovala imunosupresi 16 pacientům s PTLD po SOT. Nikdo nedosáhl kompletní remise, jeden pacient parciální. Progrese PTLD byla zaznamenána u 8 pacientů a rejekce u šesti [7].
3. Anti-CD20. V současné době je rituximab základním stavebním kamenem léčebných algoritmů prakticky všech (CD20+) PTLD z B lymfocytů. Léčba rituximabem (nejčastěji 375mg/m2 á 1 týden, celkem 4x) dosahuje již v monoterapii, resp. v kombinaci s redukcí imunosuprese, remise u cca 70 - 90% pacientů [8]. Odpověď na léčbu rituximabem je také důležitým prognostickým faktorem, který umožňuje stratifikovat pacienty. Pacienty v kompletní remisi po monoterapii lze rituximabem konsolidovat v dávkování (375mg/m2 á 3 týdny, celkem 4x), odpovědi horší než CR jsou obvykle léčeny chemoterapií [9].
4. Chemoterapie. Vzhledem k tomu, že většina monomorfních PTLD odpovídá histologicky DLBCL, jsou léčeny CHOP nebo jeho modifikacemi. Prevencí možné toxicity CHOP (u PTLD může mít až 30% mortalitu [10]) je použití sekvenčních režimů, v kterých R-CHOP navazuje na solo rituximab (viz výše). Další možností je redukce dávek cytostatik nebo modifikace CHOP režimů. Alternativou může být kombinace cyklofosfamid plus prednison, ProMACE-CytaBOM, ev. EPOCH-R. Sice retrospektivní, ale jediné srovnání EPOCH-R versus R-CHOP u PTLD vyznívá jednoznačně ve prospěch R-EPOCH. mPFS 53 měsíců versus19 měsíců, mOS 77 měsíců versus 13,5 měsíce. Režimy mají srovnatelnou toxicitu a podobné riziko rejekce štěpu [11].
5. Real life. Na našem pracovišti léčíme PTLD-DLBCL pacienty dle protokolu prospektivní studie PTLD-1[12]. Při převzetí pacienta komunikujeme s transplantačním centrem možný rozsah redukce imunosuprese. Oproti originálním schématům nečekáme na efekt vysazení imunosuprese a záhy začínáme s rituximabem 375mg/m2. Mandatorní je intenzivní hydratace a alkalinizace jako prevence TLS, antiinfekční profylaxe a podpora G-CSF. Rituximab je podáván v týdenním intervalu celkem 4krát. Po indukční fázi provádíme restaging, dle jehož výsledku a dalších parametrů stratifikujeme pacienty do 3 skupin. Nízké riziko: pacienti v kompletní remisi. Střední riziko: pacienti, kteří nedosáhli CR. Vysoké riziko: progredující pacienti po transplantaci srdce nebo plic. Pacienti s nízkým rizikem jsou konsolidováni dalšími čtyřmi rituximaby v 21denním intervalu. Střední riziko, což je naprostá většina pacientů, je léčena čtyřmi cykly R-CHOP. U vysokého rizika zvažujeme únosnost pacienta intenzifikaci nad rámec R-CHOP (např. platinové režimy, ev. ASCT).
6. Refrakterní/relabovaná onemocnění. PTLD jsou vzácná onemocnění, kterým chybí prospektivní studie a jednotné léčebné protokoly. Situace je ještě svízelnější v podmínkách relapsu onemocnění. Jen malá část pacientů je únosná klasickým salvage protokolům s platinovými režimy

plus ASCT. Analýza EBMT registru z roku 2021 popisuje 21 PTLD pacientů, kteří podstoupili ASCT. Z nich 3 zemřeli v souvislosti s ASCT a 3-leté OS bylo 61% [13]. Ve FNKV jsme transplantovali 3 pacienty s PTLD, z nichž jeden zemřel v aplázii, jeden rok po ASCT a pouze jedna pacientka zůstává v remisi 10let po ASCT.

7. CAR-T. Trendem současné hematologie jsou manipulace T lymfocytů ve smyslu bispecifických protilátek a CAR-T. Byť se jedná o léčby nadějné a velmi efektivní, v podmínkách PTLD jsou problematické. Většině PTLD pacientů je i v průběhu hematologické léčby podávána imunosuprese, která účinnost těchto strategií vážně limituje. Největší retrospektivní studie 22 pacientů sice reportuje relativně nadějné výsledky CAR-T (dvouleté PFS 35% a OS 58%) [14], další kazuistiky i naše zkušenost s dvěma PTLD pacienty ale spíše potvrzují obavy.
8. Další histologie. Monomorfní PTLD s jinou histologií než „DLBCL“ jsou výrazně vzácnější, tvoří 20-30% případů. Tito nemocní jsou léčeni RIS v kombinaci s protokoly pro daná onemocnění u imunokompetentních standardních pacientů. Burkittův lymfom PTLD je léčen RIS plus R-CHOP nebo klasické „Burkittovské“ protokoly, vždy s CNS profylaxí. T-NHL-PTLD jsou léčeni RIS, odpovídající chemoterapií a/nebo BV. Plazmablastický lymfom -PTLD je léčen RIS a CHOP nebo DA-EPOCH. Pacienti s Hodgkinovým lymfomem-PTLD profitují z RIS v kombinaci s radioterapií, chemoterapií (typicky ABVD) nebo BV [15]. Většina pacientů s „PTLD myelomem“ má detekovaný MGUS ještě před transplantací a většina je EBV negativních. Prognóza těchto pacientů je podobná klasickému myelomu a podobně jsou i léčeni [16].

Závěrem lze říci, že PTLD představují vzácnou, ale vážnou komplikací transplantací solidních orgánů a hematopoetických buněk. Jejich patogeneze i biologické chování jsou heterogenní, ale jejich podobnost s maligními proliferacemi u imunokompetentních jedinců z nich činí cenný model pro studium mechanismů lymfomageneze obecně. Léčba PTLD je obtížná jak pro nedostatek kvalitních dat, tak pro komplikovanost pacientů danou komorbiditami a imunosupresí.

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E10
NOVINKY V DIAGNOSTICE A LÉČBĚ DLBCL- POST-ASH

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Difuzní velkobuněčný B-lymfom představuje nejčastější typ nehodgkinských lymfomů. Primoterapie DLBCL je stále založena na imunochemoterapii, buď R(ituximab)-CHOP nebo Pola(tuzumab)-R-CHP[1]. V primoterapii starších či komorbidních pacientů byla dosud za zlatý standard považována redukovaná imunochemoterapie R-mini-CHOP, která ale vede k přežití bez progrese (PFS) ve dvou letech pouze u cca poloviny pacientů[2]. Bispecifické protilátky glofitamab a odronextamab byly schváleny v léčbě relabovaného či refrakterního DLBCL[3-5].

Na konferenci ASH2025 byly prezentovány výsledky několika studií, které analyzovaly efektivitu (a toxicitu) „chemo-free" nebo „chemo-light" přístupů založených na anti-CD20/anti-CD3 bispecifických protilátkách (v monoterapii či v kombinačních režimech) v primoterapie starších či komorbidních pacientů. Studie fáze II „R-Pola-Glo" testující trojkombinaci rituximab, polatuzumab a glofitamab (abstract 61) ukázala významnou účinnost a velmi dobrou snášenlivost této chemo-light kombinace s 1-letým PFS a OS 85%, resp. 90%. Léčba byla navíc účinná u pacientů bez ohledu na většinu dosud používaných prognostických faktorů. Podobně studie fáze II MorningSun testující monoterapii bispecifickou protilátkou mosunetuzumab na relativně malé skupině 49 pacientů ukázala 1-leté přežití bez progrese u 71% pacientů (abstrakt 62). Studie EPCORE-DLBCL-3 testující epcoritamab v monoterapii či v kombinaci s lenalidomidem v primoléčbě starších či komorbidních pacientů vedla k 1-letému přežití bez progrese u 54% (abstract 63). Naproti tomu kombinace epcoritamabu a imunochemoterapie R-mini-CHOP vedla k 2-letému přežití bez progrese u 76% pacientů (abstract 64). Z dosud publikovaných dat vyplývá několik společných poznatků. Zdá se, že ve srovnání se „standardní" imunochemoterapií R-miniCHOP jsou chemo-free, či chemo-light režimy účinnější. Bispecifické protilátky tak v budoucnu mohou představovat základní složku potenciálních kombinačních režimů. Naproti tomu anhracyklinová

cytostatika (doxorubicin) nemají v léčbě starších či komorbidních pacientů žádnou budoucnosti. Zatímco role cílené chemoterapie, zejména polatuzumabu, zejména v kombinaci s bispecifickými protilátkami, se zdá být velice slibná, role anti-CD20 protilátky rituximab v kombinačních režimech s bispecifickými protilátkami musí být objasněna v rámci prospektivních klinických studií. Je tudíž možné, že u starších či komorbidních pacientů s DLBCL se dostaneme nejen do éry „chemo-free/light", ale i do éry post-rituximabové.

DLBCL je skupina geneticky heterogenních onemocnění[6-9]. Je důležité připomenout, že prognostické faktory standardně užívané u pacientů s DLBCL, například IPI, byly etablovány v éře klasické imunochemoterapie (zejména R-CHOPu). S nástupem nových cílených léčiv na jedné straně a imunoterapeutických modalit založených na aktivaci T-lymfocytů na druhé straně dochází k zásadní proměně významu dosud používaných prognostických faktorů a nástup nových faktorů v kontextu inovativních léčebných modalit a kombinací[10-12].

Na konferenci ASH2025 byla prezentována nová verze LymphGen klasifikace DLBCL (skupina NCI/Stoudt/Wright) založená na integraci mutačního profilu (genetiky) a genové exprese (transkriptomiky) nazvaná LymphGen-Sig (abstrakt 49). Na rozdíl od původní LymphGen klasifikace, která byla schopná spolehlivě klasifikovat pouze cca 60% všech DLBCL, nová LymphGen-Sig klasifikace umí zařadit prakticky 100% všech nově dg. pacientů s DLBCL do jednoho ze 4 základních subtypů. V rámci „konkurenčního" klasifikátoru DLBClass (skupina Dana Farber/Shipp/Chapuy) bylo prezentováno rozdělení C5 subtypu do dvou samostatných subklusterů: C5A s typickými amplifikacemi chromozomů 3 a 18, a C5B s typickými mutacemi MYD88, CD79B, TBL1XR1 a PIM1. Oba subtypy se navíc liší i kompozicí nádorového mikroprostředí (abstract 50). V rámci edukační přednášky o DLBCL bylo prezentováno schéma nové studie National Clinical Trial Networ (NCTN) Intergroup LymphMATCH pro pacienty s nově dg. DLBCL (IPI 2-5). V rámci této studie budou pacienti po podání 1.cyklu terapie (R-CHOP nebo Pola-R-CHP) rozděleni do 5 genetických podtypů (C1/BN2, C2/A53, C3/EZB, C4/ST2 a C5/MCD) a následně (od 2.cyklu terapie) randomizováni do standardního či experimentálního léčebného ramene. Pro skupiny C1/BN2, C2/A53 a C5/MCD bude standardní rameno Pola-R-CHP, zatímco pro skupiny C3/EZB a C4/ST2 bude standardem R-CHOP. Experimentální ramena budou navíc zahrnovat specifický inhibitor dle určeného genetického podtypu (např. ibrutinib, EZH2 inhibitor, JAK2 inhibitor a další). Odlišný koncept prezentoval Mark Roschewski z NCI v rámci studie NCT04002947, ve které pacienti s nově dg. DLBCL dostali před standardní imunochemoterapií monoterapii acalabrutinibem po dobu 14 dnů (abstrakt 56). Pacienti, u kterých došlo ke zmenšení tumoru dle CT alespoň o 25%, dostali následně imunochemoterapii v kombinaci s acalabruinibem. Studie potvrdila účinnost acalabrutinibu v podskupině MCD. Studie dále ukázala významnou účinnosti acalabrutinibu i v dalších podskupinách DLBCL. V rámci predikce odpovědi na anti-CD20/anti-CD3 bispecifické protilátky byl potvrzen význam složení nádorového mikroprostředí[13]. Pacienti s podtypem GCB/immune-hot měli delší přežití bez progrese ve srovnání s ostatními podtypy (tj. GCB/immune-cold a oběma ABC podtypy) (abstrakt 3542). Zdá se, že zatímco genetická klasifikace DLBCL může poměrně přesně predikovat citlivost (či rezistenci) jednotlivých definovaných subtypů DLBCL na vybrané cílené léky (typicky například ibrutinib ve skupině MCD/C5), citlivost na imunoterapeutické léčebné modality založené na aktivaci T-lymfocytů je do značné míry asociována s expresí cílových povrchových molekul (typicky CD19 pro CAR19 T-lymfocyty, CD20 pro anti-CD20/anti-CD3 bispecifické protilátky) a dále se složením nádorového mikroprostředí (immune-hot versus immune-cold pro bispecifické protilátky).

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E11
PRAKTICKÝ PŘÍSTUP K LÉČBĚ CHRONICKÉ LYMFOCYTÁRNÍ LEUKEMIE: SOUČASNÉ MOŽNOSTI A JEJICH OPTIMÁLNÍ VYUŽITÍ

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Chronická lymfocytární leukemie (CLL) prošla v posledním desetiletí zásadní proměnou léčebného paradigmatu. Zavedení inhibitorů Brutonovy tyrosinkinázy (BTK) a inhibitoru BCL-2 venetoklaxu vedlo k výraznému prodloužení přežití bez progrese i celkového přežití napříč všemi skupinami rozdělenými dle prognostických rizikových faktorů a prakticky vytlačilo chemoimunoterapii z rutinní klinické praxe. Současná léčba již není uniformní, ale je založena na biologické stratifikaci onemocnění – zejména na přítomnosti aberací TP53 a mutačním stavu IGHV – a na individualizaci podle komorbidit, tolerance a preferencí pacienta.

Indikace k léčbě a biologická stratifikace

Navzdory terapeutickému pokroku zůstávají indikace k zahájení léčby definovány kritérii aktivity dle iwCLL a samotná diagnóza CLL léčbu nevyžaduje. Zásadním krokem před zahájením terapie je biologická charakterizace

onemocnění, zejména vyšetření delece 17p, mutace TP53 a mutačního stavu IGHV, které dnes představují nezbytný základ racionální volby léčebné strategie.

Tyto parametry si zachovávají prediktivní význam i v éře cílené léčby. Přítomnost aberací TP53 je spojena s nižší hloubkou a kratším trváním odpovědi u časově omezených režimů a podporuje preferenci kontinuální BTK inhibice. Naopak pacienti bez poruchy TP53, zejména s mutovaným IGHV, představují optimální kandidáty pro časově omezené režimy směřující k dosažení hluboké remise.

První linie léčby: kontinuální BTK inhibice

Kontinuální léčba BTK inhibitory představuje standardní přístup zejména u pacientů s vysokým biologickým rizikem a u nemocných preferujících dlouhodobou perorální terapii. Ibrutinib jako první zástupce této skupiny prokázal v klíčových studiích superioritu vůči chemoimunoterapii. Ve studii ECOG-1912 vedla kombinace ibrutinib–rituximab k významnému snížení rizika progrese nebo smrti oproti režimu FCR u mladších fit pacientů, zatímco studie RESONATE-2 potvrdila dlouhodobou kontrolu onemocnění u starších nemocných.

Limitací ibrutinibu se však ukázala off-target toxicita, zejména kardiovaskulární komplikace a krvácivé projevy. Tyto skutečnosti vedly k vývoji BTK inhibitorů druhé generace. Akalabrutinib ve studii ELEVATE-TN významně prodloužil PFS oproti kombinaci chlorambucil–obinutuzumab a ve studii ELEVATE-RR prokázal non-inferiorní účinnost vůči ibrutinibu při nižší kardiovaskulární toxicitě. Zanubrutinib ve studii SEQUOIA dosáhl vysoké účinnosti i u pacientů s delecí 17p. Tyto výsledky etablovaly druhou generaci BTK inhibitorů jako preferovanou volbu pro kontinuální léčbu.

Časově omezená léčba založená na venetoklaxu

Paralelně se rozvinul koncept časově omezené terapie založené na inhibitoru BCL-2. Kombinace venetoklax–obinutuzumab umožňuje dosažení hlubokých remisí s vysokým podílem nedetekovatelné minimální reziduální nemoci (MRN). Studie CLL14 stanovila tento režim jako standard u starších a komorbidních pacientů; při dlouhodobém sledování byl medián PFS výrazně delší než u chemoimunoterapie a většina pacientů dosáhla nedetekovatelné MRN po ukončení léčby.

Hlavním omezením tohoto přístupu zůstává nižší dlouhodobá kontrola onemocnění u pacientů s poruchou TP53. Naopak jeho zásadními výhodami jsou omezená délka terapie, nižší kumulativní toxicita, minimální riziko vzniku rezistentních mutací a absence nutnosti dlouhodobé expozice léčivu, což má klinický i ekonomický význam.

Kombinační režimy BTK a BCL-2

Kombinace BTK a BCL-2 inhibice představuje další krok ke zvýšení hloubky odpovědi při zachování časově omezené léčby. Studie CAPTIVATE a GLOW prokázaly vysoký podíl MRN pod mezí detekce a dlouhodobou kontrolu onemocnění, zatímco studie CLL13 ukázala, že triplet venetoklax–obinutuzumab–ibrutinib dosahuje dosud nejlepších výsledků v první linii u fit pacientů.

Na ASH 2025 byla prezentována časná data studie fáze II hodnotící trojkombinaci nekovalentního BTK inhibitoru pirtobrutinibu, venetoklaxu a obinutuzumabu u biologicky vysoce rizikové populace. Přestože jde o časná data s omezeným sledováním, bylo dosaženo velmi hlubokých odpovědí s vysokým podílem MRN pod mezí detekce a bez dosud pozorované MRN rekurence, což naznačuje potenciál MRN-řízených, časově omezených strategií i u vysoce rizikových nemocných.

Přímé srovnání léčebných paradigmat: studie CLL17

Randomizovaná studie fáze III CLL17, prezentovaná na ASH 2025, přinesla první přímé porovnání kontinuální BTK inhibice a časově omezených režimů založených na terapii venetoklaxem v první linii. Při mediánu sledování přibližně 34 měsíců dosáhly časově omezené režimy non-inferiority vůči kontinuálnímu ibrutinibu z hlediska PFS.

Podle předpokladů kompletní remise a nedetekovatelná MRN byly výrazně častější u časově omezených režimů s venetoklaxem, zatímco při kontinuální BTK inhibici byly tyto cíle dosahovány jen výjimečně. Celkové přežití zůstává zatím srovnatelné; dostupné subanalýzy však naznačují, že u pacientů s TP53 aberacemi si kontinuální BTK inhibice může zachovávat výhodu. Studie CLL17 tak poskytuje klíčový prospektivní důkaz srovnatelné účinnosti obou paradigmat a podporuje preferenci časově omezené léčby u pacientů bez TP53 aberací.

Relabující a refrakterní CLL

Volba léčby při relapsu je zásadně ovlivněna předchozí expozicí cílené terapii. Studie MURANO prokázala výrazně delší PFS při časově omezené léčbě venetoklax–rituximab oproti chemoimunoterapii. Retreatment venetoklaxem může vést k nové léčebné odpovědi u části pacientů, obvykle však s kratším trváním.

Po selhání kovalentních BTK inhibitorů se uplatňují nekovalentní BTK inhibitory. Pirtobrutinib dosáhl vysoké účinnosti i u pacientů s mutací BTK C481 a představuje nový standard v této klinické situaci; probíhající studie naznačují možnost jeho využití i v časnějších liniích.

Pokročilé a experimentální přístupy

U pacientů s duální refrakteritou vůči BTK i BCL-2 inhibitorům zůstává prognóza nepříznivá. CAR-T buněčná terapie umožňuje dosažení dlouhodobých remisí přibližně u 40–50 % nemocných. Velmi slibné časné klinické výsledky vykazují BTK degradéry, které představují inovativní terapeutický koncept schopný překonávat klasické mechanismy rezistence vůči inhibitorům BTK; jejich dlouhodobý klinický přínos a bezpečnostní profil však zatím nelze spolehlivě hodnotit, neboť chybí data z delšího sledování.

Závěr

Současná léčba CLL je založena na racionální volbě mezi kontinuální a časově omezenou cílenou terapií. Rozhodujícími faktory jsou biologické riziko, komorbidity a preference pacienta. Kombinační režimy a MRN-řízené strategie představují další krok směrem k funkčnímu vyléčení části nemocných, zatímco optimalizace sekvenování léčby a dlouhodobé řízení toxicity zůstávají klíčovou výzvou následujících let.

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E12
HODGKINŮV LYMFOM V ROCE 2025: KLÍČOVÁ SDĚLENÍ

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Léčba klasického Hodgkinova lymfomu (cHL) prochází v posledních letech zásadní proměnou. Do 1. linie terapie se postupně dostávají inovativní léčiva, zejména inhibitory PD-1 a brentuximab vedotin (Br), které rozšiřují možnosti dosažení remisí při potenciálně nižší toxicitě léčby. Spolu s rostoucí prevalencí onemocnění a vysokým podílem dlouhodobě přežívajících nemocných se tak

terapeutické cíle posouvají od samotné kurability k minimalizaci akutní i pozdní toxicity a k individualizované léčbě založené na biologických a klinických rizikových faktorech. Tyto otázky jsou zásadní u starších pacientů a nemocných s relabujícím/refrakterním (R/R) průběhem, u nichž zůstávají možnosti efektivní a zároveň dobře tolerované terapie omezené. Novinky z ASH 2025 tyto trendy potvrzují - byly zde představeny aktualizované výsledky studií hodnotících moderní imunochemoterapeutické režimy, spolu s novými daty týkajícími se role PET vyšetření v éře inhibitorů PD-1, validace současných prognostických modelů a strategií léčby pro R/R onemocnění. Následující přehled shrnuje vybrané poznatky z ASH 2025, které mne zaujaly.

Aktualizovaná data z klíčových studií u pokročilých stadií v 1. linii: Pilířem 1. linie u pacientů s pokročilým stadiem zůstává režim BrECADD, jehož dlouhodobá účinnost i snížená toxicita byly potvrzeny ve finální aktualizaci studie HD21. Poměr rizik (HR) pro PFS byl 0,64 (95 % CI 0,44–0,93) ve prospěch režimu BrECADD. Při mediánu sledování 5 let bylo 5leté období bez progrese (PFS) 93,6 % v BrECADD rameni oproti 90,6 % u eBEACOPP, přičemž výrazný přínos v 5letém PFS byl pozorován u PET2-negativních pacientů: 96,2 % vs. 92,4 % (HR 0,46; 95 % CI 0,25–0,83). Celkové přežití (OS) bylo výborné v obou ramenech (98 %) (abs. 152). V evropském prostředí se BrECADD definitivně řadí k preferovanému intenzivnímu režimu u mladých a výkonnostně zdatných pacientů s pokročilým stadiem. V post-hoc analýze se ukázalo, že ATB profylaxe i pegylovaný G-CSF významně snižují riziko febrilní neutropenie a závažných infekcí, zejména v 1. cyklu u režimu BrECADD. Autoři proto doporučují alespoň v 1. cyklu BrECADD zavést rutinní ATB profylaxi (abs. 3626). U pokročilých stadií přineslo ASH 2025 posílení dat pro režim nivolumab-AVD (N-AVD). Při mediánu sledování 3 let přetrvávala výhoda PFS ve prospěch režimu N-AVD (HR 0,48; 95 % CI 0,34-0,69; p < 0,0001). Tříleté PFS bylo 91 % po léčbě N-AVD ve srovnání s 82 % po léčbě BV-AVD. U nemocných 18-60 let bylo 3leté PFS 91 % po N-AVD oproti 85 % po Br-AVD (HR 0,61, 95 % CI 0,38-0,96). U nemocných > 60 let byly rozdíly výraznější - 3leté PFS 82 % po N-AVD vs. 58 % po Br-AVD (HR 0,33, 95 % CI 0,14-0,77) (abs. 151). Na základě delšího 3letého PFS, a to zejména u pacientů > 60 let, považujeme režim N-AVD za preferenční léčbu této starší věkové skupiny. V rámci studie byla zkoumána kvalita života mezi oběma léčebnými režimy. Výsledky ukázaly horší únavu na konci léčby u ramene N-AVD bez klinicky významného rozdílu, ale naopak významně nižší neuropatii po 1 roce ve srovnání s Br-AVD (abs. 2738). Srovnatelné výsledky se studií S1826 potvrdila retrospektivní multicentrická studie z běžné klinické praxe. Při mediánu sledování 10,2 měsíce bylo 1leté PFS 90,6 % u nemocných ≥ 60 let a 95,5 % u pacientů < 60 let (p = 0,044) (abs. 3622).

Prognóza nemocných: Mezinárodní prognostické skóre (IPS) bylo historicky používáno k odhadu 5letého přežití u pacientů s nově diagnostikovaným pokročilým cHL, jeho prognostická síla je však v éře rostoucího využití inovativních léčiv omezená. Mezinárodní konsorcium HoLISTIC (Hodgkin Lymphoma International STudy for Individual Care) vyvinulo nové prognostické modely - A-HIPI pro pokročilá stadia a E-HIPI pro časná (příznivá a nepříznivá) stadia onemocnění u pacientů léčených režimem ABVD. Externí validace indexu A-HIPI ve studii S1826 prokázala jeho lepší schopnost predikce PFS a OS ve srovnání s tradičním IPS, kdy prognostická hodnota zůstala zachována i u pacientů léčených imunoterapií (abs. 154). Na tato data navázala retrospektivní analýza konsorcia HoLISTIC, která porovnala PET-adaptované režimy eBEACOPP a ABVD ve 4 studiích u nově diagnostikovaných pacientů s pokročilým stadiem a potvrdila, že index A-HIPI je nezávislým prognostickým faktorem napříč léčebnými strategiemi, včetně režimu eBEACOPP (abs. 129). Prognostický význam indexu E-HIPI byl potvrzen externí validací ve studiích německé skupiny HD14 a HD17 u nemocných s časným nepříznivým stadiem. U nemocných léčených režimy eBEACOPP, ABVD nebo jejich kombinací v rámci PET-adaptovaných postupů byla diskriminační schopnost modelu srovnatelná s původními vývojovými i validačními kohortami (abs. 1845). Data prezentovaná na ASH 2025 tak ukazují, že indexy A-HIPI a E-HIPI si zachovávají konzistentní výkonnost napříč různými léčebnými přístupy, což podporuje jejich využití jako relativně na terapii nezávislých nástrojů pro stratifikaci rizika. Další výzkum je zapotřebí k hodnocení E-HIPI u pacientů s časným příznivým stadiem.

Role PET/CT při hodnocení léčebné odpovědi: Základem diagnostiky zůstává PET/CT, které slouží k určení vstupního rozsahu lymfomu a v současné době i k hodnocení léčebné odpovědi. V éře chemoterapie bylo PET zásadní pro tzv. „response-adapted" strategie, avšak nová data ze studie S1826 naznačují změnu. Interim PET (iPET) po 2 cyklech N-AVD má nižší prediktivní hodnotu než dříve. Podle této analýzy zůstává 3leté PFS u iPET-pozitivních pacientů stále 84 % v nivolumabovém rameni (93% u iPET-negativity), což ukazuje, že časná PET-pozitivita při imunoterapii může odrážet zánětlivou aktivaci, nikoliv perzistenci nádoru. Podobně i PET na konci léčby může být falešně pozitivní. Studie ukazuje, že PET pozitivity (DS 4–5) na konci léčby je nutné interpretovat s opatrností - až 68 % pacientů s PET-pozitivitou na konci léčby zůstává bez progrese (abs. 1851). Tento posun vede k závěru, že PET Lugano kritéria jsou při imunoterapii nedostatečná a měla by být doplněna citlivějšími nástroji, zejména stanovením cirkulující nádorové DNA (ctDNA). Význam PET vyšetření dále hodnotí data ze studie S1826, která prokázala, že dynamika ctDNA predikuje riziko selhání léčby přesněji než iPET i PET na konci léčby. Pacienti s výrazným poklesem nálože ctDNA již po 2. cyklu dosahovali 3letého PFS srovnatelného s PET-negativními nemocnými, zatímco u pacientů s minimálním poklesem nálože byly výsledky výrazně horší (3leté PFS 38 %). Na konci léčby měla ctDNA nejvyšší prognostickou hodnotu (3leté PFS 32 % vs. 89 % u $ctDNA^+$ vs. $ctDNA^-$) a v multivariantní analýze byl její prognostický vliv silnější než u PET vyšetření (abs. 351).

Deeskalace terapie: Na základě příznivého bezpečnostního profilu pembrolizumabu a předchozích zkušeností s PET-adaptovanými strategiemi byla testována kombinace Br, pembrolizumabu a adriamycinu s dakarbazinem (BrP-AD) s možností deeskalace léčby u pacientů s iPET negativitou. Všichni nemocní dosáhli kritérií pro deeskalaci, což vedlo ke snížení expozice cytostatikům bez zaznamenaných progresí. PET-adaptovaná deeskalace režimu BrP-AD tak prokázala dobrou toleranci a slibnou účinnost v léčbě 1. linie u pokročilého stadia. Delší sledování a větší soubory jsou nutné k potvrzení těchto výsledků (abs. 153). Dále byla představena studie, která hodnotí, zda nízkodávkovaný nivolumab (40 mg) v kombinaci s AVD v 1. linii u pokročilého stadia zachovává vysokou účinnost při současném snížení toxicity oproti současnému standardu péče (abs. 1855).

Léčba R/R cHL: V oblasti R/R HL byla prezentována aktualizovaná 5letá data studie fáze II hodnotící salvage léčbu kombinací pembrolizumabu a režimu GVD (gemcitabin, vinorelbin, lipozomální doxorubicin), po níž následovala autologní transplantace (ASCT). Tato strategie vedla k vysoké míře dosažení CR (95 %) s 5letým PFS 91 % a OS 94 % (abs. 1850). Režim pembro-GVD je nyní testován v konceptu řízení léčby podle dynamiky ctDNA. Studie poprvé zkoumá, zda lze u pacientů, kteří po 2 cyklech dosáhnou PET-negativity a zároveň nedetekovatelnou ctDNA, zcela vynechat ASCT. Studie je průlomová, protože využívá ctDNA jako rozhodovací nástroj k určení, kteří pacienti ASCT podstoupí. Pokud se očekávané výsledky potvrdí, může tento přístup zásadně změnit roli ASCT v léčbě cHL (abs. 3630). Mezi novinky ASH 2025 patřila i dlouhodobá aktualizace studie CHARIOT, která hodnotila efektivitu autologních CD30-CAR-T buněk u těžce předléčených 12 pacientů s R/R cHL po selhání ≥ 3 linií léčby včetně Br a inhibitorů PD-1. Časné výsledky byly přitom velmi povzbudivé: ORR dosáhla 75 % a CR 50 %. Klíčovým limitem ale zůstává jejich trvání - medián PFS činil pouze 7,1 měsíc a 3leté PFS bylo 17 % (abs. 1844). V oblasti buněčných terapií byla představena 1. analýza kombinace CD30-CAR-T s nivolumabem po selhání 1. linie léčby. Přestože soubor je malý (n=13), léčba byla vysoce účinná: ORR dosáhla 92 %, CR 77 %, 2leté PFS a OS byly 67 % a 90 % (abs. 5938). Studie fáze I s novým anti-CD30 konjugátem PF-08046044 (35C) prokázala u silně předléčených pacientů (většinou po Br a PD-1) vysokou účinnost (ORR 76 %, CR 17 %) (abs. 155).

Závěr: Hodgkinův lymfom vstupuje do éry, kdy imunochemoterapie se stává preferovanou léčbou pro 1. linii u pokročilých stadií. Zajímavé bude sledovat výsledky studií s tímto konceptem u časných stadií, kde je zatím standardem chemoradioterapie. Klasické hodnocení léčebné odpovědi pomocí PET založené na hodnocení Deauville skóre je v éře imunoterapie stále méně dostačující, protože ztrácí prediktivní hodnotu ve srovnání s citlivějšími metodami,

jako je ctDNA. Pro zachování klinické relevance PET bude klíčová integrace kvantitativních parametrů (ΔSUV, TMTV) s molekulárními biomarkery. Salvage léčba R/R průběhu lymfomu dosahuje dosud nejvyšší účinnosti a objevují se nové anti-CD30 konjugáty po selhání standardní léčby.

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SBORNÍK ABSTRAKTŮ

PŘEDNÁŠKOVÁ SDĚLENÍ

(ABSTRAKTY O01 – O13)

SBORNÍK ABSTRAKTŮ

O01

MULTIPLE RECIPROCAL INTERACTIONS WITHIN B-CELL RECEPTOR AND AKT SIGNALING PATHWAYS REGULATE RESPONSE TO EZH2 INHIBITION IN GERMINAL CENTER-DERIVED DIFFUSE LARGE B-CELL LYMPHOMA

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Introduction. Enhancer of Zeste Homolog 2 (EZH2), which trimethylates Histone 3 at lysine 27 (H3K27me3), is frequently affected by somatic gain-of-function (GOF) mutations in follicular lymphoma (FL) and germinal center B-cell–like diffuse large B-cell lymphoma (GCB-DLBCL). Tazemetostat, a highly specific EZH2 inhibitor (EZH2i), is FDA-approved for relapsed or refractory FL with EZH2 mutation but has shown only limited efficacy in DLBCL. It has been reported that EZH2i synergizes with inhibitors of B-cell receptor (BCR) signaling in DLBCL. Since GCB-DLBCL cells depend on tonic (antigen-independent) BCR signaling, which exclusively triggers the PI3K/AKT pathway, we investigated how EZH2i and EZH2 GOF affect tonic BCR signaling.

Methods. We used a spectrum of 10 GCB-DLBCL lines, some modified to allow comparison of unmutated and GOF-mutated EZH2 (Y646S or Y646F). We used CRISPR-Cas9 based technology for knock in (KI) and knock out (KO) and Sleeping Beauty transposon vectors for overexpression. A genetically encoded Förster resonance energy transfer-based reporter was used to measure AKT activity. Key findings were evaluated using primary normal B-lymphocytes and a mouse model having EZH2 GOF mutation.

Results. Sensitivity of GCB-DLBCL cell lines to the EZH2i tazemetostat was highly variable, but correlated with the degree of their dependence on BCR signaling. EZH2i treatment produced dose-related increases in phosphorylation of multiple BCR signaling mediators. The most EZH2i resistant cell lines have the highest dependence on BCR signaling, and the most prominent BCR signaling activation following EZH2i. Moreover, BCR KO sensitized the highly BCR dependent SUDHL-6 line to EZH2i, but not the BCR-independent line DB. KI of a Y188F mutation into the BCR co-receptor protein CD79A, known to block tonic BCR signaling, sensitized SUDHL-6 cells highly to EZH2i; this showed that EZH2i resistance was specifically mediated by tonic BCR signaling.

As expected, H3K27me3 was increased by expression of EZH2 GOF mutations, and reduced or eliminated by EZH2i. Importantly, expression of EZH2 GOF mutations led to robust increases in AKT activity and coincided with increased H3K27me3. As the likely cause for this increase in AKT activity, the abundance of PTEN protein was decreased by induction of EZH2 Y646S, associated with a significant increase of PTEN promoter methylation. Additionally, using PTEN KO and overexpression models, we detected negative feedback between AKT and EZH2 activity. AKT activation decreased EZH2 expression and the level of H3K27me3, both mediated at least partially by mTOR. Overexpression of Y646S EZH2 increased H3K27me3 and decreased PTEN protein also in normal human B cells. Using a mouse model with Ezh2 Y641F mutant allele for conditional expression in CD19+ B cells, we confirmed our observations *in vivo*. CD19+ B cells of mutant mice displayed increased pAKT levels associated with decreased PTEN.

In vitro, we detected broad synergism of EZH2i with FDA-approved or clinically tested inhibitors of BCR signal-mediating kinases SYK, BTK, and PI3Kδ. Mechanistically, EZH2i sensitized cells to AKT inhibition (direct or mediated by upstream BCR signal inhibition). Therefore, the mechanism of detected synergy is at least partially driven by the capability of EZH2i to increase dependence of cells on AKT activity and consequent increased sensitivity to its inhibition.

Conclusion. Reciprocal negative relationships affecting EZH2i resistance were found between EZH2, PTEN, and AKT, comprising a regulatory network with contributions from BCR signaling. GOF mutations increased the quantitative effect of EZH2 but were not essential to the network. Our findings provide further rationale for combining EZH2 inhibition with inhibitors of other targets, such as BCR signaling mediators, in treating lymphoma.

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O02

REPRESSION OF MIR-29 VIA MYC LEADS TO INCREASED CD40 SIGNALING IN TRANSFORMED FOLLICULAR LYMPHOMA

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Follicular lymphoma (FL) patients are at risk of disease transformation to high-grade lymphoma (tFL). While several genetic alterations have been implicated in tFL, the role of microenvironmental interactions and epigenetic regulation by non-coding RNAs remains poorly understood.

We performed the first matched profiling of short non-coding RNAs (microRNAs) and mRNAs in paired FL and tFL samples (n=10 pairs). This identified differential expression of 1075 mRNAs and 19 miRNAs, including repression of the *miR-29* family in tFL (*miR-29a/b/c*, **FIG.A**). *miR-29s* are known to regulate CLL aggressiveness (Sharma et al, Blood, 2021), and we further focused on this miR family. Subsequent analysis uncovered that MYC activity is uniformly induced in tFL (GSEA, FDR=0.016) and represses *miR-29* by directly binding to its promotor, and in lymphoma cell lines MYC silencing (siRNA) led to *miR-29s* induction (P<0.05). RNAseq in FL-tFL pairs revealed changes in multiple molecular pathways potentially controlled by *miR-29s,* including CD40 signaling being strongly activated in tFL (GSEA, IPA). CD40 pathway is a major pro-proliferative factor in normal and FL lymph nodes. A reanalysis of scRNAseq data (Roider et al, 2019) revealed that CD40L was amongst the 10 most active ligands in tFL **(FIG.B)**. Increased CD40 pathway activity in tFL contrasted with the reduced CD4+ and CD8+ T-cell numbers in tFL niches (IHC in 10 FL-tFL pairs, CIBERSORTx from FL-tFL RNAseq). To directly identify *miR-29* targets, we performed RNA profiling in 2 cell lines engineered for *miR-29c* overexpression, revealing 20 putative *miR-29* targets downregulated in both cell lines. This included *TRAF4*, which has been previously linked to CD40 signaling propensity. In cell lines, *miR-29c* overexpression leads to ~50% reduction of TRAF4 levels **(FIG.C)** via direct *miR-29c* binding to *TRAF4* 3'UTR. Importantly, cell lines constitutively overexpressing *miR-29c* or transfected with synthetic *miR-29c* (1000 nM) were less responsive (lower pIKKa/b) to recombinant CD40L **(FIG.C)** or HS-5 cells engineered for CD40L expression. Altogether, MYC-mediated *miR-29* repression results in increased TRAF4 and higher CD40 signaling propensity. Importantly, TRAF4 levels were increased in tFL compared to paired FL (n=11 pairs, **FIG.D)** and in *de novo* DLBCL compared to FL (n=30 each). Ki67 correlated positively with TRAF4 levels and negatively with *miR-29s* levels (FL/tFL n=46), and TRAF4 and MYC levels were concurrently induced in tFL (R=0,8, P=0.004)**.** Lower levels of all *miR-29s(a/b/c)* were associated with shorter OS and PFS in FL (n=185, for *miR-29c* in **FIG.E**), and this was also significant in a multivariate analysis (age, FLIPI, Hgb, LDH, Ann Arbor, B sympt.). Lower *miR-29c* levels were also associated with shorter OS in a validation FL cohort (n=92, **FIG.F)** from an R-CHOP arm of a first-line therapy trial (NCT00006721), but not in DLBCL (n=174).

The first whole-genome miRNA profiling in tFL showed that MYC represses *miR-29s* levels, leading to increased TRAF4 levels and subsequently stronger CD40 signaling propensity (**FIG.G**). This likely represents an adaptive response to reduced CD40L availability from T-cells in the tFL niches. Moreover, low levels of *miR-29c* can be used as an FFPE-based biomarker of unfavorable FL prognosis.

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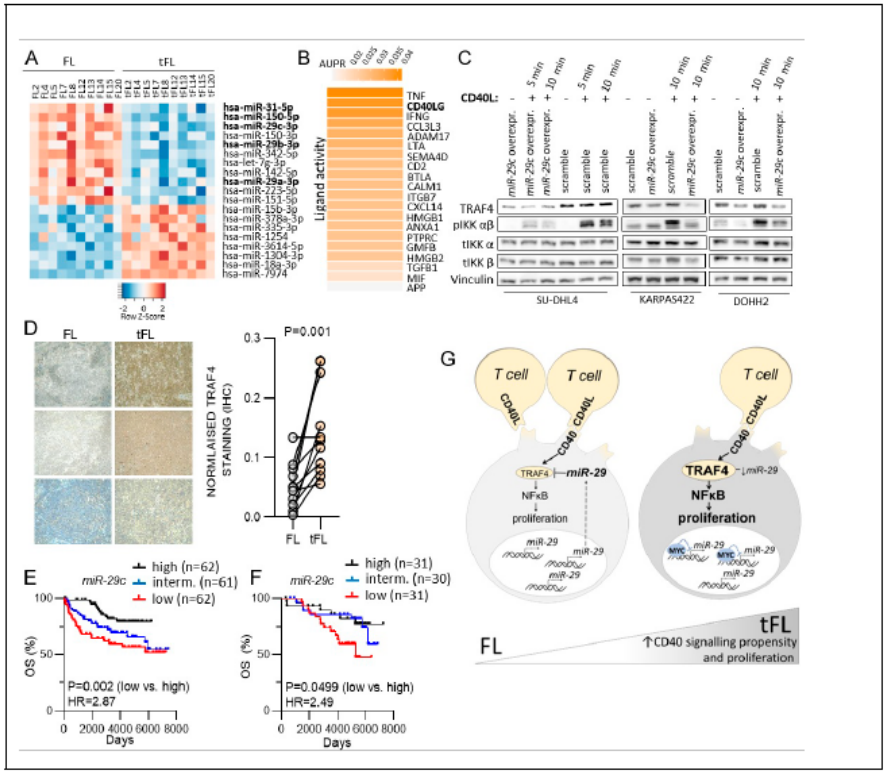
SBORNÍK ABSTRAKTŮ



O03

CLONAL DYNAMICS OF T CELLS AND TREATMENT OUTCOMES IN PEDIATRIC LYMPHOCYTIC VARIANT EOSINOPHILIC SYNDROME

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Lymphocytic variant hypereosinophilic syndrome (L-HES) is a rare form of HES driven by IL-5 overproduction from aberrant or clonal T cells, most commonly CD3⁻ or CD3lowCD4⁺. It typically presents in adults, with pediatric cases poorly characterized. Since 2006, we have systematically screened pediatric patients with unexplained eosinophilia referred to our center for the presence of aberrant CD3⁻CD4⁺ T-cell populations.

Methods

T-cell receptor (TR) gene rearrangements were assessed in bulk samples or sorted aberrant T-cell populations. In two cases with confirmed clonal TR rearrangements, clone-specific qPCR assays were developed for monitoring. Whole-exome sequencing (WES) was performed to exclude germline primary

immunodeficiencies and to assess somatic variants in sorted aberrant T cells. Extended immunophenotyping was performed in all patients.

Results

We identified four pediatric patients with persistent aberrant CD3⁻CD4⁺ T-cell populations.

Patient 1 had a severe L-HES phenotype with eosinophilia up to 30×10⁹/L and clonal CD3⁻CD4⁺ population that comprised 74% of lymphocytes. She suffered from debilitating atopic dermatitis unresponsive to multiple immunosuppressive regimens and underwent allogeneic hematopoietic stem cell transplantation (HSCT) at age 15. Post-HSCT complications included CMV reactivation and sepsis, but she achieved full donor chimerism and long-term remission. Residual clone was intermittently detectable by qPCR but not by flow cytometry for 14 years after transplant. WES revealed somatic variants predicted as potentially deleterious in genes described with T-cell lymphoproliferations (e.g. TET2, PRKCB).

Patient 2, diagnosed at age 3, presented with food-triggered allergic reactions and eosinophilia. A CD3⁻CD4⁺ population (1.2% of lymphocytes) was tracked by clone-specific qPCR. She responded well to corticosteroids and later mepolizumab (anti-IL5), with clinical improvement and stable residual eosinophilia. The clonal T-cell population persists (5.7% of lymphocytes in 9/2024). WES showed acquired changes in sorted CD3⁻CD4⁺ population, but most variants were located outside coding regions.

Patient 3, currently 17, has a severe, treatment-resistant systemic disease with psoriasiform dermatitis since age of 3, growth failure, and hepatosplenomegaly. Diagnosed at age 10, she had a small CD3⁻CD4⁺ population (0.22% out of lymphocytes). Following poor response to multiple immunosuppressants, she received mepolizumab combined with other biologics (e.g., ustekinumab, secukinumab, baricitinib), which improved eosinophilia and skin symptoms. The treatment regimen required frequent adjustments due to infections and adverse events.atient 4 is a pediatric heart transplant recipient, originally treated for critical congenital aortic stenosis, has developed persistent, unexplained eosinophilia 6 years after heart transplant. Despite the presence of an atypical CD3⁻CD4⁺ T-cell at level 0.1% of lymphocytes and sustained peripheral eosinophilia (up to 1.34 ×10⁹/l), the patient remains in good clinical condition with stable cardiac parameters and no signs of end-organ damage.

Conclusions

Clonal or aberrant CD3⁻CD4⁺ T-cell populations can underlie pediatric eosinophilia of unknown origin. Anti-IL5 therapy was effective in forms with smaller aberrant populations, while HSCT achieved long-term remission in a severe case. Continued monitoring is essential to guide individualized treatment. In cases 1 and 2, the aberrant clonal T-cell population was likely the primary driver of the disease. In the latter two cases, it remains a matter of debate whether this population is a secondary effect of long-term immunosuppression.

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O04

JAK2 R1063H GERMLINE VARIANT IN AML: ASSOCIATION WITH ONCOGENIC IDH1/2 DRIVERS RESULTING IN COOPERATIVE LEUKEMOGENESIS AND THERAPEUTIC IMPLICATIONS

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Background

Aberrant JAK2-STAT signaling is implicated in the pathogenesis of myeloid malignancies, including myeloproliferative neoplasms (MPNs) and acute myeloid leukemia (AML). The germline JAK2 R1063H weak gain-of-function variant was initially identified in hereditary erythrocytosis and later reported in MPN/AML patients. However, its role in the pathogenesis of myeloid malignancies remains unclear. Approximately 20% of AML patients harbor somatic mutations in the isocitrate dehydrogenase (IDH) genes, IDH1 or IDH2. These mutations drive excessive oncometabolite 2-hydroxyglutarate (2-HG) production, leading to epigenetic dysregulation and impaired hematopoietic differentiation.

Aims

This study explores the frequency, cooperative effects, and therapeutic implications of the JAK2 R1063H germline variant in AML, focusing on its interaction with the somatic IDH2 R140Q mutation.

Methods

We analyzed 400 AML samples from the Czech Republic (120 primary and 280 from a validation cohort) for the presence of the JAK2 R1063H variant using targeted next-generation sequencing. Functional studies were performed in Ba/F3-EPOR cells expressing JAK2 R1063H and IDH2 R140Q variants alone or in combination. These studies assessed proliferation, survival, cell cycle progression, histone methylation, DNA damage, and gene expression. 2-HG levels were quantified by mass spectrometry, and the effects of ruxolitinib (a JAK1/2 inhibitor) and enasidenib (an IDH2 inhibitor) were tested in engineered Ba/F3 cells and primary patient cells ex vivo.

Results

The JAK2 R1063H germline variant was found in 18 AML patients with 2.25% minor allele frequency - MAF, which is higher than the 1.67% MAF in healthy controls (data on healthy controls from the 1000 Czech Genomes Project). The variant was enriched in patients with somatic IDH1/2 mutations (8/74 cases), particularly IDH2 R140Q. Functional studies showed that JAK2 R1063H and IDH2 R140Q co-expressing cells had significantly higher 2-HG levels compared to controls and cells expressing the single mutants along. Consequently, the cell model with JAK2 R1063H/IDH2 R140Q had increased trimethylated H3K9 and H3K4. This was associated with accumulation of cells in the G2/M phase and elevated γH2AX levels indicating enhanced DNA damage. RNA sequencing analyses revealed impaired homologous recombination and altered mitochondrial metabolism in JAK2 R1063H/IDH2 R140Q co-expressing cells. This resulted in compromised proliferation and excessive apoptosis upon growth factor withdrawal. Combined treatment with ruxolitinib and enasidenib showed a cooperative effect in normalizing the 2-HG levels in JAK2 R1063H/IDH2 R140Q co-expressing cells, reduced proliferation, and induced cell death. In colony formation assays with primary patient samples, the JAK2 R1063H allele enhanced sensitivity to ruxolitinib, and combination therapy with enasidenib effectively suppressed colony formation, promoted differentiation, and induced hemoglobinization in JAK2 R1063H/IDH2 R140Q AML patients' colonies.

Summary/Conclusion

We confirm a higher frequency of the germline JAK2 R1063H variant in AML. Functionally, we demonstrate the cooperation between the IDH2 oncogene and the JAK2 R1063H germline variant. Our findings also propose a potential therapeutic strategy using a combination of ruxolitinib and enasidenib for personalized treatment of IDH2 R140Q-mutant AML patients harboring the germline JAK2 R1063H variant.

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O05

CHRONIC MYELOID LEUKEMIA (CML) TREATMENT DISCONTINUATION AFTER A TWO-STEP DOSE REDUCTION. THE FIRST RESULTS OF THE PHASE 2 STUDY HALF

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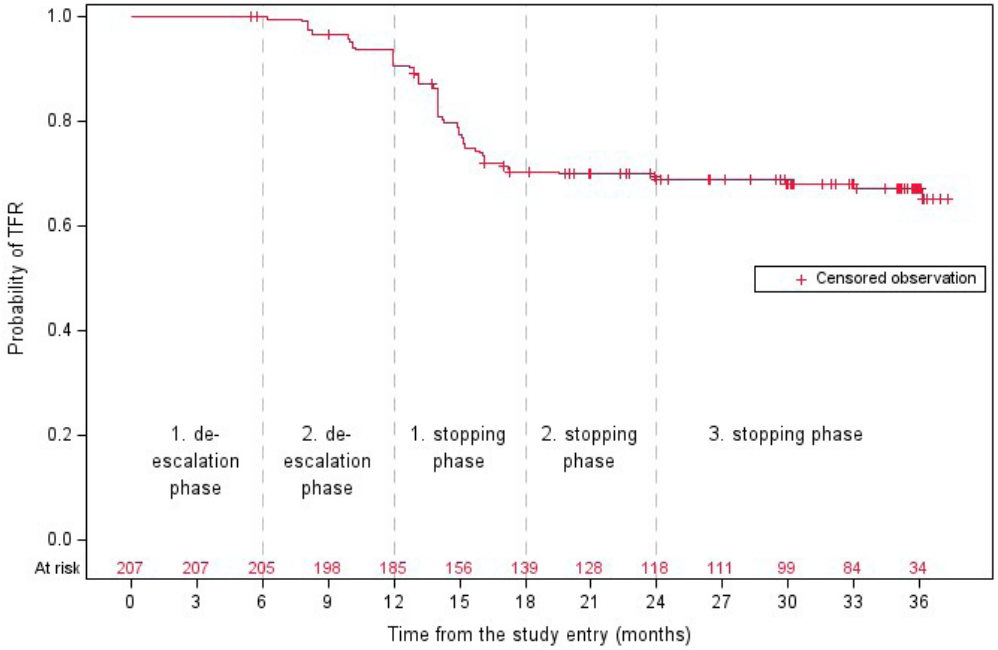
Background:

The tyrosine kinase inhibitor (TKI) discontinuation strategy has become a safe management approach in clinical practice for eligible patients who meet the necessary criteria. However, several important aspects observed in clinical trials involving the abrupt discontinuation remain unresolved, such as the biological mechanisms underlying early and rapid molecular relapses, as well as withdrawal syndrome.

Aims:
The objectives of the HALF clinical trial were to assess the rate of patients achieving the treatment-free remission (TFR), to identify predictive factors for molecular relapses, and to describe the dynamics of side effects after stopping.

Methods:
During the years 2020-2023, the HALF trial was offered to eligible patients in the Czech Republic: treatment duration for at least 4 years, and MR4 duration for at least 2 years. After enrollment, the TKI dose was reduced to the half of the standard dose. After a half year, this reduced dose was administered every other day for another half year. Then, the treatment was stopped. Several clinical and laboratory parameters were collected before and after stopping. The trigger for TKI reintroducing was the confirmed loss of major molecular response (MMR).

Results:
We enrolled 207 patients with median age 61.8 years (24-86) and with median TKI duration 8,6 years (4-20). Imatinib was the most frequent first line treatment (79%). TFR rate at 15 months was 70.4%. With this dose reduction approach, none of the so far studied parameters showed significant correlation ($p<0.05$) with the TFR rate: age at diagnosis; age at enrollment; sex; basophils, eosinophils, blasts and thrombocytes at diagnosis; risk scores (Sokal, Hasford, ELTS); transcript type; length of TKI therapy or MR4; type of TKI; CD4+, CD8+, T-regs cells, B and NK cell; and the presence of clonal hematopoiesis at enrollment. At month 15, the following parameters significantly ($p<0.05$) decreased (creatinine, Na, Cl, AST, creatine kinase, amylase, lipase, adiponectin) or increased (leukocytes, thrombocytes, Hb, urea, K, Ca, P, ALP; but also total and LDL cholesterol, triglycerides). At the same time point, these side effects present at enrollment with the frequency of at least 5% significantly ($p<0.05$) decreased: arthralgia, myalgia, skin rash, nausea, peripheral edemas, diarrhea, muscle cramps, fatigue; but in some patients also newly developed: arthralgia (8.9%), myalgia (4.7%), skin rash (1%), peripheral edemas (2.1%), diarrhea (0.5%), muscle cramps (4.7%), fatigue (4.7%). Withdrawal-related musculoskeletal symptoms were mostly mild and transient. Three patients received steroids, one returned to the TKI; others received oral magnesium (n=9) or analgesics (n=7). Assessment of *BCR::ABL1* kinetics during both reduction phases showed that a linear trend analysis can help to classify patients for TFR success. Patients with increasing *BCR::ABL1* levels in either phase have a higher risk of subsequent loss of MMR.



O06

DNA-BASED MRD MONITORING ENHANCES RISK STRATIFICATION DURING TKI DOSE REDUCTION IN CML: EVIDENCE FROM THE CLINICAL TRIAL

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Background:
In chronic myeloid leukemia (CML), DNA-based measurable residual disease (MRD) analysis - either combined with RNA MRD (Machova Polakova et al. Leukemia 2020) or with characterization of DNA-positive cell subtypes (Pagani et al. Blood 2023) - has demonstrated predictive value for treatment-free remission (TFR). These strategies form the basis of the TFR traffic light stratification model, previously described in patients undergoing direct tyrosine kinase inhibitor (TKI) cessation.

Aim:
This study evaluated the applicability of the DNA-/RNA-based TFR traffic light model in a structured, two-step TKI dose de-escalation protocol prior to cessation, as implemented in the HALF trial (NCT04147533).

Methods:
Between 2020 and 2023, 95 of 207 patients enrolled in the HALF trial underwent genomic breakpoint characterization and optimization of *BCR::ABL1* DNA digital PCR assays. To date, DNA and RNA MRD assessments were performed in 83 patients, generating 1,222 paired samples. MRD was monitored throughout the two de-escalation phases: (1) halving the TKI dose for six months, followed by (2) alternate-day dosing for an additional six months. Patients maintaining major molecular response (MMR) proceeded to TKI cessation.

Results:
At the end of phase 1, 83 patients were stratified as follows: 29 double-negative (green), 26 DNA^{+}/RNA^{-} (yellow), and 28 double-positive (red). After phase 2, MRD status shifted to 31 green, 19 yellow, and 33 red. Only red-group patients relapsed during phase 2 (n=7).

By 24 months post-enrolment, MRD dynamics continued to evolve. In the yellow group, 11/19 progressed to double-positive (4 relapsed); 1 reverted to green. Among green patients, 6 converted to red (3 relapsed), and 2 to yellow. In the red group, 11 remained unchanged; 14 relapsed; and 1 improved to yellow.

Stratification by MRD status at 12 months significantly predicted molecular recurrence-free survival (MRFS) at 18 months (i.e., 6 months post-TKI cessation): red group, 39% MRFS (HR: 9.71; 95% CI: 2.87–32.78; p=0.00025); yellow group, 87% MRFS (HR: 1.68; 95% CI: 0.34–8.34; p=0.56); green group, 90% MRFS. This stratification remained consistent at 36 months (i.e., 24 months post-TKI cessation): red group, 36% MRFS (HR:

8.10; 95% CI: 2.77–23.69; *p*=0.00013); yellow group, 74% MRFS (HR: 2.12; 95% CI: 0.57–7.91; *p*=0.26); green group, 87% MRFS.

Conclusion:
Persistent RNA positivity (double-positive MRD) is significantly associated with molecular relapse both during phase 2 of TKI dose reduction and after TKI cessation. DNA MRD analysis is essential to distinguish truly MRD-negative (green) patients from those with DNA⁺/RNA⁻ status (yellow), who carry an intermediate risk of relapse. Importantly, in yellow-group patients, the emergence of RNA expression during either phase of dose reduction indicates an increased risk of relapse. In such cases, we recommend returning to the previous TKI dose rather than proceeding to cessation. For green-group patients, the appearance of DNA positivity should prompt close monitoring, and any subsequent RNA positivity should be considered a warning sign to resume reduced-dose or full-dose TKI therapy. Overall, the two-step TKI de-escalation strategy, when combined with integrated DNA/RNA MRD monitoring in RNA-negative patients through regular follow-up, may reduce the risk of molecular relapse and improve patient selection for safe TKI discontinuation. Supported by: Ministry of Health of the Czech Republic NU22-03-00136, MH CZ-DRO (IHBT 0002373), National Institute for Cancer Research Project (Programme EXCELES, ID Project No. LX22NPO5102) - Funded by the EuropeanUnion - Next Generation EU

O07

ELEVATED CIRCULATING TUMOR CELL (CTC) NUMBERS ASSOCIATE WITH A HIGHLY PROLIFERATIVE (PR) AND GENOMICALLY COMPLEX PHENOTYPE IN NEWLY DIAGNOSED MULTIPLE MYELOMA (NDMM) PATIENTS

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Background
CTCs have emerged as a key prognostic factor for predicting clinical outcomes in NDMM. However, it is unclear if high CTC counts represent a surrogate of tumor burden or might be driven by distinct genomic drivers.

Aims
To define genomic and transcriptomic features associated with CTC burden.

Methods
We interrogated 540 baseline patients from CoMMpass (IA22) with CTC information available. CTCs were studied by the CellSearch System. Whole-exome/genome (WGS/WES) and RNA-seq data of bone marrow

(BM) plasma cells (PC) were available for 85% and 51%. An external dataset (n=114) with CTCs estimated by next-generation flow (NGF) was used for validation, with RNA-seq and WGS of BM PCs available for 52% and 9.6%.

Results

Elevated CTCs at baseline showed shorter progression-free (PFS) and overall survival (OS). Notably, patients with ≤10 CTCs exhibited outstanding OS. Refractory and early progressing patients (ie, ≤18 months) had higher CTC counts at baseline compared to the other patients (p=.036). High CTC levels were significantly associated with high BM infiltration (ρ=.4; p<.001) and high-risk clinical features such as ISS3 (p<.001) and high LDH (p=.017). Longitudinal CTC assessments revealed a bigger CTC burden reduction in those patients responding favorably (p<.001). Considering the genomic classification proposed in [Maura, JCO 2024], complex genomic subgroups (ie, NSD2_Gain/Amp1q_Del13q, CCND1_Complex, and MAF_HyperAPOBEC; p≤.02) were associated with high CTCs. Thus, we also observed a strong correlation between elevated CTCs and high-risk genomic features such as chromothripsis (p<.001), gain/amp1q (p<.001), APOBEC mutagenesis (p=.02), MAF translocations (p=.004), and RB1(p<.001). External validation also revealed an association between high CTC counts and complex genomic profile. Analyzing recurrent structural variant hotspots, we identified enriched focal gains of ITGAE in patients with high CTCs (n=10). As to clinical outcomes, a cutoff of 1000 CTCs showed an independent association with PFS and/or OS when combined, for instance, with chromothripsis (p=.002/p=.05), APOBEC (p<.001/p=.016), gain/amp1q (p<.001/p=.7), or RB1 (p<.001/p=.2). To explore transcriptomic patterns linked to CTCs, we analyzed RNA-seq data using a linear model adjusted for BM infiltration, identifying 210 genes positively correlated with CTCs (e.g., NRAS and TENT5C) enriched in proliferation and cell cycle functions (eg, chromosome segregation or spindle organization). The validation set confirmed the enrichment in cell cycle-related functions. Other 452 genes negatively associated with CTCs (e.g., CXCL12 and CCL25), enriched in migration and immune activation (eg, leukocyte migration or macrophage activation). Hence, CTCs strongly correlated with published PR indexes (ie, Zhan, Blood 2006 or Skerget, Nat Gen 2024; p<.001) or the PC leukemialike signature (Bruinink, JCO 2022; p<.001). Yet, some patients still had high PR values independent of CTC counts, showing reduced PFS (p≤.001), high LDH or ISS3 (p≤.01), and increased amp/gain1q, del8p, or del13p.

Summary/Conclusion

While BM from patients with low CTC counts reflected NDMM patients with low PR profile (~70-75% patients), elevated CTCs appear to reflect disease with increased circulating potential, higher PR activity and genomic complexity, and reduced adhesion molecules' expression. The addition of CTC enumeration to genomic classification may further improve the risk stratification of MM patients.

SBORNÍK ABSTRAKTŮ

O08

LONGITUDINAL IMMUNE MONITORING IN PATIENTS TREATED WITH CD19 AND BCMA CAR-T CELLS USING STANDARDIZED 8 COLOR FLOW CYTOMETRY PANEL REVEALS SUBSTANTIAL DIFFERENCES AMONG PRODUCTS KINETICS AND PERSISTENCE

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Background: Chimeric antigen receptor T cells (CAR-T) represent a groundbreaking therapy in hematologic malignancies. Currently approved products target CD19 (axi-cel, brexu-cel, tisa-cel, liso-cel) and BCMA (cilta-cel, ide-cel). CAR-T expansion was linked to both clinical efficacy and toxicity, however, comparative data across CAR-T products remain limited, particularly longitudinal data on CAR-T kinetics and persistence of both CAR-T and non-CAR-T subsets and its clinical relevance.

Aims: To assess and compare longitudinal kinetics of CAR-T and non-CAR-T immune subsets among different CD19 and BCMA CAR-T products and to determine clinical relevance of immune monitoring in CAR-T treated patients.

Methods: In this multicenter study, 727 peripheral blood (PB) samples from 123 patients treated with axi-cel (sample/patient counts: 377/71), cilta-cel (177/26), tisa-cel (96/14), and brexu-cel (77/12) were analyzed. Samples were collected at baseline (Day 0 [D0]), then D7, D14, D21, D28, and monthly to Month 15 (M15). Specifically designed and standardized 8-color flow cytometry panel (CD3, CD4, CD8, CD19, CD20, CD45, CD16+CD56, and CAR) was used in routine practice.

Results: Diagnoses included B-cell non-Hodgkin lymphoma (B-NHL) (71% [89/123]), multiple myeloma (MM) (21% [26/123]), and B-cell acute leukemia (ALL) (7% [8/123]). Median age was 63, 71, and 40 years for B-NHL, MM, and ALL respectively. Axi-cel was used in 80% (71/89) of B-NHLs. CRS occurred in 91%, 76%, and 75% with grade ≥2 observed in 54%, 28%, and 24%. ICANS occurred in 46%, 40%, and 38% ,with grade ≥2 observed in 30%, 8%, and 12% of B-NHL, MM, and ALL cases. CR/VGPR was achieved in 70%, 96%, and 100% cases and 26%, 0%, and 12% progressed (PD). Median follow-up of was 364 days. Median CD45+ leukocytes per sample was 1.74×10^5 and median limit of detection (LOD) was 0.0117%.

First, CAR-T product expansion was compared among the products. Axi-cel and brexu-cel counts (cells/μL PB) peaked on D7, while tisa-cel and cilta-cel peaked on D14. Cilta-cel exhibited markedly higher peak counts (median: 599.1), compared to axi-cel (91.4), tisa-cel (86.8), and brexu-cel (35.3).

Moreover, persistence in PB was assessed for axi-cel and cilta-cel, which had sufficient sampling. Axi-cel persisted at low levels often near LOD (median 0.8/μL) in 45% (17/38) to 41% (7/17) of patients from M4 to M13-15, while cilta-cel was undetectable from M3 onwards (16/16). Axi-cel patients showed limited B-cell recovery (5% to 35% from M4 to M13-15), while B-cells recovered in 100% of cilta-cel patients on M3 and sustained.

Then, CD4/CD8 kinetics in the CAR-T pool were compared between axi-cel and cilta-cel. Axi-cel had initial low CD4+ proportions followed by CD4+ increase in persistence (D7–D21 30.9% vs M4–M15 85.2%; p=0.001), whereas cilta-cel showed early CD4+ dominance declining over time (D7 77.8% vs D28 43.0%; p<0.001).

In order to assess the association of CAR-T cell expansion and clinical outcomes, we used our largest cohort of B-NHL patients treated with axi-cel (N=71). As anticipated, CAR-T expansion at D7 was linked to toxicity and efficacy. Increased number of CD8+ CAR-T cellsassociated with CRS ≥2 (p=0.048), with CD4+ showing statistical trend (p=0.06). Total CAR-T counts were elevated in CRS and ICANS ≥2 (p<0.012). CD4+ and CD8+ CAR-T counts were higher in CRS ≥2 (p<0.02), and CD8+ counts increased in ICANS ≥2 (p=0.005). Patients achieving CR had higher D7 CD8+ CAR-T counts (p=0.049); total and CD8+ counts at D14 were also elevated (p<0.024). Using a cutoff (Maxstat) of 129 CAR-T/μL at D7, low expansion predicted worse PFS (1-year PFS: 59% vs. 100%, HR 0.12, p=0.015) and lower CR rates (57% vs 82%, p=0.08).

Conclusion: This is the first study comprehensively comparing CAR-T and non-CAR-T cell kinetics across different CAR-T products targeting CD19 and BCMA using a specialized flow cytometry panel. Distinct peak kinetics and persistence patterns were observed, with cilta-cel showing higher peaks and rapid disappearance by M3, accompanied by B-cell recovery, while axi-cel tends to persist for longer time period in a subset of patients, with prolonged B cell aplasia. In axi-cel patients, greater CAR-T expansion was associated with higher toxicity but also improved response and PFS.

O09

IMMUNE AND DNA-DAMAGE RESPONSES IN THE INTESTINAL STROMA OF PATIENTS PRESENTING WITH LOWER GASTRO-INTESTINAL SYMPTOMS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Introduction

Infection and immune-driven inflammation of the gut often result in severe intestinal dysfunction or even end-organ disease. Intestinal dysfunction negatively affects outcome of allogeneic hematopoietic stem cell (HSCT) recipients. Cellular senescence (CS) represents a stress response mechanism. It is considered as a program of innate immunity and exhibits immunomodulatory characteristics. The study of DNA-damage and CS markers in nucleated cells of the intestinal stroma including innate and adaptive immunity cellular effectors infiltrating the intestinal stroma was performed. Correlations between DNA-damage and immune responses and the effect of common pre- and peri- transplant risk factors responses were analyzed.

Methods
Using the multiplex immunofluorescence (Lunaphore CometTM) we analyzed a tissue microarray marked with 34 antibodies targeting immune cells, CS, apoptosis and inflammation from 27 colonic biopsies of 24 patients who developed lower gastro-intestinal (GI) symptoms after allogeneic HSCT. CD3 and CD68 markers were used to identify T- lymphocytes and macrophages infiltrating intestinal stroma, respectively. Promyelocytic leukemia protein (PML) and p53-binding protein 1 (53BP1) nuclear bodies as DNA-damage markers and CS marker p16INK4a were analyzed in all nucleated cells and in CD3 and CD68 cellular subsets of the intestinal stroma. Nuclear markers were quantified per nuclei count and per nuclear area. Immune cells were quantified per cell count and per stromal area. Mean fluorescence intensity was measured for every marker.

Results
Median time of GI symptoms onset was 64 days (12-903). GI symptoms stage III-IV were present in 75 % of recipients. The median time of onset of GI symptoms stage III-IV was 45 days (range 12-134), whereas the time of onset of GI symptoms stage I-II was 126 days (range 64-903) ($p<0.01$). Acute graft versus host disease (GVHD) was diagnosed in 71 % of patients. Overlap syndrome and chronic GVHD developed in 21 and 30 % of patients, respectively. Nuclear PML foci count positively correlated with the count of nuclear 53BP1 foci ($R=0.53$, $p<0.01$). Importantly, positive correlation was found between the count of 53BP1 foci and p16INK4a positivity ($R=0.50$, $p<0.01$). Increased PML nuclear foci count correlated with augmented infiltration with CD68+ macrophages and CD3+lymphocytes ($R=0.46$, $p=0.02$, $R=0.45$, $p=0.02$), respectively. Also, increased 53BP1 nuclear foci correlated with enriched infiltration with CD68+ macrophages and CD3+lymphocytes ($R=0.61$, $p<0.01$, $R=0.39$, $p=0.04$), respectively. Increased counts of 53BP1 foci were observed in patients who developed GI symptoms later than the median time of onset ($p=0.07$). Also, patients presenting with mild GI symptoms (stage I-II) had increased counts of 53BP1 nuclear foci ($p=0.02$). There was a positive correlation found between the time of lower GI symptoms onset and the number of CD3+ T-cells positive for p16INK4a ($p=0.03$). Patients classified as late onset acute GVHD/overlap syndrome had increased expression of p16INK4a in the intestinal stromal cells ($p=0.08$). Combined immunosuppression was associated with augmented counts of PML ($p=0.03$) and 53BP1 ($p=0.06$) nuclear foci in CD3+ lymphocytes, respectively. Moreover, in CD3+ lymphocytes, 53BP1 nuclear foci were increased in HLA- mismatched recipients ($p<0.01$).

Conclusion
Organ-intrinsic immune reconstitution favors successful HSCT outcome. DNA-damage response and CS elicit immunomodulatory effects implying innate immune responses that may provide transitional substitute for restoring adaptive immunity but may become deleterious in long time perspective. Deciphering their roles in the context of HSCT-related complications may unravel novel treatment venues.

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SBORNÍK ABSTRAKTŮ

O10

OPTIMIZING RISK STRATIFICATION IN PATIENTS AGED 80+ YEARS WITH LARGE B-CELL LYMPHOMA: COMPARISON OF PROGNOSTIC INDICES IN THREE POOLED INTERNATIONAL COHORTS

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Introduction

The International Prognostic Index (IPI) is widely used to stratify risk in large B-cell lymphoma (LBCL) across all ages, with age-adjusted (aa)IPI applied to younger patients. While there have been efforts to develop prognostic indices (PIs) tailored to older patients, few are adopted in clinical practice due to their complexity and reliance on functional assessments, which are not routinely collected in real-world registry datasets. Recently proposed SENIOR-IPI (Dubois, ASH 2024) was developed for older patients with LBCL receiving anthracycline (A)-based therapy. We assessed the performance of

standard and novel PIs in patients aged 80+ years and externally validated the SENIOR-IPI.

Methods

We harmonized and pooled data of patients aged 80+ years with newly diagnosed LBCL from a national registry and two prospective cohorts: NiHiL (Czech Republic, enrolled 2010–2023), LEO (USA; n=222, 2015–2020), and MER (USA; n=77, 2010–2015), forming a cohort of 819 individuals. We included 536 individuals with complete data on IPI components and albumin, including 485 treated with chemotherapy and 397 with A-based regimens (primary cohort). Following PIs were evaluated: IPI, NCCN-IPI, (aa)IPI, and SENIOR-IPI (age per year, aaIPI components, LDH >3x ULN as extra point, and albumin, all weighted six-fold; extranodal involvement excluded due to lack of prognostic significance). Multivariable hazard ratios (HR) were estimated using Cox proportional hazards models; discriminative ability was assessed using C-index and calibration plots. The primary endpoint was overall survival (OS); event-free survival results were consistent (230/357 events were deaths) and are not reported here.

Results

Among the 397 A-treated patients (median age 82 years, range 80–99), ECOG performance status (PS) 2–4 was observed in 35% of patients, clinical stage III–IV in 67%, elevated LDH in 65% (8% with >3x ULN), >1 extranodal (EN) site in 32%, and albumin levels < 3.5 g/dL in 27%. In multivariable modeling, only age per year (HR=1.07, *P*<0.01), and clinical stage (HR=1.36, *P*=0.048) were independent predictors of OS, whereas ECOG PS (HR=1.27, *P*=0.12), trichotomized LDH by ULN (HR=1.23, *P*=0.08) and albumin (HR=1.17, *P*=0.35) were not significant.

SENIOR-IPI had the highest discrimination (C-index 0.631) in A-treated patients, outperforming IPI (0.617), NCCN-IPI (0.591), and aaIPI (0.620), with consistent results across cohorts: NiHiL (0.633), LEO (0.619), and MER (0.621). SENIOR-IPI stratified patients into low- (L-Risk, 0–9 points, 28%), low-intermediate (L-Int, 10–19 points, 42%), high-intermediate (H-Int, 20–29 points, 23%), and high-risk (H-Risk, 30–40 points, 7%) categories, with OS medians of 6.3, 3.8, 2.4, and 1.4 years, respectively (*P*<0.01), and significant separation between L-Int and H-Int (HR=1.47, *P*=0.02). By comparison, IPI categorized patients as L-Risk (12%), L-Int (24%), H-Int (27%), and H-Risk (37%), with median OS of 8.7, 4.6, 4.8, and 1.9 years, resp. (*P*<0.01). aaIPI yielded following distributions: 13%, 29%, 37%, and 21%; and median OS: 8.7, 5.3, 3.4, and 1.5 years; *P*<0.01. NCCN-IPI stratified patients into three groups: L-Int (13%), H-Int (49%), and H-Risk (39%), with respective OS medians of 8.7, 4.1, and 2.5 years (*P*<0.01). All PIs showed modest overestimation of mortality (calibration slopes: SENIOR-IPI 1.36; IPI 1.24; aaIPI 1.30; NCCN-IPI 1.26; intercept: -0.97, -0.87, -0.92, -0.87, resp.).

In the entire cohort (n=536), SENIOR-IPI showed highest C-index (0.651 vs IPI 0.622, aaIPI 0.631, NCCN-IPI 0.606), and stratified patients into L-Risk (26%, 2-year OS: 6.4 years), L-Int (40%, 3.2 years), H-Int (24%, 1.5 years), and H-Risk (10%, 0.6 years). Calibration slope was 1.18 (intercept -0.33). Among patients not receiving chemotherapy, SENIOR-IPI also performed best (C-index 0.759 vs IPI 0.738, aaIPI 0.757, NCCN-IPI 0.728).

Conclusion

SENIOR-IPI enables effective risk stratification of patients aged 80+ years with LBCL into four prognostically distinct groups, outperforming traditional indices (IPI, NCCN-IPI, aaIPI) in patients receiving A-based therapy, as well as in the entire cohort and in patients not receiving chemotherapy. While its discrimination is superior, modest miscalibration suggests that future recalibration may improve precision. These findings support SENIOR-IPI as a clinically relevant prognostic tool for the elderly LBCL patient population.

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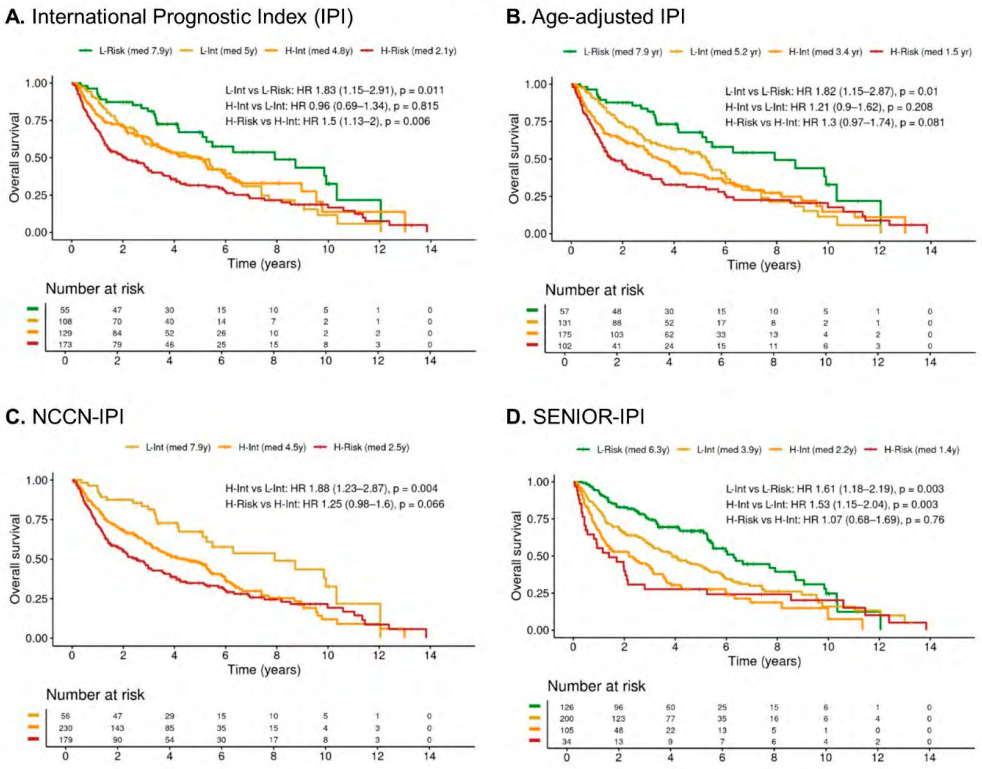
SBORNÍK ABSTRAKTŮ

Figure. Survival curves by prognostic indices.



O11

ANTHRACYCLINES INDUCE EPIGENETIC CHANGES IN ALDH1A THAT PROMOTE CHEMORESISTANCE IN AML, REVEALING A POTENTIAL TREATMENT STRATEGY CO-TARGETING ALDH1A INHIBITION WITH HYPOMETHYLATING AGENTS

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Background: Relapse/Refractory Acute Myeloid Leukemia (R/R AML) is a condition where patients do not achieve complete remission after chemotherapy and thus develop chemo-resistance. Aldehyde dehydrogenase 1A (ALDH1A) plays a critical role in leukemic cell survival by maintaining stemness, proliferation, and chemoresistance through aldehyde detoxification and retinoic acid synthesis. Elevated ALDH1 expression has been linked to poor prognosis in acute myeloid leukemia (AML), but the underlying mechanisms remain poorly defined.

Methods: Molecular and cellular techniques, including GFP-ALDH1 knock-in models, site directed mutagenesis, ChIP-seq, luciferase reporter assays, RT-qPCR, protein expression analysis, capillary electrophoresis-based protein analysis, ALDH enzymatic tests, ATP measurements. drug interaction studies,

and colony formation assays were employed. Experiments were conducted using human AML cell lines, murine MLL-AF9 leukemia models, murine and human AML mouse models, and R/R AML patient samples. In addition, ALDH1 expression was analyzed in an independent cohort of 116 AML patients and using data from the BEAT AML public database.

Results: While analysing ALDH1 in an independent cohort of 116 AML patients and integrated data from the BEAT AML public database, we observed that refractory AML blasts exhibited significantly higher ALDH1 levels than non-refractory cases, particularly in patients with prior myelodysplastic syndrome (MDS), MDS/myeloproliferative neoplasms, or therapy-related AML. Longitudinal analysis revealed that as the chemotherapy cycles progressed, there was an upregulation of ALDH1 on circulating blasts, thus suggesting an ALDH1-high phenotype induced by the treatment. High ALDH1A1 expression strongly correlated with reduced overall survival, particularly in R/R AML and patients with prior anthracycline exposure. To evaluate the role of anthracyclines in the transcription of ALDH1A1 and ALDH1A2, we carried out genetic studies using GFP-ALDH1 knock-in models, site-directed mutagenesis, ChIP-seq, luciferase reporter assays, RT-qPCR, and protein expression analysis. Our results demonstrated that anthracyclines directly activate ALDH1A1 and ALDH1A2 transcription via drug-inducible enhancer elements, regulated by JNK and STAT transcription factors. This was further supported by a combinational treatment of ALDH1 inhibition with Daunorubicin in a KG1a mouse model. We then evaluated the role of ALDH1A in promoting therapeutic resistance by performing functional studies using capillary electrophoresis-based protein analysis, ALDH enzymatic tests, and ATP easurements. These results demonstrated the role of ALDH1A in oxidative stress adaptation, and promoting therapeutic resistance. We then carried out drug response assays to determine the effect of ALDH1A inhibition in combination with 10 standard-of-care chemotherapeutic drugs in a panel of AML cell lines, we observed that ALDH1A inhibition in combination with hypomethylating agents has a synergistic role on AML cell lines. The effect of ALDH1A inhibition in combination with hypomethylating agents was further explored using colony formation assays and the synergistic interaction of ALDH1A inhibition with 5-Azacytidine was further supported by transplanting murine MLL-AF9 leukemic cells in C57BL/6J mice. To further understand the synergistic effect of ALDH1A inhibition with hypomethylating agents on primary R/R AML, we performed colony formation assays. Lastly, we determined the molecular mechanism of ALDH1A inhibition by capillary electrophoresis which further revealed that ALDH1A inhibition disrupts key proliferative pathways and induces extrinsic apoptosis via the Noxa/Mcl-1 axis, remaining effective in R/R AML.

Conclusion: Our findings identify ALDH1A as a key driver of adaptive resistance in anthracycline-exposed AML, highlighting its potential as a therapeutic target. Preclinical in vivo models and patient-derived R/R AML samples demonstrate that ALDH1A inhibition enhances anthracycline sensitivity when sequentially administered. Alternatively, co-targeting ALDH1A and DNA methylation offers a promising salvage strategy in R/R AML cases. These results support the clinical development of ALDH1A inhibitors for high-risk AML subsets.

O12

BULKY DISEASE AND/OR VERY HIGH LDH DEFINE A HIGH-RISK LBCL SUBGROUP WITHIN IPI 1–2, BUT ONLY IN YOUNGER PATIENTS

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Introduction

The International Prognostic Index (IPI) is a widely used tool for risk stratification of newly diagnosed large B-cell lymphoma (LBCL) patients. However, some patients experience refractory disease or relapse even within the low or low-intermediate risk groups (IPI 0–2). To improve risk discrimination, several modifications to the IPI have been proposed. Recent data suggest that patients within IPI 1–2 with bulky disease and/or very high lactate dehydrogenase (VH-LDH) levels have outcomes similar to those with IPI 3 (Maurer, ASH 2023), and could therefore be grouped with the intermediate-high and high-risk (H-Risk) IPI categories. This approach is already being incorporated into clinical trials (NCT06356129). However, external validation outside the US cohort is limited. We aimed to independently validate whether LBCL patients with IPI 1–2 and bulky disease and/or VH-LDH have survival outcomes comparable to those with IPI 3.

Methods

Consecutive patients with newly diagnosed systemic LBCL registered in the NiHiL project (NCT03199066) between 2010–2020 were identified. Inclusion criteria were clinical stage II–IV, age 18–80 years, IPI 1–5, complete baseline data, first-line R-CHOP-like therapy (n=2,303). Bulky disease was defined as ≥7.5 cm, and VH-LDH levels as >1.3× upper limit of normal per prior report. IPI 1–2 H-Risk was defined by the presence of bulky and/or VH-LDH within the IPI 1–2 group. The primary endpoint was event-free survival (EFS) comparison between IPI 1–2 H-Risk versus IPI 1–2 low-risk (L-Risk) and IPI 3 groups. Hazard ratios (HRs) were estimated using Cox proportional hazards models. Age-stratified subgroup analysis was performed in patients aged ≤60 years and >60 years.

Results

Among 2,303 eligible patients, the median age was 67 years (IQR 59–72), with 71% being >60 years old, 48% were female, 78% had stage III–IV disease, and 34% had ECOG performance status 2–4. The IPI distribution was 36% IPI 1–2 (n=828), 29% IPI 3 (n=664), and 35% IPI 4–5 (n=811). Among

IPI 1–2 patients, 440 (53%) were classified as L-Risk and 388 (47%) as H-Risk. The comparison of H-Risk versus L-Risk IPI 1–2 groups revealed the following differences: the H-Risk patients were younger (median age 59 vs 66 years, *P*<0.01), had more frequently elevated LDH (70% vs 21%, *P*<0.01) and bulky disease (77% vs 0%, *P*<0.01, by definition), as well as a shorter diagnosis-to-treatment interval (24 vs 35 days, *P*<0.01).

With a median follow-up of 8.1 years, 2-year EFS was 86% in the IPI 1–2 L-Risk group, 75% in IPI 1–2 H-Risk, and 65% in IPI 3; corresponding 5-year EFS rates were 73%, 69%, and 54%; with no significant differences between H-Risk vs L-Risk group (HR=1.08, 95% CI 0.87–1.35, *P*=0.47), while the EFS of IPI 3 patients was inferior when compared to IPI 1–2 H-Risk (HR=1.58, 95% CI 1.31–1.91, *P*<0.01).

Due to the observed age imbalance between L-Risk and H-Risk groups, we performed an age-stratified analysis. Among patients aged ≤60 years (n=659), 361 had IPI 1–2, including 150 (42%) L-Risk and 211 (58%) H-Risk, while 203 (31%) had IPI 3. In this subgroup, 2-year EFS was 89% in L-Risk, 74% in H-Risk, and 65% in IPI 3; 5-year EFS was 81%, 69%, and 58%, respectively. Patients in the H-Risk group had significantly inferior EFS compared to L-Risk (HR=1.53, 95% CI 1.04–2.24, *P*=0.03), whereas the difference between IPI 1–2 H-Risk and IPI 3 was of borderline statistical significance (HR=1.35, 95% CI 1.00–1.84, *P*=0.05).

Among patients between 60 and 80 years (n=1,644), 467 (28%) had IPI 1–2, including 290 (62%) L-Risk and 177 (38%) H-Risk, and 461 (28%) had IPI 3. The 2-year EFS was 84% in L-Risk, 76% in H-Risk, and 64% in IPI 3; 5-year EFS was 70%, 68%, and 52%, respectively; with no difference between L-Risk and H-Risk groups (HR=1.00, 95% CI 0.75–1.38 *P*=0.98), while IPI 3 was associated with worse outcomes compared to H-Risk (HR=1.59, 95% CI 1.24–2.05, *P*<0.01).

Conclusion

The external validation in the NiHiL project demonstrated that IPI 1–2 H-Risk DLBCL patients had significantly better EFS than those with IPI 3 and did not have different EFS compared to IPI 1–2 L-Risk. Thus, we were unable to confirm the findings reported in the US cohort (Maurer, ASH 2023), raising questions about the clinical utility of this stratification. However, in patients aged ≤60 years, the H-Risk group had significantly worse outcomes than L-Risk, supporting the potential prognostic relevance of this stratification in younger patients.

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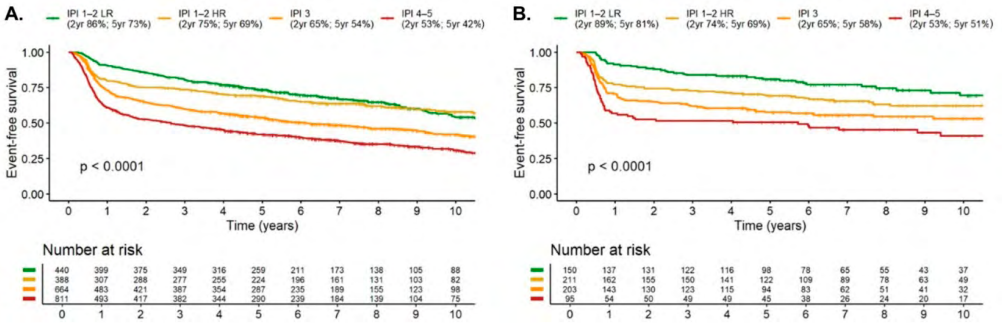
Figure. Survival curves of the entire patient population (n=2,303; **A**) and of patients ≤60 years old (n=659; **B**).



SBORNÍK ABSTRAKTŮ

O13

TAFASITAMAB (TAFA) PLUS LENALIDOMIDE (LEN) AND RITUXIMAB (R) FOR PATIENTS WITH RELAPSED OR REFRACTORY FOLLICULAR LYMPHOMA (R/R FL): RESULTS FROM THE PHASE 3 INMIND STUDY

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Background: FL is characterised by episodes of remission and relapse, with patients (pts) requiring multiple lines of treatment (tx). Chemoimmunotherapy is often used frontline but yields shorter response duration with successive lines of tx. Immunotherapy is preferred for R/R FL but there is a need to improve durability. Len+R is approved after ≥1 prior line of tx and frequently used. Tafa, a humanised CD19-targeting monoclonal antibody (mAb), has been previously approved in combination with len for R/R DLBCL based on the L-MIND study.

Aims: inMIND (NCT04680052) is a phase 3, double-blind, randomised, placebo (pbo)-controlled, multicentre international trial evaluating efficacy and safety of adding tafa to len+R in pts with R/R FL or R/R MZL. The trial was only powered to assess PFS in pts with FL.

Methods: Pts ≥18 y with R/R CD19$^+$ and CD20$^+$ FL (grade [gr] 1-3A) and ECOG PS ≤2, requiring tx after ≥1 prior systemic therapy including an anti-CD20 mAb, were randomised 1:1 to receive tafa or pbo with len+R for up to twelve 28-day cycles. Primary endpoint was investigator-assessed PFS (after 174 events occurred). Additional endpoints included PET-CR rate (FDG-avid pts), OS, PFS (independent review committee [IRC], prespecified subgroups), ORR, DOR, safety, TTNT.

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Results: 548 pts with FL were randomised: tafa arm, n=273; pbo arm, n=275; pt characteristics were similar between arms: median age 64 y (range, 31-88); 55% male; 79% intermediate- or high-risk FLIPI; 83% high tumour burden per GELF criteria. Median number of prior lines of tx was 1 (range, 1-10), 45% had ≥2 prior lines, 32% had disease progression within 24 m of diagnosis (POD24), and 43% were refractory to prior anti-CD20 mAb. Rates of baseline hypogammaglobulinaemia were low (tafa arm, 7%; pbo arm, 3%); mean levels of IgG, IgA, and IgM did not significantly change in the study. At data cutoff, pts in tafa and pbo arms had received a median of 12 and 11 tx cycles, 19% and 15% were still on tx, and 81% and 84% had discontinued tx, primarily due to tx completion (54% and 43%) or disease progression (11% and 31%). At median follow-up of 14.1 m, adding tafa to len+R significantly lowered risk of progression, relapse, or death vs pbo (median investigator-assessed PFS, 22.4 vs 13.9 m; hazard ratio [95% CI], 0.43 [0.32, 0.58]; *P*<0.0001). Benefit with tafa was confirmed by IRC and observed regardless of POD24 status, refractoriness to prior anti-CD20, and number of prior lines of therapy, and shown across additional study endpoints **(Table)**. Similar rates of tx-emergent adverse events (TEAEs) (99% vs 99%), gr 3 or 4 AEs (71% vs 70%) and serious AEs (36% vs 32%) occurred with tafa and pbo; the most common gr 3 or 4 AE was neutropenia (40% vs 38%), and TEAEs led to discontinuation in 11% and 7% of pts. Overall, 15 pts (6%) in the tafa arm and 23 (9%) in the pbo arm died on study, including 5 (2%) vs 17 (6%) due to progression and 6 (2%) in each arm due to fatal AEs.

Summary/Conclusion: Tafa added to len+R resulted in significant and clinically meaningful improvement in PFS, representing a 57% reduction in risk of progression, relapse, or death in pts with R/R FL; benefit was observed in all subgroups analysed. Although OS data are immature, a trend favouring tafa was observed. The safety profile was manageable and consistent with expected toxicities. Tafa+len+R can be administered in community and academic settings and represents a potential new standard of care option for pts with R/R FL.

Table. Primary and select secondary and exploratory outcome results (investigator assessed in the ITT population, unless indicated otherwise)

Variable	Tafa+len+R (n=273)	Pbo+len+R (n=275)
Primary endpoint		
Median PFS, months	22.4	13.9
Hazard ratio (95% CI)	0.43 (0.32, 0.58)	
P value	<0.0001	
Select secondary and exploratory endpoints		
Median PFS by IRC, months	NR	16.0
Hazard ratio (95% CI)	0.41 (0.29, 0.56)	
Nominal *P* value	<0.0001	
Pts with FDG-avid positive PET scan at baseline, n	251	254
PET-CR rate,* %	49.4	39.8
Odds ratio (95% CI)	1.5 (1.04, 2.13)	
P value	0.0286	
ORR†, %	83.5	72.4
Odds ratio (95% CI)	2.0 (1.30, 3.02)	
Nominal *P* value	0.0014	
Median DOR, months	21.2	13.6
Hazard ratio (95% CI)	0.47 (0.33, 0.68)	
Nominal *P* value	<0.0001	
Median TTNT, months	NR	28.8
Hazard ratio (95% CI)	0.45 (0.31, 0.64)	
Nominal *P* value	<0.0001	
OS, hazard ratio (95% CI)	0.59 (0.31, 1.13)	
Subgroup and post hoc analyses		
Median PFS in pts with POD24, months	19.2	11.3
Hazard ratio (95% CI)	0.43 (0.27, 0.69)	
Median PFS in pts refractory to anti-CD20 mAb, months	15.0	8.6
Hazard ratio (95% CI)	0.44 (0.30, 0.65)	
Median PFS in pts with ≥2 prior therapies, months	22.4	11.5
Hazard ratio (95% CI)	0.41 (0.28, 0.61)	
Median DOCR‡, months	NR	NR
Hazard ratio (95% CI)	0.59 (0.29, 1.18)	

*PET-CR rate is defined as the proportion of patients who achieved complete metabolic response at any time after start of treatment as per Lugano classification among the patients with a positive PET scan at baseline. †Per Lugano 2014 classification. ‡Post hoc analysis.
CI, confidence interval; DOR, duration of response; DOCR, duration of complete metabolic or radiologic response; FDG, fluorodeoxyglucose; IRC, independent review committee; ITT, intent-to-treat; len, lenalidomide; mAb, monoclonal antibody; NR, not reached; ORR, overall response rate; OS, overall survival; pbo, placebo; PET-CR, positron emission tomography-complete response; PFS, progression-free survival; POD24; progression of disease within 24 months of initial diagnosis; pts, patients; R, rituximab; tafa, tafasitamab; TTNT, time to next treatment.

SBORNÍK ABSTRAKTŮ

POSTEROVÁ SEKCE

(POSTERY P01 – P84)

SBORNÍK ABSTRAKTŮ

P01

L-ASPARAGINASE ALTERS REDOX HOMEOSTASIS IN ACUTE LYMPHOBLASTIC LEUKEMIA

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BACKGROUND

Oxidative stress is recognized as a hallmark of various cancers which enhances antioxidant defenses leading to chemo-resistance development. In acute lymphoblastic leukemia (ALL), the expression level of the cystine (CC) transporter, responsible for delivering a key substrate for the main antioxidant glutathione, has been shown to negatively correlate with resistance to cytostatic drug, L-asparaginase (ASNase) (Ferguson et al. 2022).

AIMS

This study elucidates the role of ASNase in maintaining redox homeostasis under conditions of altered CC levels and impaired transport in ALL.

METHODS

Viability of cells was measured by absolute cell number using flow cytometer and viability staining. ROS levels were determined using H_2DCF-DA dye. Metabolic fluxes were investigated using LC-MS based stable isotope tracing (SIT) using labelled $^{13}C_3$-Serine and $^{13}C_6$,$^{15}N_2$-Cystine.

RESULTS

Firstly, we demonstrated that CC depletion is lethal in a wide range of ALL cell lines *in vitro*. However, this effect is not general across all leukemia types, as shown by the resistance observed in the K562 myeloid leukemia cell line. Surprisingly, co-treatment with ASNase mitigated the effect of CC depletion in ALL cells. These results were recapitulated using treatment with erastin, a CC transporter inhibitor.

We employed state-of-the-art technology, SIT to investigate metabolic changes following CC depletion and ASNase treatment in ALL cells. Specifically, we examined *de novo* cysteine synthesis through the reverse trans-sulfuration pathway (RTSP). Our objective was to determine whether an active RTSP could, *in situ*, compensate for the absence of extracellular CC by redirecting the metabolic fate of methionine and serine. We confirmed the presence of this compensatory mechanism in K562 cells, which exhibit low basal RTSP activity that is upregulated upon CC depletion. In contrast, MOLT4 (ALL) cells lacked active RTSP. However, analysis of SIT data revealed an alternative mechanism potentially underlying the interaction between CC depletion and ASNase treatment. Specifically, ASNase treatment increased both total and *de novo* synthesized glutathione (GSH) under CC-limited conditions. Furthermore, inhibition of GSH synthesis by BSO diminished the protective effect of ASNase against CC depletion-induced cell death.

Using labeled CC, we demonstrated that ASNase enhances CC influx. Additionally, ASNase treatment independently reduced ROS levels and, notably, maintained this effect even in the presence of CC depletion, despite the fact that CC depletion alone elevates ROS levels. Based on these findings, we propose that the increase in GSH levels and CC influx may explain the mitigating effects of ASNase on CC depletion in ALL, warranting further investigation.

Clinically, ASNase depletes two amino acids, asparagine (N) and glutamine (Q). To elucidate the mechanisms that prevent CC depletion-induced lethality, we separately mimicked these enzymatic activities. Our findings indicate that depletion of either N or Q enhances cell survival, correlating with changes in ROS levels.

CONCLUSIONS

We have shown that cystine depletion which functionally resembles low expression of the cystine transporter, enables the activation of rescue mechanisms of ALL cells after ASNase treatment. This study highlights redox homeostasis as one of the key players of chemoresistance and enhances our understanding of leukemia cell behavior.

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P02

MONOCYTIC SWITCHING IN DUX4-POSITIVE BCP-ALL: CHALLENGES IN MRD ASSESSMENT AND PROGNOSTIC IMPACT

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Background

In our previous studies, we identified a BCP-ALL subtype, switching ALL (swALL), characterized by a transition to the monocytic lineage during the initial treatment. These 'switched blasts' exhibit increased myeloid markers (CD14, CD33) and decreased lymphoid markers (CD19, CD34) (Slamova et al., Leukemia 2014; Novakova et al., Haematologica 2021). SwALL is frequent in DUX4-rearranged (DUX4r), PAX5-P80R-mutated, and ZNF384-rearranged cases, potentially causing discordance between flow cytometry (FC) and PCR-based minimal residual disease (MRD) findings.

Objectives

We analyzed DUX4r cases diagnosed in Czechia (1996–2024) to evaluate prognosis, clinical behavior, and FC-MRD accuracy compared to PCR-MRD. Additionally, we explored strategies to detect both monocytoid and B-blasts.

Methods

Our cohort included 82 patients with DUX4r (age 2–18 years, median 8.5; 37 females, 45 males) diagnosed between 1996– 2024, treated under various BFM protocols: ALL-BFM95 (n=3), ALL-IC-BFM-2002 (n=10), ALL-BFM2000 Interim (n=8), ALL-BFM2009 (n=31), ALL-BFM2017 (n=30). RNA sequencing/ gene expression profiling was performed in ALL-BFM2009/2017 in all patients belonging to B other group enabling assessment of prevalence of DUX4r and selectively in earlier protocols for swALL cases.

The switching phenomenon was defined as the presence of an intermediate B-monocytoid population, i.e. gradual CD19 decrease with a concurrent increase in CD14/CD33 or higher side scatter between d0 and d+33 (Slamova et al., Novakova et al.). Since 2007, we have prospectively monitored swALL using a standardized FC panel (CD45, CD14, CD33, CD19, CD34, CD10, and CD20).

Results

Among BCP-ALL patients treated under BFM2009/2017 protocols, DUX4r was identified in 6.7% (61/902 cases), with 77% (47/61) classified as swALL. The prognosis of all DUX4r did not significantly differ between swALL and other DUX4r patients, with five-year EFS of 90.8% ± 5.3% and 76.9% ± 15.2%, respectively ($p > 0.05$).

A total of eleven clinical events were observed: three toxic deaths, two extramedullary BCP relapses in the uterus and testes, and five bone marrow BCP relapses; notably, in the case of swALL - one patient experienced an AML relapse with identical Ig/TR rearrangements. Extremely poor prognosis was observed among four patients with early isolated bone marrow relapse (0% EFS).

At day 15, the sensitivity (assessed by comparing to Ig/TR rearrangements that served as a gold standard) of B cell-oriented FC MRD detection was 90%, with a specificity of 100%. However, by day 33, sensitivity significantly declined to 56%, while specificity remained consistently high at 100%. Quantitatively, comparisons of MRD medians indicated that both B lymphoblasts and monocytoid cells contributed to Ig/TR-based MRD levels.

Conclusion
The DUX4r genotype exhibits significant immunophenotypic plasticity during induction treatment. When FC-MRD includes both lymphoid and myeloid markers, transdifferentiation is detected in >77% of DUX4r cases. SwALL is associated with higher MRD at d15 and d33 but does not impact prognosis. Most relapses occur with a B-lymphoid phenotype; only one patient relapsed with a monocytic phenotype while retaining identical Ig/TR rearrangements.

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P03

SURFACE PROTEIN PROFILING OF CHILDHOOD B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA

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Introduction:
B-cell precursor leukemia is the most common childhood cancer, with an overall survival rate of over 90% due to stratification, response monitoring, and advanced supportive care.Minimal Residual Disease (MRD) monitoring approaches need adaptation for patients receiving targeted therapies or undergoing lineage switch.

Methods:
In this study, using full-spectrum cytometry, we evaluated CDmaps (Kužlková et al. Front Immuno 2022) - the expression of surface markers - (n=305) on BCP ALL cells obtained from thawed samples at diagnosis (n=15) or relapse (n=3).In this pilot phase, the following genotypes were included: DUX4r (6), ETV6::RUNX1 (11), BCR::ABL (1).One sample of CD19 negative relapse was analyzed (ETV6::RUNX1). We barcoded samples with anti-HLA-I antibodies for high-throughput analysis and used backbone antibodies to differentiate blast cells from non-malignant B cells. The marker expression was evaluated as a percentage of positivity and quantified as antibody bound per cell using the BD-Quantitation kit. Additionally, RNA-seq data from diagnostic samples were used to support our findings.

Results:
We compared marker expression in CDmaps with fresh material from diagnosis or relapse using conventional flow cytometry (34 out of 305).

No significant differences were found between fresh and thawed samples. We confirmed a specific expression signature in DUX4r (CD371pos, CD10 heterogeneous, CD34 bright). DUX4r cases showed higher expression of CD146, CD84, and CD140a ($p < 0.02$). In a CD19-negative relapse patient, we identified the expression of other B cell markers - CD22, CD24, CD72, and CD81.

Conclusions:
While barcoding works in other projects, BCP-ALL presented some challenges. Despite this, high-throughput flow cytometry is effective for protein profiling and MRD marker identification in childhood leukemias. Promising markers will be further validated.

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P04

HIGH METABOLIC ACTIVITY IN LEUKEMIC CELLS IS ASSOCIATED WITH MORE AGGRESSIVE PHENOTYPE AND REDUCED TREATMENT EFFICACY

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Background: Despite significant advances in the treatment of childhood acute lymphoblastic leukemia (ALL), with a 90% survival, the cure rate for relapsed patients drops to 60–70%. Current risk stratification, which is mainly based on genomic profiles and treatment response including minimal residual disease (MRD) levels and prednisone response, still fails to accurately predict treatment outcomes in a number of patients. Our study was based on the assumption that the phenotypic characteristics of leukemic cells play a crucial role in determining leukemia's response to treatment.

Aims: Evaluation of the metabolic phenotype of leukemic cells to determine its prognostic value and the role in therapeutic response.

Methods: We determined the metabolic profile of primary cells isolated from 60 patients with B-cell precursor ALL (BCP-ALL), focusing on glycolytic parameters: glycolysis, glycolytic capacity (GC), glycolytic reserve, and total glycolytic activity (TGA). Next we performed whole-transcriptome sequencing (WTS) in the same patient cohort. Results were correlated with genomic

characteristics, MRD levels, risk stratification, prednisone response, and event-free survival (EFS) as well as with *ex vivo* detected L-asparaginase sensitivity (ASNase). In addition, leukemic cells from ten patients were transplanted into NSG/NOD SCID mice.

Results: Our findings indicate that glycolytic function distinguishes high-risk (HR) from standard-risk (SR) group. A high glycolytic (HG) state of leukemic cells was significantly associated with HR stratification, whereas a low glycolytic (LG) state correlated with stratification into the SR group ($p < 0.01$ for glycolysis, $p = 0.04$ for GC, and $p = 0.01$ for TGA). High sensitivity to ASNase correlated with low glycolytic profile and vice versa ($p < 0.01$ for glycolysis, $p = 0.04$ for GC, and $p = 0.01$ for TGA), while MRD levels and prednisone response show no correlation. Genetic analyses revealed that specific metabolic phenotypes are associated with particular genetic aberrations: ETV6::RUNX1–positive patients predominantly exhibited an LG phenotype, whereas patients with unfavorable genetic markers (e.g., KMT2Ar) demonstrated HG activity. Transcriptomic analysis confirmed that HG activity corresponds to a "hypermetabolic state" characterized by enhanced TCA cycle/OXPHOS, altered redox homeostasis, and nucleotide metabolism. HCA of differentially expressed genes based on glycolytic function grouped patients into two clusters: Cluster I, enriched for SR patients (16/21), and Cluster II, enriched for HR patients (10/11) and those with events according to EFS (9/10). The predictive value of risk based on these gene expression differences was significantly higher than expected by chance ($p = 0.016$). In PDX models, engraftment occurred more rapidly in those transplanted with HG cells (n=5, average of 8.4 weeks) compared to LG cells (n=5, average of 18.8 weeks). Moreover, treatment simulating the induction phase of ALL therapy, completely eradicated leukemic cells in PDX models derived from LG ALL, while a significant number of leukemic cells persisted in PDX models from HG ALL patients, consistent with observed MRD levels detected in the those patients.

Conclusion: Glycolytic activity at diagnosis is associated with disease progression and treatment response and is characteristic of certain genetic subtypes. Our findings highlight the metabolic heterogeneity in leukemic cells, indicate that the metabolic phenotype contributes to the response to chemotherapy drugs, and open up new therapeutic options using metabolism targeting drugs.

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P05

INVESTIGATING PREDNISONE-INDUCED SIGNALING PATHWAY CHANGES IN PEDIATRIC T-ALL

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T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive malignancy of T-cells. Despite comparable initial prognoses to other childhood ALL subtypes, approximately 25% of T-ALL patients relapse, resulting in poor outcomes. Therefore, targeted therapies for resistant and high-risk pediatric T-ALL are urgently needed. Prednisone response on day 8 of therapy is a critical prognostic indicator, with prednisone good responders (PGR) having better outcomes than prednisone poor responders (PPR). However, the mechanisms underlying glucocorticoid resistance remain unclear.

We previously identified a correlation between *ex vivo* IL-7 induced STAT5 phosphorylation and *in vivo* prednisone response in 16 pediatric T-ALL samples. Therefore we aimed to further investigate the relation between

signaling pathways and prednisone sensitivity. We developed a 44-parameter mass cytometry panel to analyze PI3K, Notch, JAK/STAT, and MAPK pathways, along with pro- and anti-apoptotic markers. We examined 22 paired T-ALL samples (12 PGR, 10 PPR) at diagnosis and day 8 of treatment, using probability binning to identify makers significantly changed under induction therapy (Chi-Squared T >0).

Our data suggest heterogeneous of the response pattern of samples from the PGR group. We have observed both, signaling and apoptotic markers to be down-regulated (n=10) or up-regulated (n=10). Several markers (pCREB, Notch1, pS6, BIM, p-p38 and pSrc) were in some samples up-regulated (n=4,2,4,1,1 and 1, respectively) and in other down-regulated (n=2,1,1,2,1 and 1, respectively). No marker was deregulated in at least 50% of PGR samples. To the contrary, two markers (pCREB and pS6) were up-regulated in several samples (n=1 and 2, respectively) while 13 markers were down-regulated in samples from the PPR group. Three markers (pRb, pCREB and CDK6) were down-regulated in at least in 50% of PPR samples (50%, 60% and 50%, respectively). Down-regulation of pRb, pCREB and CDK6 is slightly less frequent in PGR samples (25%, 17% and 33%, respectively), pCREB is more frequently up-regulated in PGR samples (33%). Anti-apoptotic protein BCL-2 is up-regulated (33%) in PGR samples, while down-regulated (40%) in PPR samples.

Our data reveal heterogeneous response patterns in PGR samples, with both up- and down-regulation of signaling markers. In contrast, PPR samples predominantly exhibited down-regulation of signaling, proliferation, and apoptotic markers, including pRb, CDK6, and pCREB. Notably, anti-apoptotic protein BCL-2 was up-regulated in PGR samples but down-regulated in PPR samples.

In conclusion, pediatric T-ALL cells demonstrate significant inter-individual heterogeneity. Our findings suggest that prednisone-resistant cells tend to exhibit decreased signaling pathway activity *in vivo*. Hereby we prove the feasibility of single-cell mass cytometry for the in-depth study of T-ALL signaling and the description of the dynamic heterogeneity of T-ALL cellular machinery.

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P06

IMPLEMENTATION OF EXTENDED MOLECULAR DIAGNOSTICS IN THE CLINICAL MANAGEMENT OF ADULT ALL PATIENTS

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Objectives

Approximately 50 adult patients are newly diagnosed with acute lymphoblastic leukemia (ALL) in the Czech Republic each year. With increasing age, the proportion of prognostically unfavorable subtypes, such as BCR::ABL1-positive and BCR::ABL1-like ALL, rises.

In 2023, as part of the Czech Leukemia Group for Life, we launched a multicenter initiative to advance molecular diagnostics in adult ALL. Modern genomic techniques based on massively parallel sequencing enable precise classification of B- or T-ALL subtypes.

Methods
The extended diagnostic approach included transcriptome sequencing, panel sequencing, and digital MLPA. Additionally, we performed *ex vivo* drug sensitivity testing on primary T-ALL patient cells for response to venetoclax and dasatinib.

Results
Within the first year of extended diagnostics, 26 ALL patients were analyzed at diagnosis (16 B-ALL, 4 MPAL, 6 T-ALL). Molecular subtypes were successfully identified in all B-ALL cases (including ZNF384 rearrangements, KMT2A rearrangements, BCR::ABL1-like subtypes. In all T-ALL cases and 3 out of 4 MPAL cases, pathogenic somatic mutations were detected. *Ex vivo* drug testing was performed in 14 cases (T-ALL, MPAL, or relapsed/refractory B-ALL), revealing sensitivity to venetoclax in 9 cases, while only one case demonstrated sensitivity to dasatinib.

Notably, extended diagnostics clarified the diagnosis in a patient with a typical immunophenotypic profile (cytCD3+, CD1a+, CD2+, CD5+, CD7++, CD10+, CD33+, CD99+, CD19-, cCD22-, cCD79a-, CD11b, cCD13-, CD13-, CD14-, CD64-, CD117-, MPO-) of cortical T-III ALL (EGIL 1995). RNA sequencing identified a *PICALM::MLLT10* fusion, while digital MLPA revealed a monoallelic deletion of *IKZF1, IKZF2, LMO2, TP53, NF1*, and *SUZ12*. Panel sequencing detected somatic mutations in *EZH2* (R646C), *NOTCH1* (L2390_Q2391insGX), *TP53* (M237I), and *PHF6* (E189X). Despite the flow cytometry profile suggesting T-ALL, molecular data indicated a diagnosis closer to acute leukemia with ambiguous lineage. *Ex vivo* testing demonstrated venetoclax sensitivity, which was confirmed by remission following venetoclax initiation after failure of standard chemotherapy.

Conclusion
Extended molecular diagnostics, in combination with standard laboratory methods (cytogenetics, flow cytometry, myelogram), enhances subtype classification, refines risk stratification, and informs treatment decisions. It also supports the selection of targeted therapies in relapsed/refractory ALL cases.

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P07

CLINICAL VALUE OF ADVANCED GENOMIC TESTING IN ADULT PHILADELPHIA-NEGATIVE B-ALL: RESULTS FROM THE CZ NATIONAL LEUKEMIA STUDY GROUP (CELL)

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Introduction

B-cell precursor acute lymphoblastic leukemia (B-ALL) in adults is a biologically and clinically heterogeneous disease. Historically, prognostication has relied on so-called standard risk factors, including age, white blood cell count at diagnosis, immunophenotype, and cytogenetic abnormalities. Recently, advances in genomic sequencing have enabled the precise classification of molecular subtypes, including BCR::ABL1-like, DUX4-rearranged (DUX4r), PAX5-altered (PAX5-alt), and ZNF384-rearranged (ZNF384-r) ALL. Additionally, deletion of the IKZF1 gene—encoding a key lymphoid transcription factor— combined with deletions of PAX5 and CDKN2A/CDKN2B, and in the absence of ERG gene deletion, defines the IKZF1plus genotype, which has been linked to poor prognosis, particularly in BCR::ABL1-positive ALL. We evaluated the clinical significance of these recently identified subtypes, in the context of measurable residual disease (MRD) status.

Patients and Methods

The study cohort included 75 adults diagnosed with BCR::ABL1-negative B-ALL. The median age was 44.9 years (range 18–73), and the median leukocyte count at diagnosis was 8.8×10^9/L (range 0.6–635). Immunophenotyping revealed 46 cases (61%) of common B-ALL, 20 (27%) pro-B, and 9 (12%) pre-B. Central nervous system (CNS) infiltration was identified in 9 patients (12%). Patients received an induction treatment either with standard CELL chemotherapy protocols (n=39), or with targeted therapies as part of the Blina-CELL (NCT04554485, n=29) and EWALL-INO (NCT03249870, n=7) clinical trials. Early treatment response was assessed after two blocks of intensive therapy, at week 10 or 11. A poor response was defined as refractory or MRD $>1\times10^{-4}$, an intermediate response as detectable MRD $<1\times10^{-4}$, and a good response as MRD negativity. Standard diagnostic approaches were complemented by transcriptome sequencing, targeted panel sequencing, whole-exome sequencing, and digital MLPA. In addition, extended clinical and statistical analyses were conducted within the CELL.

Results

Molecular subtypes were represented as follows: low hypodiploid 18 (24.0%), BCR::ABL1-like 8 (10.7%), ZNF384-r 7 (9.4%), KMT2A-r 6 (8.0%), hyperdiploid 5 (6.7%), DUX4-r 4 (5.4%), TCF3::PBX1 3 (3.9%), hypodiploid 2 (2.7%), PAX-alt 2 (2.7%), ETV6::RUNX1 1 (1.3%), MEF2D-r 1 (1.3%), MYC-r 1, PAX5 p.P80R 1 (1.3%), ZEB2 p.H1038R)/IGH::CEBPE 1 (1.3%), not otherwise specified 15 (20.0%). IKZF1plus genotype was detected in 14 (20%) subjects (not tested in 4 patients). Five-year overall survival (OS) and progression-free survival (PFS) was 69% (59%–81%) and 60% (50%–73%),

respectively. Treatment response was classified as poor in 16 patients (21%), intermediate in 12 (16%), and good in 47 (63%). The highest rates of MRD negativity were observed in patients with the following subtypes: TCF3::PBX1 (100%, n=3), hyperdiploid (80%, n=5), PAX5 p.P80R (100%, n=1), PAX5-alt (100%, n=2), and ZEB2 (p.H1038R)/IGH::CEBPE (100%, n=1). Despite high MRD burden (good response in only 25%, n=4), patients with DUX4-r had excellent overall survival (OS 100%). In contrast, patients with BCR::ABL1-like ALL had both poor MRD response (good response in only 37%) and inferior survival (OS 62.5%). However, there was a trend toward improved OS and PFS in those treated within clinical trials involving frontline immunotherapy. Patients without the IKZF1plus genotype were 4.24-times more likely to achieve MRD negativity at week 10/11 (p=0.027) and showed improved survival outcomes (OS: 76% vs. 58%, p=0.021; PFS: 63% vs. 50%, p=0.10). Patients treated with frontline targeted therapy in the Blina-CELL or EWALL-INO protocols showed a trend toward better molecular response, with a 1.51-times higher probability of achieving MRD negativity compared to those on standard protocols.

Conclusions
Advanced genomic profiling enhances risk stratification by identifying molecular subtypes, including the IKZF1plus genotype, with distinct MRD responses and survival outcomes in BCR::ABL1-negative adult B-ALL. Integration of these findings into clinical practice may improve personalized treatment strategies.

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P08

ACUTE TOXICITIES DURING INDUCTION AND FIRST CONSOLIDATION TREATMENT OF YOUNG ADULTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA: COMPARISON OF ALL CELL 2023 JUNIOR AND GMALL 07/2003 PROTOCOLS – A SINGLE CENTER EXPERIENCE

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Background. Since 2023, adult patients with acute lymphoblastic leukemia (ALL) in Czech Republic are treated according to ALL CELL 2023 Junior (CELL) protocol. Compared to the previously used GMALL 07/2023 (GMALL), CELL includes two doses of pegasparaginase during Induction (Days 20 and 42 vs. Day 20), reduction of cytarabine (omitted Days 42-45) and cyclophosphamide (omitted Day 46) and delayed start of 6-mercaptopurine (Day 49 vs. Day 33), Consolidation I remained unchanged. This study aimed to compare acute toxicities during Induction and Consolidation I in young patients treated with CELL and GMALL at the Institute of Hematology and Blood Transfusion in Prague.

Methods. Twelve patients younger than 40 years (median age 23.1; 19.2-37.8) were treated with CELL (June 2023-December 2024). The control group included all 11 young adults (median age 33.6; 20.6-39.8) treated with GMALL between January 2020 and May 2023. Toxicities were graded using CTCAEv5.0, and statistical analyses were performed using Fisher's exact test.

Results. The most common toxicities during Induction were hepatopathy, coagulopathy, and febrile neutropenia. ALT and GGT elevation (grade ≥ 3) occurred significantly more often in GMALL Induction II compared to CELL (63.6% vs. 0.0%; p=0.001, and 36.4% vs. 0.0%; p=0.037, respectively). Bilirubin, AST, and ALP elevation in both inductions were more frequent in GMALL, but not significant. In Induction I, hypertriglyceridemia (grade 1-2) was

observed in 41.7% of CELL patients, compared to none in GMALL (p=0.037), no patient had grade ≥ 3; in Induction II grade ≥ 3 hypertriglyceridemia was present in both groups with no significant difference. Similarly, there were no differences in pancreatic enzymes elevation. Asparaginase-associated pancreatitis occurred in 2/11 GMALL and 1/12 CELL patients. Thromboembolism was reported in one GMALL (grade 2) and three CELL patients (two grade 2, one grade 3). Febrile neutropenia was more common in GMALL, but not significant (Induction I: 54.5% vs. 41.7%, p=0.68; Induction II: 81.8% vs. 50.0%, p=0.19). Other severe toxicities were more frequent in GMALL, including candidemia (3/11), mucositis (3/11), tonsillitis (1/11), and cholangitis (1/11), while mucormycosis (1/12) and mucositis (4/12) were observed in CELL. Pegasparaginase was omitted in 2/11 GMALL and reduced to one dose in 3/12 CELL patients; 6-mercaptopurine was withheld in 6/11 GMALL and 1/12 CELL patient. In Consolidation I, hepatopathy and mucositis were the most frequent adverse events with similar incidence in both protocols. The average hospitalization length for Induction was 7 days shorter in CELL (52.8 vs. 59.8 days in GMALL), while no difference was observed in Consolidation I.

Conclusion. The ALL CELL 2023 Junior Induction protocol, incorporating two doses of pegasparaginase alongside reduced cytarabine, cyclophosphamide, and delayed start of 6-mercaptopurine, did not increase the risk of acute toxicities, including asparaginase-associated pancreatitis or thromboembolism, compared to GMALL. Furthermore, severe toxicities were less frequent in CELL. The introduction of pegasparaginase activity monitoring and complex genomic profiling has enabled a more personalized approach, including TDM-based dose adjustments. Importantly, CELL resulted in a shorter hospitalization stay during induction.

P09

ANALÝZA GENOMOVÝCH ABERACÍ U DOSPĚLÝCH PACIENTŮ S AKUTNÍ LYMFOBLASTICKOU LEUKEMIÍ POMOCÍ SEKVENOVÁNÍ DLOUHÝM ČTENÍM

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Úvod: Patogeneze akutní lymfoblastické leukemie (ALL) je spojena s výskytem různých genomových aberací, které jsou standardně analyzovány kombinací karyotypizace, fluorescenční *in-situ* hybridizace (FISH) a real-time PCR (RT-PCR) (PMID:38295337). Mezi nové slibné molekulárně-biologické přístupy, umožňující komplexní charakterizaci nádorové populace, patří celogenomové sekvenování dlouhým čtením (long-read whole genome sequencing; LR-WGS).

Metodika: Pomocí LR-WGS bylo na přístroji PromethION (ONT; průměrné pokrytí 31-48x) analyzováno 5 dospělých pacientů s ALL diagnostikovaných na IHOK FN Brno. U 4 z těchto pacientů byly s využitím běžných laboratorních vyšetření prokázány klinicky významné chromozomové aberace. U posledního pacienta nebyla metodami FISH a RT-PCR kauzální přestavba určena. Výsledky rutinního screeningu byly u všech 5 pacientů doplněny také o analýzu početních změn (CNV) a genových mutací pomocí sekvenačního panelu LYNX.

Výsledky: Přítomnost rekurentních chromozomových přestaveb byla metodou LR-WGS prokázána u všech 5 vyšetřených ALL pacientů. U 4 pacientů byly v souladu s cytogenetickou a RT-PCR analýzou detekovány fúze genů *BCR::ABL1*, *KMT2A::MLLT1*, *KMT2A::AFF1* a *SET::NUP214*. U posledního pacienta LR-WGS dodatečně odhalilo translokaci t(2;21)(q31.1;q22.12) (176090938;34847164), která nebyla rutinním vyšetřením rozpoznána. V případě CNV analýzy potvrdilo LR-WGS 8/8 delecí (0,02-22,17 Mb, včetně *IKZF1* delece) a 0/1 amplifikací (1,7 Mb) stanovených panelem LYNX. Příčinou selhání LR-WGS při detekci amplifikace bylo zřejmě její nízké procentuální zastoupení (proporce ~ 30 %). LR-WGS dále správně rozpoznalo 6 klinicky významných variant určených LYNX analýzou v genech *NOTCH1*, *JAK1*, *JAK3*, *EP300* a *SH2B3*. Jedna varianta v genu *IL7R* typu delece-inzerce byla metodou LR-WGS detekována v jiném rozsahu než panelem LYNX.

Závěr: Metoda LR-WGS umožňuje souběžné stanovení početních i strukturních chromozomových změn včetně genových mutací. V případě přestaveb LR-WGS přímo definuje zlomová místa, čímž zpřesňuje výsledky rutinních analýz a bezprostředně tak charakterizuje cílové oblasti pro sledování minimální zbytkové nemoci.

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P10

BREXUCABTAGENE AUTOLEUCEL V LÉČBĚ AKUTNÍ LYMFOBLASTOVÉ LEUKEMIE – PILOTNÍ ANALÝZA CELL A SROVNÁNÍ S DATY MEZINÁRODNÍCH SOUBORŮ

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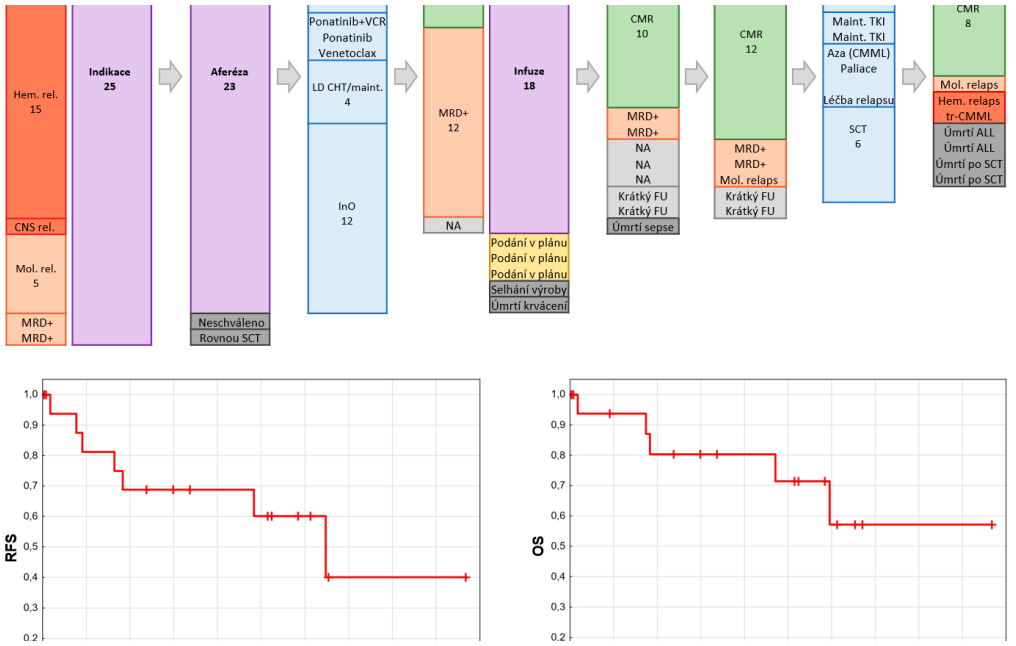
Úvod:
Brexucabtagene autoleucel (brexu-cel) představuje po blinatumomabu a inotuzumabu ozogamicinu další zásadní posun v léčbě dospělých pacientů s B-prekurzorovou akutní lymfoblastovou leukemií (B-ALL). Cílem naší práce bylo zhodnotit dosavadní výsledky léčby a toxicitu brexu-celu u českých pacientů a porovnat je s publikovanými daty mezinárodních souborů.

Metody:
Analyzovali jsme data dospělých pacientů s B-ALL indikovaných v Česku k léčbě brexu-celem od prosince 2022 do srpna 2025. Hodnoceny byly charakteristiky pacientů a nemoci před aferézou, typ bridging terapie, léčebné odpovědi po infuzi, výskyt komplikací a přežití. Výsledky byly srovnány s publikovanými daty klinického hodnocení ZUMA-3 a registrů z reálné klinické praxe (RWE) ROCCA, CIBMTR a DESCAR-T.

Výsledky:
K léčbě bylo indikováno celkem 25 pacientů, aferéza byla provedena u 23 a infuze podána 18 nemocným. Všichni pacienti dosáhli kompletní hematologické remise (CR) již po bridging terapii často obsahující inotuzumab ozogamicin. Po infuzi brexu-celu bylo dosaženo kompletní molekulární remise (CMR) u 80 % hodnotitelných nemocných. Medián sledování činil 12,1 měsíců. Délka přežití se zdála být mírně lepší než v ZUMA-3 i RWE souborech; ve 12 měsících byl odhad přežití bez relapsu (RFS) 69 % a celkového přežití (OS) 80 %. Výskyt závažných forem toxicity (CRS a ICANS grade ≥3) byl překvapivě nízký, jen 6 % (1 pacient). Celková časná mortalita do 28. dne od podání brexu-celu činila 6 % (1 pacient, sepse). Přibližně třetina pacientů následně podstoupila alogenní transplantaci krvetvorných kmenových buněk.

Závěr:
První české zkušenosti s brexu-celem u B-ALL potvrzují vysokou účinnost a příznivý bezpečnostní profil této terapie v reálné praxi. Ve srovnání s již publikovanými daty jsme pozorovali nižší toxicitu a s limitací krátké doby sledování i trend k lepšímu přežití. To může souviset s širším využitím inotuzumabu ozogamicinu v bridging terapii, a tedy i s nižší nádorovou náloží před infuzí brexu-celu, důslednější profylaxí a promptní reakcí na projevy časné toxicity.



P11

CD123-SPECIFIC CAR-T CELLS PRODUCED BY LINEAR DNA-BASED PIGGYBAC TRANSPOSON SYSTEM – INITIATION OF PHASE I CLINICAL TRIAL IN PATIENTS WITH AML

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Background
Chimeric antigen receptor-modified T cells represent a novel and highly effective therapeutic approach for hematological malignancies. However, their

high costs result mainly from the manufacturing process utilizing recombinant viral vectors which critically complicates their further development and broad availability.

Aims

To develop a novel approach for rapid and cost-effective non-viral production of clinical-grade CAR T cells which utilizes polymerase-chain-reaction (PCR) produced linear piggyBac transposon DNA and in-vitro transcribed (IVT) transposase mRNA. To fully validate this technology, we initiated a clinical trial with CD123-specific Chimeric Antigen Receptor-T Cells (CAR123) in patients with refractory AML.

Methods

To produce the vectors, a large-scale PCR using high-fidelity polymerase was used to generate the linear piggyBac transposon DNA of approx. 5kb length. This sequence contains a ubiquitin promoter-regulated CD123 CAR (with 4-1BB-zeta intracellular signaling domain) joined via T2A self-cleaving motif with CD20 protein. The PCR product was separated by HPLC, yielding 100% pure DNA, and the sequencing revealed no mutations. The transposase mRNA was similarly synthesized from a plasmid template using T7 RNA polymerase followed by DNAse treatment and by precipitation with LiCl to generate pure mRNA. To generate CAR123 T cells, PBMCs obtained from the blood were electroporated with this linear DNA and mRNA. The transfected T cells were then polyclonally activated with anti-CD3/CD28 antibodies and expanded in the presence of cytokines IL-4, IL-7, and IL-21 which enable the preservation of stem-cell memory phenotype, furthermore, the expansion of CAR123 was enhanced by addition of irradiated mixed allogeneic PBMCs (a “feeder”). This manufacturing process is GMP-compliant and was certified for the initiation of a clinical trial.

Results

First, linear DNA and mRNA production were feasible, and no issues regarding identity and purity were noticed. Next, the production of CAR123 T cells was highly effective as 1 µg of linear DNA yielded approx. 100-150 million CAR-T cells per one electroporation of 10 million of PBMCs from healthy donors (n=5). The median percentage of CAR+ cells was 31% (range, 23-36%), median CD4/CD8 ratio was 0.72 (range, 0.64-0.82). Median 68% (range, 54-83%) of CAR+ had central memory phenotype (CD62L+CD45RA+) and the vector copy number was within 2-5 range. Produced CAR123 displayed efficient cytotoxicity in vitro against target cell lines THP-1 and SKM-1. Furthermore, CAR123 T cells were effective in NSG mice transplanted with AML cell line SKM-1.

Next, the successful manufacturing enabled the initiation of a clinical trial “CART123 T Cells in Relapsed or Refractory CD123+ Hematologic Malignancies: a Dose Escalation Phase I Trial“ (NCT06765876). A first patient has been enrolled and received a dose of 0,5x10e6 CAR-T/kg. In this patient, we observed expansion of CAR123 in the blood, and elimination of AML blast. This clinical trial is ongoing and additional patients are undergoing the therapy.

Summary/Conclusions

The linear DNA transposon platform enables a rapid GMP certification and is especially suitable for the cost-effective production of novel experimental gene-engineered T-cell therapeutics. We are currently enrolling AML patients to this clinical trial with CAR123 T cells at dose-escalating scheme to establish the maximum tolerated dose.

P12

FLT3 INHIBITORS MIDOSTAURIN AND GILTERITINIB ENHANCE CYTARABINE ACCUMULATION BY AFFECTING OCTN1 AND ENT1 TRANSPORTERS IN AML CELLS

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Background
Acute myeloid leukemia (AML) is among the deadliest malignancies, with only 30 % of patients achieving 5-year overall survival. The standard 7+3 induction therapy, consisting of cytarabine (Ara-C) and an anthracycline, remains the cornerstone of treatment in fit patients. FLT3-targeting inhibitors, such as midostaurin (MID) and gilteritinib (GIL) represent an important component of therapy in AML patients with the most frequent unfavourable genetic abnormalities in AML. Ara-C uptake into leukemic cells is mediated by nucleoside transporters, particularly ENT1 (*SLC29A1*), while OCTN1 (*SLC22A4*) has also been implicated in its transport. In contrast, MRP4 (ABCC4) has been reported to facilitate Ara-C efflux. A significant correlation between OCTN1 expression and patient survival has been previously reported. The link between FLT3 inhibition and Ara-C transport remains unclear and has not yet been fully investigated.

Aims
The aim of this study was to evaluate *SLC22A4* and *SLC29A1* not only in their predictive role in cytarabine-based therapy but also further investigate the link between their expression/function and effects of FLT3 inhibitors.

Methods
We explored this by isolating mononuclear cells from the blood of 32 newly diagnosed AML patients and 10 gilteritinib-treated patients. Gene expression of transporters was analyzed using droplet digital PCR and qRT-PCR in MV4-11 cells exposed to FLT3 inhibitors (MID and GIL) and in AML patients treated with GIL. Functional assays, including ASP+ uptake and [3H]-cytarabine accumulation, were used to evaluate drug transport efficiency. Additionally, cellular distribution and protein expression of OCTN1 and ENT1 were evaluated in MV4-11 cells following exposure to MID and GIL using immunofluorescence microscopy and Western blotting. Furthermore, cell cycle analysis was performed by flow cytometry.

Results
We observed differential expression of OCTN1 and ABCC4 in AML patient samples, with significant variability among individuals, except for ENT1. Interestingly, significantly higher levels of *SLC22A4* were observed in FLT3-ITD-positive patients. In MV4-11 cells, inhibition of FLT3 with MID and GIL led to significantly enhanced intracellular accumulation of Ara-C, which could be completely abolished by addition of OCTN1/ENT1 inhibitors. In contrast, a downregulation of ENT1 and OCTN1 encoding genes was observed in MV4-11 cells after acute (24 h) exposure to MID and GIL. In gilteritinib-treated AML patients, longitudinal analysis showed a decreasing trend in both ENT1 and OCTN1 expression over 7 and 30 days of treatment. This discrepancy between gene expression and uptake activity can be

explained by the revealed changes in the intracellular distribution of OCTN1 and ENT1 following MID and GIL treatment.

Summary/Conclusion
Our findings suggest that FLT3 inhibitors significantly enhance intracellular cytarabine accumulation, most likely by affecting the intracellular trafficking of OCTN1 and ENT1 transporters in AML cells. Gaining a deeper understanding of how FLT3 inhibitors regulate these transporters could pave the way for novel therapeutic strategies, ultimately improving treatment outcomes for this AML subtype.

The study was supported by the Czech Health Research Council (project No. NU23-03-00562).

Keywords: flt3 inhibitor, Ara-C (cytarabine), AML

P13

IDENTIFICATION OF CELLULAR MECHANISMS THAT CAN LEAD TO DRUG RESISTANCE TO FLT3 INHIBITOR GILTERITINIB IN AML

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Flt3 inhibitors currently represent important part of the AML pharmacotherapy in patients with FLT3-ITD/TKD, nevertheless, resistance to midostaurin, but also to gilteritinib (GLT) has been already reported. Identification of cellular changes inducedby GLT and molecular mechanisms that AML cells utilize to activate survival mode and that lead to cellular resistance is crucial for ensuring maximal treatment efficacy.

In this study, we aimed to generate AML cell lines resistant to GLT and identify mechanisms contributing to the cellular resistance to this FLT3 inhibitor. Additionally, we evaluated, if any of the most profound deregulated processes can beidentified in AML patients following treatment with Xospata (gilteritinib) at the recommended dosage. Applying stepwise selective pressure with increasing GLT concentrations, we generated two gilteritinib-resistant cell lines: (1)HL-60 leukemic cell line “HL-60 G75” and (2) MV4-11 cell line “HL-60 g45”. Transcriptomic and proteomic profiles were analysed in both resistant cell lines and their corresponding drug-sensitive variants. The most

deregulated genes/proteins have been identified and further evaluated in AML patients before and during treatment with Xospata.

While the HL-60 G75 cells revealed transient resistance fading out within 5 weeks of GLT depletion, the MV4-11 g45 show permanent resistance. Transcriptomic and proteomic analysis revealed clear clustering between both resistant and sensitive pairs. In total 3372 differentially expressed genes (DEGs) were identified between HL-60 G75 and HL-60 WT, while 2668DEG were found within the pair of MV4-11 g45 and MV4-11 cells. A comparison of the protein profiles revealed 174 differentially expressed proteins (DEPs) between the HL60 cell lines and 387 DEPs between the GLT resistant MV4-11 g45cells and their non-resistant variant. Although the HL-60 G75 appeared to have developed resistance by a multifactorial adaptation to gilteritinib selective pressure, MV4-11 g45 cells showed permanent resistance highly related to FLT3 inhibition and antiapoptotic pathways including MAPK cascade. Still, some processes, seemed to be shared by both cell lines. Among them, the activation of immune response mediated by myeloid leukocytes and neutrophils, deregulation of cytokine production, cell adhesion and extracellular matrix organisation were identified among top deregulated biological processes. Interestingly, we have identified > 10 genes that are involved in these processes and are deregulated also in the patientsfollowing gilteritinib (Xospata) treatment.

To sum up, we have generated and characterized two gilteritinib resistant cell lines and revealed the most deregulated genes and proteins that are responsible for the transient non-FLT3 dependent resistance and the permanent resistance in FLT3 mutated cells. We identified several genes that show changing expression pattern also in GLT treated AML patients and could lead to gilteritinib therapy failure in AML.

P14

INTEGRATIVE ANALYSIS OF THE INTERPLAY BETWEEN PERIPHERAL IMMUNE LANDSCAPE AND LEUKEMIC BLAST ADAPTATION IN ACUTE MYELOID LEUKEMIA

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Introduction and aims: With the rapid development of immunotherapeutic strategies in acute myeloid leukemia (AML), it has become increasingly important to understand the complex interactions between leukemic blasts and the surrounding immune environment. Increasing evidence indicates that immune cell composition plays a critical role in AML pathogenesis, influencing both disease progression and patient response to therapy. This study aims to comprehensively profile the peripheral blood immune landscape in AML patients and explore how the relative abundance of specific immune cell populations correlates with clinical outcome. Furthermore, by integrating these immune profiles with transcriptomic signatures of leukemic blasts, we aimed to elucidate how the leukemic clone adapts to and interacts with the surrounding immune environment to promote its survival and evade immune surveillance.

Methods: Peripheral blood samples were collected from 32 patients with non-acute promyelocytic AML at diagnosis. For cell profiling by digital cytometry, a targeted sequencing library was prepared from whole leukocyte RNA using the SureSelect CD CiberMed Heme + HiRes kit (Agilent). For whole-transcriptome library preparation (NEBNext Ultra II, NEB), ribodepleted RNA (RiboCop, Lexogen) from pure leukemic blasts was used. Both libraries were sequenced on NovaSeq (Illumina). Relative fractions of 12 lymphoid-derived cell types were calculated using the iSORT Fractions CiberMed software, with proportions normalized to the total lymphoid fraction.

Gene expression was quantified using StringTie2 (v1.3.6), and analyses were performed in R (v4.0.0), GraphPad Prism 10, and MetaScape.

Results: Using PERMANOVA analysis based on lymphoid cell fractions, patients who achieved complete remission (CR; n=15) after the first cycle of 3+7 chemotherapy and those who did not (n=17) were significantly separated (p=0.014). In responders, the relative fractions of $CD8^+$ T cells (p=0.005) and resting NK cells (p=0.019) were significantly higher compared to non-responders. The largest difference was observed for the total lymphoid cytotoxic cell fraction (LCCF; $CD8^+$ T cells, resting NK cells, activated NK cells, and γδ T cells), which was 45% on average in responders compared to 31% in non-responders (p<0.001).

Gene set enrichment analysis of 112 differentially expressed genes in leukemic blasts between high and low LCCF (H/L-LCCF) patients (divided by median) highlighted pathways in interferon signaling, cytoskeletal remodeling, and cell–microenvironment interactions, alongside tissue development processes. Several genes associated with proliferation, immune evasion, immune recognition and regulation, microenvironmental remodeling, and survival signaling, including *SPARC*, *LAG3*, *PRAME*, *SETBP1*, and *NFKBIZ* – were significantly downregulated in blasts from H-LCCF patients compared to L-LCCF. Conversely, the tumor suppressor gene *DLC1*, which is involved in cell migration and proliferation, was significantly upregulated in blasts from H-LCCF patients.

Conclusions: H-LCCF was identified as a marker of better therapy response and was associated with a distinct blast transcriptomic profile. Under strong cytotoxic surveillance, leukemic blasts exhibited reduced expression of genes involved in survival and proliferation (e.g., *SETBP1*), immune evasion and T cell inhibition (e.g., *LAG3*), T cell recognition (e.g., *PRAME*), and microenvironmental remodeling (e.g., *SPARC*), while upregulating tumor suppressors (e.g., *DLC1*). This suggests that strong immune pressure may drive transcriptional reprogramming and immune-mediated selection, leading to a less aggressive and less immunogenic leukemic phenotype. Conversely, in patients with low immune pressure, the overexpression of immune-inhibitory genes such as *LAG3* may contribute to the suppression of cytotoxic T cells and the establishment of an immunosuppressive microenvironment, resulting in reduced LCCF.

Our results highlight the interplay between leukemic blasts and the peripheral immune landscape, revealing candidate mechanisms of immune modulation and evasion in AML. Furthermore, we identified potential biomarkers of leukemic immunogenicity that may be relevant to the optimization of immunotherapeutic approaches.

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P15

EVALUATION OF NOVEL STRUCTURES AS POTENTIAL FLT3 INHIBITORS IN AML

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Background
Acute myeloid leukemia (AML) is characterized by the excessive proliferation of myeloid progenitor cells to immature myeloblasts and poor overall survival of the patients. Approximately 30 % of AML patients have FMS+-like tyrosine kinase 3 (Flt3) mutation. Recently, Flt3 inhibitors were introduced and became standard pharmacotherapy for Flt3-positive patients. Nevertheless, several

mechanisms have been described to mediate resistance to these drugs leading to therapy failure and thereby driving an intensive effort in searching for new potent and specific Flt3 inhibitors.

Aims
Firstly, we aimed to evaluate 60 newly synthesized investigative compounds as potential Flt3 inhibitors for their cytotoxic/antiproliferative effect and their specificity to the FLT3 receptor. Secondly, we wanted to characterize the cell cycle and proapoptotic behavior of the selected investigative compounds in cell lines and on AML patients' peripheral blood mononuclear cells (PBMC) samples.

Methods
MTT assay has been performed on AML cell lines THP-1, MV4-11, and MOLM-13 to determine the cytotoxicity profile of tested compounds and specificity to the Flt3 receptor. Annexin-V assay was used on MV4-11 and THP-1 cell lines as well as on PBMC samples with and without FLT3-ITD mutation. DNA cell cycle analysis with propidium iodide was performed on MV4-11.

Results
MTT assay revealed the three substances LG-2166, LG-2189, and LG-2192 with the highest antiproliferative activity on AML FLT3 mutated cell lines MOLM-13 and MV4-11 which significantly exceeded that in FLT3 non-mutated cells THP1 - specificity ratio (SR) to FLT3 mutation (MV4-11/THP1) of 155.7; 64.0; and 108.0 respectively). The proapoptotic effect of these compounds was further performed using Annexin V assay in MV4-11 and THP-1 cell lines. All three compounds showed superior proapoptotic effects induced in the mutated MV4-11 cells above that found in THP1 cells, however, significantly higher concentrations were needed to induce proapoptotic effect in PBMC samples. All three substances showed G0/G1 cell cycle arrest compared to untreated control.

Summary/Conclusion
The cytotoxic effect of 60 newly synthesized compounds was tested and 3 lead compounds were identified showing superior inhibition of cellular proliferation in Flt3 mutation-containing cells. The lead compounds showed a proapoptotic effect specific for cell-line with FLT3-ITD and G0/G1 cell cycle arrest in MV4-11. As much higher concentrations of the drugs are needed to detect proapoptotic effect in PBMC, we hypothesized that the effect of the novel inhibitors is predominantly antiproliferative.

The study was supported by the Czech Health Research Council (projects No. NU23-08-00439 and NU23-03-00562).

P16

ANALYSIS OF AML CELLS IN VITRO: SENSITIVITY TO CK1 INHIBITION VS. GENETIC BACKGROUND

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Acute myeloid leukaemia (AML) is a heterogeneous malignancy characterized by diverse genetic background, high relapse rate, and poor prognosis.

Inhibition or degradation of casein kinase 1 α (CK1α) represents a promising therapeutical approach for AML patients, currently being tested in a clinical trial (BMS-986397 degrader, patients with R/R AML and HR-MDS; NCT04951778).

We treated 48 AML bone marrow samples *in vitro* with an in-house developed CK1 inhibitor (range 0.03-100 uM). Cell viability was measured using the WST-8 assay, and inhibitor efficacy was calculated as the half-maximal inhibitory concentration (IC50). The IC50 values were compared with the mutation status of 25 prognostically relevant genes from a next-generation sequencing (NGS) panel. A lasso regression model, combined with k-fold cross-validation, was employed to identify relevant and redundant variables. The model used genetic background, patient age, and sex as input variables, with IC50 values as the outcome variable.

The final lasso model identified genes associated with both higher and lower responses to CK1 inhibition. Genes associated with higher response (lower IC50) included *FLT3-ITD, IDH1, IDH2*, and *NPM1*. Genes associated with lower response (higher IC50) included *EZH2, FLT3-TKD, PTPN11, TP53*, and *TET2*. The IC50 values predicted by the lasso model correlated significantly with the measured IC50 values (Spearman correlation, $R = 0.69$, $p < 0.001$).

Our findings underscore the diversity of treatment responses observed *in vitro*, reflecting the inherent heterogeneity of AML. While further validation *in vivo* and with larger datasets is necessary, these results emphasize the importance of tailoring therapeutic strategies to enhance patient outcomes.

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P17

PD-L1 EXPRESSION ON LEUKEMIA CELLS IS DYNAMIC AND CORRELATES WITH GLYCOLYTIC RATE

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Introduction

The immune system is crucial for elimination of residual acute myeloid leukemia (AML) cells after chemotherapy, as documented by benefits of the hematopoietic stem cell transplantation. AML cells employ various immune evasion strategies to avoid elimination by the immune system. One of the best-known mechanisms of immune escape is the expression of the Programmed cell Death Ligand 1 (PD-L1), which binds to PD-1 receptor on T cells and interferes with their activation. PD-L1 expression can be regulated via intrinsic signalling (e.g. JAK2 V617F, NPM-ALK) or extrinsically from the microenvironment (cytokines, lactate). However, the prognostic significance of PD-L1 expression in AML remains unclear, possibly due to variable experimental conditions used in different studies. We examined PD-L1 expression on primary AML cells at diagnosis, as well as potential factors influencing its modulation.

Methods and Results

PD-L1 protein surface amounts and transcript levels were analysed in primary cells freshly isolated from leukapheretic products and after 24 h of *in vitro* culture (N = 44). The cell metabolism was measured using a Seahorse device. A large increase in PD-L1 expression, both at the protein and transcript levels, was detected in about two thirds of samples after 24h cultivation. This increase correlated with cell glycolytic rate. Moreover, glycolytic primary cells induced PD-L1 expression on co-cultivated cell lines. Considering possible extrinsic regulatory mechanisms, we excluded effects of lactate and transfer of PD-L1 by exosomes. Inflammatory cytokines were analysed in 17 matched plasma

samples using the LEGENDplex™ Human Inflammation Panel 1. Surface PD-L1 levels on primary AML cells positively correlated with IL-6 and IL-18 in the plasma. STAT3 inhibition by stattic significantly reduced PD-L1 induction during the *in vitro* culture.

Conclusion

Glycolytic primary AML cells are able to induce PD-L1 expression both in autocrine and paracrine manner, via a mechanism involving STAT3. We speculate that PD-L1 expression on leukemia stem cells could be enhanced by bulk glycolytic cells, and its prognostic value at diagnosis might be limited. We anticipate that PD-L1 positivity will have negative prognostic impact if it is driven by cell-inherent mechanisms persisting under minimal residual disease conditions.

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P18

NEOCENTROMERES IN COMPLEX ACUTE LEUKEMIA KARYOTYPES AS AN INDICATOR OF CHROMOSOMAL INSTABILITY AND DISEASE PROGRESSION

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Neocentromeres are newly formed chromosomal regions that can replace the function of traditional centromeres. They are well known from human clinical studies. However, a few have also been reported in different malignancies. Their presence suggests a potential mechanism by which tumor cells may bypass conventional chromosomal segregation control pathways. Although they contribute to genomic instability, a hallmark of cancer cells, their occurrence in neoplasia, including acute myeloid and lymphoblastic leukemia (AML/ALL), appears to be rare.

We investigated complex karyotypes in bone marrow cells from 113 AML and 1 ALL patients using centromeric/multicentromeric FISH and identified four cases (3.5%) with derivative chromosomes exhibiting newly formed constrictions. The complex karyotypes were analyzed using G-banding, FISH, mFISH, mBAND, and aCGH/SNP. In all four cases, neocentromeres were detected on derivative chromosome 11. In two patients, marker chromosomes were identified as inv dup(11)(q13.3qter), with neocentromeres located at 11q13.3 in one case and in its distal region in the other. In a third case, the neocentromere was detected on a marker chromosome composed of

chromosomal material from chromosomes 1 and 11, localized to 11q23.3. In the fourth case, the derivative chromosome resulted from an amplification of the 11q23.3–11qter region.

In conclusion, we identified neocentromeres in the highly complex karyotypes of four AL patients, indicating advanced disease. In two of them, neocentromeres arose due to inverted duplications, a mechanism frequently associated with neocentromere formation. Nevertheless, chromosome 11 as a target of this phenomenon has been described sporadically. Neocentromeres can influence cell division, increase chromosomal instability, and drive clonal evolution that supports unrestricted tumor growth and resistance to treatment. Thus, they may serve as biomarkers for aggressive malignant processes and disease progression.

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P19

OPTICKÉ MAPOVÁNÍ GENOMU AKUTNÍCH MYELOIDNÍCH LEUKÉMIÍ (AML): NOVÉ POHLEDY NA STRUKTURNÍ VARIANTY A JEJICH KLINICKÝ VÝZNAM

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Základem diagnostiky a prognostické stratifikace nemocných s akutní myeloidní leukémií (AML) je komplexní analýza nádorového genomu. Moderní metodou umožňující komplexní analýzu genomu na úrovni DNA je optické mapování genomu (OGM). Metoda využívá neamplifikované, ultradlouhé molekuly DNA o velikosti 250–350kbp, na nichž jsou fluorescenčně značeny specifické 6bp sekvenční motivy (CTTAAG). Značená a linearizovaná DNA je analyzována pomocí speciálního zobrazovacího systému, jenž zaznamenává pozici jednotlivých značek na každé molekule a výsledkem je digitální záznam signálního vzoru – tzv. optická mapa DNA, která je následně porovnávána s referenčním lidským genomem. Vizualizace výsledků probíhá pomocí specializovaného softwaru Access (Bionano). Metoda OGM umožňuje detekci počtu kopií, strukturních aberací, včetně balancovaných translokací a ztráty heterozygotnosti (LOH).

Budeme presentovat výsledky analýzy provedené metodou OGM u 20 pacientů s AML (5Ž /15M, medián věku 63 let) diagnostikovaných na Interní hematologické a onkologické klinice FN Brno v letech 2023-2025. V souboru bylo 8 nemocných s normálním a 12 nemocných s abnormálním karyotypem. Nemocní byli vyšetření metodami FISH, arrayCGH a cíleným NGS panelem.

Metodou OGM byl normální nález potvrzen pouze u 1 nemocného, u 19 nemocných byly potvrzeny všechny změnu určené v klasických metodách a navíc OGM odhalilo další změny, které konvenčními metodami nebyly pozorovány. U dvou nemocných OGM nepotvrdilo cytogenetický nález dicentrických chromosomů. U čtyř nemocných odhalilo OGM ztrátu heterozygotnosti. OGM upřesnilo nálezy u nemocných s komplexním karyotypem, určilo přesná zlomová místa a geny v nich lokalizované a u 3 nemocných OGM odhalilo inzerce v genu *KMT2A* a vznik PTD. Celkem 6 nemocných bylo na základě nálezů OGM zařazeno do nové prognostické podskupiny.

Naše výsledky ukazují, že metoda OGM přináší významné informace doplňující klasická cytogenomická vyšetření, zvyšuje rozlišovací schopnost při detekci strukturních změn a může přispět ke zpřesnění diagnostiky, prognózy i výběru optimální léčebné strategie u pacientů s AML.

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P20

SQSTM1/P62 INHIBITION IMPAIRS MITOPHAGY AND CELLULAR RESPIRATION IN AML CELLS

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Introduction
Acute myeloid leukemia (AML) stem cells exhibit heightened sensitivity to mitochondrial damage compared to their healthy counterparts. Mitophagy, a key mitochondrial quality control mechanism, has emerged as a potential therapeutic target in AML. Notably, deletion or inhibition of the mitophagy adaptor SQSTM1/p62 has been shown to reduce AML cell proliferation and leukemia engraftment in murine models. In this study, we investigated the effects of SQSTM1/p62 inhibition by the small molecule compound XRK3F2 on mitophagy, cellular respiration, and viability in AML cell lines and primary AML cells.

Methods
Primary peripheral blood mononuclear cells were isolated from leukapheresis products of 20 AML patients at diagnosis. Mitophagy and mitochondrial content were assessed using optimized in-house protocols involving MitoTracker dyes and flow cytometry. Cellular respiration and glycolysis were measured using a Seahorse analyzer, reporting oxygen consumption rate (OCR) and extracellular acidification rate (ECAR), respectively. Cell viability was evaluated by flow cytometry using the propidium iodide exclusion assay.

Results
XRK3F2 effectively inhibited mitochondrial turnover in AML cell lines (N = 11) and primary cells (N = 20). While mitophagy rates were generally low in cell lines, they were highly heterogeneous among primary samples. XRK3F2-induced cytotoxicity varied and correlated with mitophagy rates in primary cells, but no clear correlation was observed in cell lines. A pronounced and rapid reduction in OCR was detected in all AML cell lines. In primary cells, XRK3F2 typically lowered the maximal respiration rate without having marked impact on basal OCR. We hypothesized that the effect of XRK3F2 on cellular respiration might be due to inhibition of lipophagy, a key source of respiratory substrates in AML cells. Consistently, inhibition of fatty acid oxidation using etomoxir largely limited cellular respiration in AML cell lines but less in primary cells.

Conclusions
SQSTM1/p62 inhibition by XRK3F2 effectively impaired mitophagy in AML cell lines and primary cells. In primary cells, XRK3F2 cytotoxicity correlated with mitophagy inhibition. In contrast, a strong reduction in cellular respiration was observed in cell lines, possibly reflecting their specific metabolic dependence on fatty acid oxidation. Given its dual role in regulating mitophagy and lipophagy, SQSTM1/p62 represents a promising therapeutic target for disrupting mitochondrial function in both immature and bulk AML cells.

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P21

COMMON FEATURES OF STRESS RESPONSE TO DRUGS TARGETING THE NUCLEUS AND MITOCHONDRIA

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Objectives: Drugs used for acute myeloid leukemia treatment usually target excessive cell proliferation and/or impaired apoptotic pathways to eradicate tumor cells. Thanks to communication among individual subcellular compartments, drugs targeting the nucleus affect mitochondria signalization and vice versa. To find common mechanisms of the inter-organelle communication, effects of Doxorubicin, which targets mainly the cell nucleus, and the Bcl2-inhibitor Venetoclax were analyzed.

Methods: The effect of Doxorubicin and/or Venetoclax on cell proliferation, viability, cell cycle, extent of oxidative stress, level of apoptotic and nucleolar proteins, and their specific phosphorylations was investigated in a panel of AML cell lines and primary AML samples using flow- cytometry, immunoblotting and confocal microscopy.

Results: Both drugs as well as their combination induced apoptosis in majority of samples. Cell sensitivity correlated with reactive oxygen species (ROS) production, decreased activity of Bcl-2 protein, and with lowered nucleolin (NCL) level. Mitosis-specific NCL phosphorylation at Threonines T76 and T84 was significantly reduced, consistently with inhibition of cell proliferation. Importantly, detailed time-course analysis of ROS production revealed the existence of a sensitive subpopulation with high ROS levels in treated cells, suggesting cell culture heterogeneity in response to the treatment.

Conclusions: Stress responses of AML cells induced by both DNA- and mitochondria-targeting drugs share common markers, in particular changes of Bcl-2 protein family, modifications of nucleolar protein NCL, and ROS production. Significant ROS induction preceded apoptosis in a specific cell subpopulation. Detailed characterization of this subpopulation could identify important therapeutic targets.

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P22

REDOX DEPENDENCY AND ANTIOXIDANT VULNERABILITY IN MLL-REARRANGED LEUKEMIA

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Introduction:
During leukemogenesis, hematopoietic progenitors undergo extensive metabolic reprogramming to sustain uncontrolled proliferation and block differentiation. However, the link between MLL-rearranged (MLL-r; “mixed-lineage leukemia”) leukemogenesis and redox homeostasis remains unclear. MLL-r leukemia, which accounts for roughly 10% of all leukemia cases, arises from MLL1 (KMT2A) gene fusions that define distinct molecular subtypes. We hypothesize that different MLL-r subtypes exhibit unique redox regulation and varying sensitivity to antioxidant metabolism disruption.

Aims:
To compare the sensitivity of MLL-r leukemia subtypes to inhibition of antioxidant systems and identify cellular responses induced by their perturbation.

Methods:
We studied MLL-AF6 (ML-2, SHI-1) and MLL-AF9 (NOMO-1, Monomac-6) leukemia cell lines, as well as murine pre-leukemic progenitors with doxycycline-inducible MLL::ENL expression, representing three MLL1 fusions. Dependence on antioxidant metabolism was assessed using specific inhibitors - buthionine sulfoximine (BSO), auranofin, and ezatiostat. Intracellular free thiols and reactive oxygen species (ROS) were quantified by flow cytometry, and LC/MS-based proteomics were applied to identify pathways activated upon antioxidant disruption.

Results:
MLL-r leukemic cells displayed markedly different sensitivities to inhibitors targeting glutathione and thioredoxin pathways. These effects were not solely determined by the MLL fusion variant but were influenced by the broader genetic background, including KRAS and TP53 mutations. Moreover, specific cellular programs were activated following MLL rearrangement, underscoring the complex interplay between oncogenic fusion identity, redox balance, and antioxidant metabolism in leukemic transformation.

P23

APL-LIKE ACUTE MYELOID LEUKEMIA WITH KMT2A-PTD AND MYC AMPLIFICATION IN AN ELDERLY PATIENT: A CASE REPORT

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Background:
Acute promyelocytic leukemia (APL) represents approximately 7–8% of acute myeloid leukemia (AML) and is characterized by life-threatening coagulopathy (fibrinolysis and thrombocytopenia) [1]. Classic APL is defined by the t(15;17)(q22;q21) translocation and *PML::RARA* fusion in ~98% of cases [2]. However, rare APL-like AML variants lack *PML::RARA* rearrangements despite exhibiting typical morphologic and immunophenotypic features, posing diagnostic and therapeutic challenges—especially in elderly patients with limited treatment tolerance.

Case Presentation:
An 86-year-old male was referred for evaluation of pancytopenia and abnormal circulating cells. He reported progressive fatigue and dyspnea over five months. Past medical history included chronic heart failure, atrial fibrillation, hypertension, prior upper gastrointestinal bleeding, peptic ulcer disease, hypercholesterolemia, and benign prostatic hyperplasia.

Laboratory testing revealed anemia (Hb 78g/L, RBC 2.37×10^{12}/L), thrombocytopenia (95×10^{9}/L), leukopenia (2.54×10^{9}/L), accompanied by nonsignificant prolongation of PT and aPTT, with normal fibrinogen and D-dimer levels. Peripheral blood smear examination demonstrated only a single atypical promyelocytic cell. Bone marrow morphology, however, showed a significant infiltration (27%) by abnormal promyelocytes exhibiting basophilic cytoplasm, prominent granulation, nuclear irregularities, and multiple Auer rods, including fagott forms. Flow cytometry demonstrated a typical APL-like phenotype: strong CD117 and CD33 expression, weak CD15 positivity, and absence of CD34 and HLA-DR. Cytoplasmic myeloperoxidase (MPO) expression was markedly elevated. Conventional karyotype was normal. FISH excluded t(15;17) and trisomy 8. Comprehensive molecular testing, including fragment analysis and RNA sequencing, definitively excluded both canonical and variant *PML::RARA* rearrangements and identifying a KMT2A-PTD e8e2 (MLL-PTD e9e3). Array-CGH provided critical confirmatory evidence for the diagnosis of APL-like AML by identifying *MYC* amplification, thereby supporting the morphologic and immunophenotypic findings in the absence of *PML::RARA* rearrangements.

ATRA (45 mg/m²/day) and arsenic trioxide (0.15 mg/kg/day) were initiated but discontinued due to QT prolongation and hepatotoxicity.

Conclusion:
This case illustrates diagnostic complexity of APL-like AML, characterized by APL phenotype but lack of *PML::RARA* and presence of KMT2A-PTD e8e2 and *MYC* amplification. Comprehensive immunophenotyping and molecular profiling are essential for accurate classification. In elderly patients, ATRA/ATO may be limited by toxicity, highlighting the need for individualized management strategies in this rare leukemia subtype.

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[2] de Thé H, Lavau C, Marchio A. Acute promyelocytic leukaemia, a paradigm for oncogene-induced human cancer. *Nat Rev Cancer*. 2017;17:495-508.

P24

PERFUSION MODEL OF MICROVASCULAR ENDOTHELIUM ACTIVATION BY AML-DERIVED SECRETED FACTORS

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Endothelial–cancer cell crosstalk has emerged as an important factor influencing disease progression and vascular complications. In acute myeloid leukemia (AML), factors secreted by AML blasts - notably inflammatory cytokines - promote vascular permeability and pro-adhesive and pro-coagulant phenotypes, contributing to vascular activation, microthrombosis, and impaired hemostasis. These conditions lead to AML-associated coagulopathy, which may clinically manifest as both thrombotic and hemorrhagic complications.

In this project, we investigated stimulation of immortalized human microvascular endothelial cells (HMEC-1) by AML-derived factors present in AML patient plasma or blast-conditioned media (CM) under static and perfusion conditions. Through integrative metabolic, proteomic, and functional analyses, we aim to elucidate the mechanisms of AML-induced endothelial dysfunction and identify early predictive biomarkers of life-threatening coagulopathies as well as potential targets to mitigate vascular complications in AML.

The Ibidi perfusion system is used for HMEC-1 culture under flow. Stimulation is induced by 30% heparinized plasma or 30% CM supplementation. Untargeted proteomic analysis is performed by mass spectrometry (MS). Cell proliferation and adhesion are assessed using electric cell–substrate impedance sensing (ECIS). The metabolic profile of HMEC-1 cells is evaluated using the Seahorse XFp Analyzer. Live-cell imaging is performed with an Olympus confocal microscope, and surface protein expression is quantified by flow cytometry.

Stimulation of HMEC-1 cells with AML patient plasma did not induce endothelial activation, as evidenced by the absence of VCAM-1 and ICAM-1 induction. In contrast, exposure to CM from AML blasts resulted in a strong ICAM-1 upregulation. Metabolic analysis revealed a glycolytic shift in CM-stimulated cells, consistent with an activated endothelial phenotype. Both control and patient plasma promoted cell proliferation, increased metabolic rates, and stimulated pro-angiogenic behavior. Preliminary MS data demonstrated significant alterations in the proteomic profile between control and CM-stimulated cells, suggesting broad molecular reprogramming upon AML-related stimulation.

P25

CD123-SPECIFIC CAR-T CELLS PRODUCED BY LINEAR DNA-BASED PIGGYBAC TRANSPOSON SYSTEM – INITIATION OF PHASE I CLINICAL TRIAL IN PATIENTS WITH AML

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Background

Chimeric antigen receptor-modified T cells represent a novel and highly effective therapeutic approach for hematological malignancies. However, their high costs result mainly from the manufacturing process utilizing recombinant viral vectors which critically complicates their further development and broad availability.

Aims

To develop a novel approach for rapid and cost-effective non-viral production of clinical-grade CAR T cells which utilizes polymerase-chain-reaction (PCR) produced linear piggyBac transposon DNA and in-vitro transcribed (IVT) transposase mRNA. To fully validate this technology, we initiated a clinical trial with CD123-specific Chimeric Antigen Receptor-T Cells (CAR123) in patients with refractory AML.

Methods

To produce the vectors, a large-scale PCR using high-fidelity polymerase was used to generate the linear piggyBac transposon DNA of approx. 5kb length. This sequence contains a ubiquitin promoter-regulated CD123 CAR (with 4-1BB-zeta intracellular signaling domain) joined via T2A self-cleaving motif with CD20 protein. The PCR product was separated by HPLC, yielding 100% pure DNA, and the sequencing revealed no mutations. The transposase mRNA was similarly synthesized from a plasmid template using T7 RNA polymerase followed by DNAse treatment and by precipitation with LiCl to generate pure mRNA. To generate CAR123 T cells, PBMCs obtained from the blood were electroporated with this linear DNA and mRNA. The transfected T cells were then polyclonally activated with anti-CD3/CD28 antibodies and expanded in the presence of cytokines IL-4, IL-7, and IL-21 which enable the preservation of stem-cell memory phenotype, furthermore, the expansion of CAR123 was enhanced by addition of irradiated mixed allogeneic PBMCs (a “feeder”). This manufacturing process is GMP-compliant and was certified for the initiation of a clinical trial.

Results

First, linear DNA and mRNA production were feasible, and no issues regarding identity and purity were noticed. Next, the production of CAR123 T cells was highly effective as 1 µg of linear DNA yielded approx. 100-150 million CAR-T cells per one electroporation of 10 million of PBMCs from healthy donors (n=5). The median percentage of CAR+ cells was 31% (range, 23-36%), median CD4/CD8 ratio was 0.72 (range, 0.64-0.82). Median 68% (range, 54-83%) of CAR+ had central memory phenotype (CD62L+CD45RA+) and the vector copy number was within 2-5 range. Produced CAR123 displayed efficient cytotoxicity in vitro against target cell lines THP-1 and SKM-1. Furthermore, CAR123 T cells were effective in NSG mice transplanted with AML cell line SKM-1.

Next, the successful manufacturing enabled the initiation of a clinical trial “CART123 T Cells in Relapsed or Refractory CD123+ Hematologic Malignancies: a Dose Escalation Phase I Trial“ (NCT06765876). A first patient has been enrolled and received a dose of 0,5x10e6 CAR-T/kg. In this patient, we observed expansion of CAR123 in the blood, and elimination of AML blast. This clinical trial is ongoing and additional patients are undergoing the therapy.

Summary/Conclusions
The linear DNA transposon platform enables a rapid GMP certification and is especially suitable for the cost-effective production of novel experimental gene-engineered T-cell therapeutics. We are currently enrolling AML patients to this clinical trial with CAR123 T cells at dose-escalating scheme to establish the maximum tolerated dose.

P26

DETERMINANTS OF OUTCOMES AFTER ALLOGENEIC TRANSPLANTATION (ALLOHCT) IN PATIENTS ≥70 YEARS OF AGE – SINGLE CENTRE ANALYSIS

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Background: AlloHCT in elderly patients with haematological malignancies is often the only curative procedure. In particular, patients ≥70 years of age are rarely referred for alloHCT due to concerns about high non-relapse mortality (NRM).

The aim of our study was to determine key factors for overall survival (OS), NRM, progression-free survival (PFS), and GVHD-relapse free survival (GRFS) survival in these patients transplanted at our center.

Methods: Retrospective unicentric analysis of 32 consecutively transplanted patients ≥70 years of age with AML (n=25), MDS (n=4), ALL, PMF, and CLL (n=1 each) in the period 02/2012-10/2023.

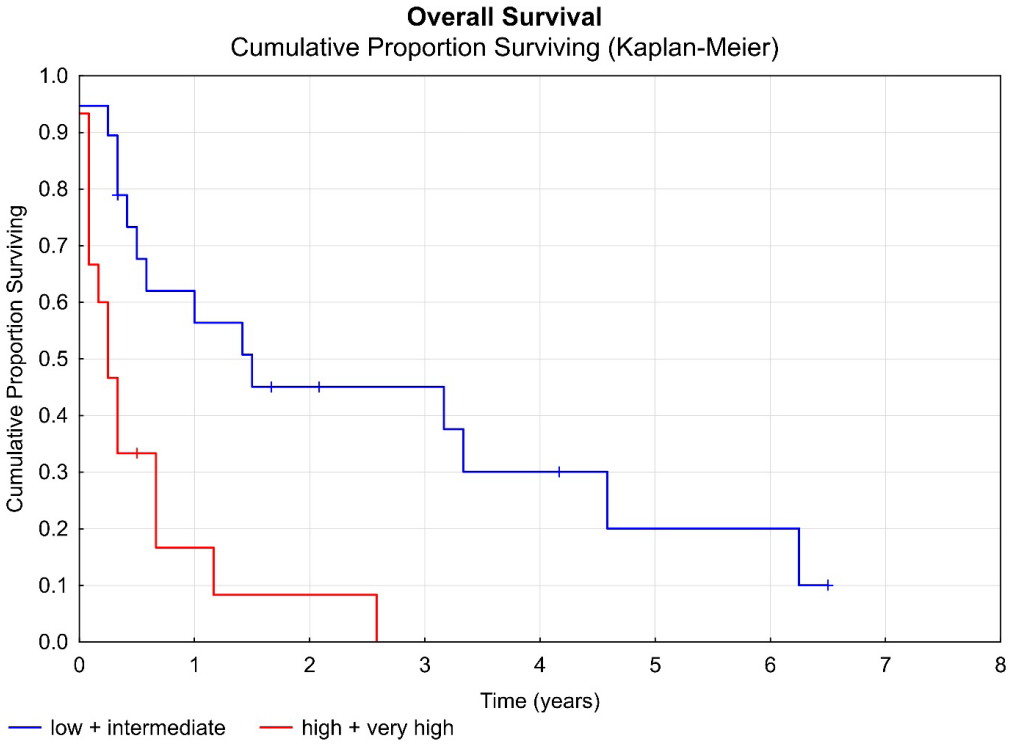
Results: Median age was 71 years (range 70-74), women 19 (59%). Donors were matched unrelated (MUD=19; 59%) and haploidentical (HID=13; 41%). Median age of donors was 31 years (range 20-53). Conditioning was reduced-intensity in all pts. Disease risk index (DRI): low/intermediate 18 (56%) patients, high/very high 14 (44%). Median comorbidity index – HCT-CI - was 2 (i.e. intermediate risk, range 0-8), in 6 (19%) patients it was >3. Median EBMT score was 4 (range 2-6). 20 (63%) pts were transplanted after 2018.

With a median follow-up of 24 months (range 5-78), 6 (19%) patients are alive. Overall NRM was 53% (17 patients), of which 7 patients (41%) died without direct relation to alloHCT. Probability of NRM at 1-year was 39% and at 3-year 65%. Relapses occurred in 10 (31%) patients. Kaplan-Meier probabilities of OS at 1y/3-years were 42% and 29%, respectively. For GRFS these figures were 29% and 18%, respectively.

Univariate analysis showed high/very high DRI as the only negative significant factor for OS (HR 3.987, 95% CI: 1.647 - 9.651, P=0.0007), PFS and GRFS (HR 4.780, 95% CI: 2.041 - 11.193, p<0.0001). and for NRM also EBMT score >4 (HR 2.385, 95% CI 1.040-7.734, p=0.0296). Transplant outcomes were not affected by HCT-CI or donor type.

In multivariate analysis, high/very high DRI remained the only significant factor for OS (HR 4.4432; 95%CI 1.616-12.158, p=0.0038) and GRFS (HR 4.4432; 95%CI 2.022-14.345, p=0.0008)

Conclusion: Almost 20% of patients ≥70 years achieved 3-year GRFS. The risk of underlying disease was crucial, not HCT-CI or other factors. Selected patients ≥70 years should not be excluded from alloHCT.



P27

THE TRANSPLANT PROGRAM QUALITY COUNCIL MEETINGS AS THE EFFECTIVE TOOL FOR THE QMS INTRODUCTION AND MAINTENANCE AND THE IMPLEMENTATION OF NEW REQUIREMENTS

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Backround: Since 1986 IHBT has had a tradition of performing HSCT in adults (annually 75-80 allogeneic applications in recent years). CAR-T therapy has been provided since 2019.

The hospital's QMS has been set up in accordance with JCI standards. Since 2016 QMS according to JACIE standards was implemented for the Transplant Program. The TP Quality Council (8 mandatory members) was established and regularly holds quarterly meetings.

Methods:
The TP QC meeting's program is divided into 4 mandatory areas.

The Clinical part data are obtained from the hospital information system (FONS Enterprise -STAPRO co.). Statistical outputs of evaluated quantitative/ qualitative parameters (e.g. SCT types/numbers, engraftment, PGF, TRM, NRM, aGVHD/ cGVHD incidence and grading) are obtained using the R language programming. The data are presented in the form of clear graphs with timeline, in which it is possible to demonstrate ev. deviations or discontinuities and identify their cause.

The activity of the Collection facility is documented in the Amadeus SW (Steiner co.). These data (type and course of performance, parameters of apheretic products, results of lab tests) together with excel records are the source of the presented graphs and tables.

The Processing facility activity is documented in the CRYUS SW (Steiner co.) and partial excel evidences. Presented reports and graphs are generated from the collected data (types/ number of procedures, product parameters, contamination lab tests results). The main attention is paid to interannual trends too.

The used electronic databases interconnection is not implemented.

Results: The March meeting (about 25 participants) is devoted to the report of results for the entire previous calendar year. The participants are introduced to the Annual TP Report draft with overall TP and QMS activities summary and the internal regular/ extraordinary audits findings overview. The evaluation of the validity of the institute's long-term mission in TP, the fulfillment of set annual professional and quality goals, and the announcement of goals reflecting new requirements for the next period are discussed. The definitive version of the TP Annual Report is handed over to the institute's top management.

Other meetings (June/ September/ December) are devoted to summarizing the interim results of the activities and the progress in solving the set tasks (procedures changes, SAE/AE impact/overlap into TP, the risk management effectiveness). These meetings were less attended (about 15 people). The addition of a short presentation (20 min) on a topic related to the administration of cell therapy products increased the interest in participation to the level of annual meetings.

Meetings are held in a hybrid manner. The minutes and all presentations are electronically available to all IHBT employees.

Conclusions: TP QC meetings have become a routine part of the TP QMS. With the cooperating contractual partners representatives participation of (Prague's MUD register, the Prague's Children's TP) these joint meetings have proven to be an opportunity for a face-to-face working discussion, which makes it possible to find feasible ways to ongoing maintaining the QMS not only in accordance with legislative and accreditation requirements, but also with daily practice.

P28

PONATINIB-BASED COMBINATION THERAPIES INCLUDING ASCIMINIB AND VENETOCLAX WERE EFFECTIVE ON PATIENT-DERIVED XENOGRAFT MODELS OF CHRONIC MYELOID LEUKEMIA IN BLAST PHASE

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Introduction: Blast phase of chronic myeloid leukemia (CML-BP) represents advanced and aggressive phase of CML, marked by high genetic heterogeneity, compromised efficacy of BCR::ABL1-targeted tyrosine kinase inhibitors (TKIs), and poor clinical outcomes. Emerging evidence indicates that combination therapies may offer more effective treatment options in this setting. This European Treatment and Outcome Study (EUTOS 2024) work aims to evaluate the efficacy of allosteric BCR::ABL1 inhibitor asciminib, 2nd generation TKI nilotinib, 3rd generation TKI ponatinib, BCL2 inhibitor venetoclax, and their combinations in patient-derived xenograft (PDX) models of CML-BP.

SBORNÍK ABSTRAKTŮ

Methods: Leukemic cells from CML-BP patients were analyzed for chromosomal abnormalities and mutations in *BCR::ABL1* kinase domain (KD) and 61 other leukemia-associated genes. *Ex vivo* drug sensitivity was conducted. CML blasts were transplanted into immunodeficient mice to establish PDX models. Animals (n=5–8 per group) were randomized into control or treatment arms and received daily oral treatment for 7 days: ponatinib (25 mg/kg b.w.), nilotinib (40 mg/kg b.w.), asciminib (30 mg/kg b.w.), venetoclax (50 mg/kg b.w.), or their combinations. Tumor growth and event-free survival (EFS) were assessed. The experimental design was approved by the institutional Animal Care and Use Committee and the Ministry of Education Youth and Sports of the Czech Republic.

Results: The PDX model BC-CML2, derived from a 70-year-old male patient with myeloid CML-BP following rapid progression on imatinib, exhibited a complex karyotype with additional Philadelphia chromosomes but no detectable somatic mutations. In line with *ex vivo* findings, the model was poorly responsive to nilotinib, asciminib and venetoclax monotherapies but responded well to ponatinib. Combination therapies—asciminib+venetoclax and especially ponatinib+venetoclax—significantly reduced tumor growth and prolonged EFS compared to controls and monotherapies ($p<0.001$). Conversely, combination of nilotinib and asciminib have limited efficacy against tumor growth and have no survival benefit.

The BC-CML3 model originated from a 58-year-old female patient with B-lymphoid CML-BP in 3rd relapse on ponatinib. Blasts carried multiple *BCR::ABL1* mutations (T315I, E255K and E459K), a *RUNX1* mutation (R201Q), and a complex polyclonal karyotype. This model was resistant to venetoclax and nilotinib alone and showed only modest responses to asciminib and ponatinib monotherapies. However, combinations of asciminib+ponatinib and ponatinib+venetoclax delayed disease progression and significantly improved EFS compared to controls ($p<0.001$) and single agents ($p<0.01$ for asciminib+ponatinib; $p<0.001$ for ponatinib+venetoclax).

The BC-CML4 model was established from a 65-year-old male patient with myeloid CML-BP relapsing after four lines of TKI therapy including ponatinib. Mutational profiling revealed polyclonal *BCR::ABL1* mutations (E255K and E255V), along with *NRAS* (G12D) and *GATA2* (R307Q) mutations, and a complex karyotype. *Ex vivo* testing showed resistance to venetoclax. Ponatinib and its combination with venetoclax significantly delayed tumor growth and extended EFS ($p<0.001$).

Conclusions: Preclinical testing in CML-BP PDX models confirmed the enhanced efficacy of combination therapies. Across all models, dual targeting with ponatinib and venetoclax was most effective. In the BC-CML3 model, harboring multiple *BCR::ABL1* mutations linked to ponatinib resistance, the asciminib+ponatinib combination showed notable activity, consistent with previous findings in cell line-derived xenograft (CDX) models and *in vitro* testing.

Support: EUTOS 2024, MH CZ – DRO (IHBT, 00023736), NW24-03-00056

P29

ALLELE-DEPENDENT ACTIVITY OF PROMOTERS OF GENES SLC22A4 AND SLC22A5 ENCODING IMATINIB TRANSPORTERS IN CHRONIC MYELOID LEUKEMIA BLAST CELLS

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Introduction: In our previous studies, we identified an association between the regulatory SNP rs460089, located in the promoter of *SLC22A4* gene (encoding a transporter involved in imatinib transport), and the response to imatinib (Jaruskova et al., JECCR 2017) as well as treatment-free remission (Machova Polakova et al., Leukemia 2024) in patients with chronic myeloid leukemia (CML). The „GG“ genotype was significantly linked to higher frequency of imatinib failure and molecular recurrence after therapy discontinuation. However, underlying biological mechanism for this association remains unclear. The initial hypothesis suggesting that „GG“ genotype leads to reduced expression of *SLC22A4* and lower intracellular imatinib concentration was not confirmed (Burda et al., Mol Metab 2024). We propose a new hypothesis that the regulatory SNP of *SLC22A4* and *SLC22A5* affect the intracellular concentrations of their natural substrates, ergothioneine (a stable and effective antioxidant) and carnitine, respectively, thereby supporting the survival of leukemic cells.

Methods: The promoter regions of *SLC22A4* and *SLC22A5* genes were analyzed using chromatin immunoprecipitation assays, luciferase reporter assays and DNA methylation analysis. Gene expression was assessed via real-time qPCR (RT-qPCR), while intracellular concentrations of carnitine and ergothioneine were quantified using mass spectrometry. The experiments were conducted using the KCL-22 myeloblast cell line („GG“ genotype), two blast phase patient-derived cell lines (PDs, „GC“) and primary PBMC cell isolated from CML patients at chronic phase at the time of diagnosis (n=12, Covering all 3 genotypes, GG, GC and CC).

Results: Luciferase assays showed higher promoter activity with the „G“ allele at the rs460089 compared to „C“. This was supported by significant increase in intracellular ergothioneine intake in KCL-22 („GG“) vs. PD („GC“) cells after 24 and 48 hours of incubation. Regardless of the allele, promoter activity decreased after JQ1 treatment (MYC expression inhibitor), confirming the rs460089 as a biologically relevant MYC binding site as predicted by previous in silico analysis. Chromatin immunoprecipitation showed MYC occupation at the rs460089 locus only in „GG“ and incubation with ergothioneine even enhanced MYC binding only in KCL-22 („GG“). Despite active chromatin marks (especially H3K4Me3 and H3K27Ac) and complete DNA demethylation at CpG within promoter (independent on rs460089 genotype), *SLC22A4* expression was very low in KCL-22 („GG“) cells and fell below the detection limit of RT-qPCR in „GC“ PD cell lines. In contrast, *SLC22A5* expression was two logs higher in both „GG“ and „GC“ genotypes. Notably, two other regulatory SNPs in *SLC22A5* promoter (exhibiting 99% Linkage Disequilibrium with rs460089) were identified. We show that *SLC22A5* promoter, including these high-LD loci, exhibited transcriptionally active chromatin state, fully demethylated CpG in promoter and MYC occupancy was detected only in KCL-22 „GG“. Carnitine incubation induced MYC expression and MYC (and its cofactor MAX) occupancy, stimulated *SLC22A5* expression and increased corresponding intracellular carnitine level only in „GG“ KCL-22 cells. Importantly, both „GC“ genotype PD cell lines showed reduced intracellular carnitine concentrations compared to „GG“ KCL-22. In relation to GG and GC cell lines analyzed,

any DNA methylation at the promoters, regardless of the rs460089 allelic configuration in all 12 CML patients.

Conclusion: Our results revealed that the rs460089 locus, which is in strong linkage disequilibrium with regulatory elements in the *SLC22A5* promoter, exerts a significant allele-specific effect on promoter activity, leading to altered *SLC22A5* transcription and accumulation of intracellular carnitine. Elevated carnitine concentrations (potentially supported by ergothioneine antioxidant activity) may enhance the survival of CML cells. Modulated carnitine-dependent metabolic pathways may potentially explain the previously observed association between rs460089 and both imatinib response and treatment-free remission (TFR) in CML. We are currently investigating whether the non-optimal response in patients with the "GG" genotype (compare to "GC") may be related to a more efficient carnitine-dependent metabolism, thereby promoting better survival of leukemic cells.

Support: MH CZ - DRO (IHBT 00023736)

P30

RESIDUAL NORMAL HEMATOPOIETIC STEM CELLS PREDICT EARLY HEMATOLOGICAL TOXICITY IN CHRONIC MYELOID LEUKEMIA

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Objectives

During tyrosine kinase inhibitor (TKI) treatment, *BCR::ABL1pos* clone is suppressed, and normal hematopoiesis is reconstituted from surviving normal hematopoietic stem cells (HSCs). The question is whether patients with less HSCs encounter worse hematopoietic reconstitution with more hematological adverse events. From our earlier experiments on leukemic stem cell identification, we were able to simply extract data on HSC frequencies.1 The aim here was to correlate these HSC data with the occurrence of hematological adverse events (hAE).

Methods

In a group of 48 consecutive patients, diagnosed with chronic phase chronic myeloid leukemia (CP CML), we assessed hAEs (leukopenia: < 4 × 10^9 leukocytes/L, thrombocytopenia: < 150 × 10^9 platelets/L, and anemia < 120 g/L for women and < 130 g/L for men) and the resulting TKI treatment reductions or interruptions (TKI R/Is), within the first 9 months of treatment. HSCs were identified in bone marrow as CD34+CD38- cells negative for leukemic stem cell markers: CD26, CD25, IL1-RAP, CD56, CD93, CD69, and quantified as: i) the percentage of HSCs in CD34+CD38- fraction (HSC %), ii)

the number of HSCs in mononuclear cell fraction (HSC n/MNC), and iii) the number of HSCs per ml of bone marrow (HSC n/ml).

Results

TKI R/Is events predominantly occurred early in treatment, with 9/10 events arising within the first three months. A comparison with HSC data from diagnosis revealed significant differences between patients experiencing TKI R/Is and those without. Specifically, the TKI R/Is group exhibited substantially lower HSC n/MNC (7.2×10^{-5} vs 3.6×10^{-4}, $p < 0.01$) and HSC n/ml counts (5.2×10^{2} vs 1.8×10^{4}, $p < 0.01$). However, no statistically significant difference was observed in the HSC percentage (44.1% vs 71.1%, $p = 0.10$). Interestingly, no relationship was found for individual hAE and the HSC data. We also asked whether refinement to a more primitive CD90+ HSC fraction could provide further benefit for TKI R/Is prediction, however, this was not proven. Also, HSC quantification in PB provided no link with TKI R/Is.

Conclusions

Flow cytometric analysis of hematopoietic stem cells in bone marrow at chronic-phase chronic myeloid leukemia diagnosis can predict hematological toxicity risk. Prophylactically reducing initial TKI dosage may mitigate this toxicity in high-risk patients identified by this method.

References:

Culen M., et al. Quantification of chronic myeloid leukemia stem cells at early follow-up shows prognostic value. 26th ESH-iCMLf, 27-29 Sept 2024

P31

EVALUATING REVERSE TRANSCRIPTASE EFFICIENCY IN CML: CONSEQUENCES FOR DEEP MOLECULAR RESPONSE CLASSIFICATION AND TREATMENT-FREE REMISSION DECISIONS

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Background

In chronic myeloid leukemia (CML), deep molecular response (DMR) is defined as molecular response 4 (MR^4) or deeper. If *BCR::ABL1* is undetectable the molecular response is determined by the number of control gene (CG) copies - typically *ABL1* or *GUSB* - detected by RT-qPCR. RT-qPCR remains the gold standard for monitoring measurable residual disease (MRD). Sensitivity thresholds are set as follows: MR^4 requires 10,000–31,999 *ABL1* copies (or 24,000–76,999 *GUSB* copies, reflecting its 2.4-fold higher expression); $MR^{4.5}$ requires 32,000–99,999 *ABL1* copies or 77,000-239,999 *GUSB* copies; and MR^5 requires ≥100,000 *ABL1* or ≥240,000 *GUSB* copies. The efficiency of reverse transcriptase (RT) enzymes used during cDNA synthesis can significantly impact transcript quantification and thereby MR classification.

Aim

As part of the European Treatment and Outcome Study for CML 2024 (EUTOS 2024) initiative, this study aimed to evaluate the influence of various RT enzymes on assay sensitivity and MR classification - an important

consideration for treatment discontinuation decisions, as outlined in the European LeukemiaNet (ELN) 2025 guidelines.

Methods
Samples from 12 CML patients in deep molecular response (DMR) - 7 with major and 5 with atypical *BCR::ABL1* transcripts - were analyzed. Equal amounts of RNA, within the range recommended by the respective manufacturers, were reverse-transcribed using seven different reverse transcriptase (RT) enzymes: SuperScript II (SSII), GoScript, MMLV, High Capacity, VILO, Clara, and GoTaq. Quantification of *ABL1*, *GUSB*, and *BCR::ABL1* transcripts was performed using standardized RT-qPCR protocols. Therefore, the only variable in this analysis was the choice of RT enzyme.

Results
Using SSII, the expected *GUSB/ABL1* expression ratio was confirmed (mean 2.57, SD 0.35; range 2.12–3.16), yielding consistent MR classification: 1 sample at $MR^{4.5}$, 11 at MR^{5} for both CGs. High Capacity produced comparable results in 10 samples, with 2 classified at *GUSB*-MR^{5} but *ABL1*-$MR^{4.5}$ (mean ratio 3.17, SD 0.53). MMLV showed slightly higher *GUSB* expression (mean ratio 3.42, SD 1.15) resulting in the classification of 5 samples as *GUSB*-MR^{5} but *ABL1*-$MR^{4.5}$, while 6 remained at MR^{5} for both CGs and 1 sample at $MR^{4.5}$ for both CGs.

VILO and GoTaq exhibited markedly higher *GUSB/ABL1* ratios - 10.64 (SD 2.71) and 7.08 (SD 1.49), respectively - causing inconsistent MR classification across most samples. GoScript produced similar *ABL1* and *GUSB* levels (mean ratio 1.3, SD 0.18) but overall lower transcript yields, leading to lower sensitivity. Clara showed highly variable performance and inconsistent *GUSB/ABL1* ratios (SD 3.19, range 1.31-10.5), with 6/11 samples yielding false-negative *BCR::ABL1* results. These findings indicate that *ABL1* copy numbers are often insufficient, reflecting a lower analytical sensitivity. *GUSB* also showed suboptimal performance in most cases, with the exception of VILO, which tended to overestimate *GUSB* expression.

Conclusions
This study highlights the critical impact of reverse transcriptase choice on MR classification in CML, particularly in cases of undetectable *BCR::ABL1* . Only SuperScript II, High Capacity, and MMLV produced consistent and ELN-compliant results, including assessment of DMR. In contrast, GoTaq and GoScript generally produced insufficient copy numbers of both *ABL1* and *GUSB*, resulting in reduced analytical sensitivity. VILO also yielded low *ABL1* copy numbers but tended to slightly overestimate *GUSB* expression. Clara, due to inconsistent control gene transcription and a high risk of false-negative MRD results, appears unsuitable for clinical use in this context. We recommend that testing laboratories should validate their cDNA synthesis protocols to ensure that measured CG copy numbers fall within ELN-defined MR ranges, especially when evaluating patients for treatment-free remission.

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P32

FUNCTIONAL ANALYSIS OF A NOVEL GERMLINE JAK2 VARIANT ASSOCIATED WITH THROMBOCYTOSIS

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Janus kinase 2 (JAK2) mediates signaling from cytokine receptors, namely MPL (thrombopoietin receptor) and EPOR (erythropoietin receptor) in hematopoietic cells. Changes in JAK2 activity markedly affect hematopoiesis via cell proliferation and differentiation. While the somatic JAK2 Val617Phe variant is known as the leading molecular cause of myeloproliferative neoplasms (MPN), the role of germline JAK2 variants remains unclear.

A previously undescribed germline variant, Val1043Phe, associated with thrombocytosis has been identified during NGS screening of MPN patients. The second allele encoded a frameshift mutation probably leading to loss of function.

The impact of the Val1043Phe change was studied using exogenously expressed JAK2 in HEK293T and HeLa cells. Activation of downstream signaling pathways was analyzed by western blot with phosphospecific STAT5, STAT3 and ERK antibodies. Western blot band intensity was normalized according to transfection efficiency determined by flow cytometry.

JAK2 Val1043Phe induced markedly lower activation of downstream signaling compared to the wild-type variant if expressed without exogenous receptors. In HeLa cells, significantly lower signaling capacity was observed even with EPOR cotransfection. The maximal activity measured in the presence of EPO in cells cotransfected with JAK2+EPOR was equal in terms of STAT5 phosphorylation. In contrast, STAT3 phosphorylation remained significantly lower for the Val1043Phe variant.

Structural modeling showed that Val1043, which is situated within the kinase domain, forms part of the catalytic cleft, an essential element for substrate binding. Val-to-Phe substitution may result in spatial deformation of this binding structure and reduce the probability of correct substrate localization in the cleft.

Our results suggest that the Val1043Phe change reduces JAK2 kinase activity, probably due to suboptimal conformation of the catalytic cleft, modulating JAK2 affinity to individual substrates. This results in low spontaneous activity of the Val1043Phe variant as well as altered balance between STAT5 and STAT3 signaling under conditions of ligand-induced JAK2 activation.

P33

FUNCTIONAL CHARACTERIZATION OF A NOVEL GERMLINE JAK2 R989FS MUTATION

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Janus kinase 2 (JAK2) mutations are key drivers of Philadelphia chromosome-negative myeloproliferative neoplasms (MPNs), with somatic *JAK2* V617F mutation, present in ~99% of cases, being the most prevalent. However, the studies of rare and novel *JAK2* variants may reveal previously unrecognized pathophysiological mechanisms of myelopoiesis. Here, we describe the functional impact of a novel germline frameshift mutation, *JAK2* R989fs, found in a 24-year-old woman with a clinical phenotype resembling an atypical MPN. The patient, after suffering cerebral stroke, was found to have unexplained thrombocytosis (platelets 557 × 10^3/μL; range 150-450 × 10^3/μL), borderline hematocrit (45.4%; range 35-45%), and normal hemoglobin (14.5 g/dL; range 12-15,5 g/dL) with normal leukocytes and differential with decreased transferrin saturation at 17%, while taking iron supplementation. Her erythropoietin (EPO) was low-normal range at 7 and 8 mU/mL (normal range 4-27 mU/mL); however, her thrombopoietin was increased at 158 pg/ml (~2x level of upper normal range).

The *JAK2* R989fs variant, a 2-nucleotide deletion at positions c.2967_2968 (exon 22) with allelic burden at 46,9%, was identified through next generation sequencing and confirmed to be heterozygous in granulocyte DNA; its germline origin was confirmed in her fibroblasts. The same mutation was found to be inherited from her mother and was also identified in her older sister,

who had hemoglobin: 14.8 g/dL, hematocrit: 45.3%, platelets: 411 × 10^3/μL; however, possible iron deficiency was not excluded. Her brother was found not to carry the mutation and had normal blood counts. Her mother, maternal great grandmother and maternal uncle had a history of repeated thrombosis, but their blood counts were not available. The *JAK2* R989fs mutation results in a premature stop codon, predicting a truncated protein of 990 amino acids (~114 kDa), lacking the distal C-terminal portion of the kinase domain. The expression of the truncated JAK protein was confirmed by Western blot analysis of HEK293 cells transfected with expression plasmid bearing *JAK2* R989fs mutation.

To investigate the structural consequences of the R989fs truncation, we performed AlphaFold3 modeling of both wild-type and R989fs-mutant JAK2 proteins. The truncation occurs at Val990, within the JH1 kinase domain, leading to the loss of key regulatory motifs including the DFG motif (Asp994–Phe996–Gly997), activation loop tyrosines (Tyr1007 and Tyr1008), and portions of the C-lobe required for regulatory inhibition and substrate specificity. Despite this, the truncated kinase retains the ATP-binding VAIK (Lys882) and HRD (His974–Asp976) motifs, and the core kinase cavity, suggesting retained catalytic potential. Structural comparison showed a more compact and potentially more stable conformation, possibly due to loss of flexible regulatory regions. Notably, the absence of the activation loop may result in constitutive activity, broader substrate specificity, and reduced sensitivity to competitive inhibitors.

Her early peripheral erythroid progenitors (BFU-Es) grew independently of EPO (hallmark of polycythemia vera (PV)) comparable to two *JAK2* V617F positive PV controls and one *JAK2* V617F positive essential thrombocythemia control. In an *in vitro* liquid erythroid expansion of mononuclear cells differentiated into erythroid progenitors (PMID:17976518), she had increased proportion of $CD71^+$ and $CD235a^+$ erythroid cells, compared to controls, but comparable to *JAK2* V617F-positive cells from PV, suggesting accelerated differentiation of *JAK2* R989fs mutant erythroid cells. *JAK2* R989fs erythroid progenitors had greater proliferative capacity than PV *JAK2* V617F-positive cells, while apoptosis rates remained unaltered. In *in vitro* erythroid expansion, phospho-STAT5 (p-STAT5) levels were elevated on day 16 of erythroid expansion although not surpassing the levels observed in the *JAK2* V617F-positive control. She achieved complete hematologic remission with ropeginterferon alpha (100 μg administered every two weeks), and p-STAT5 levels in her erythroid cells were normalized.

We report that the novel germline *JAK2* R989fs truncated variant encodes a constitutively active JAK2 kinase that enhances *in vitro* proliferation and differentiation of erythroid progenitors, with increased

P34

DESIGNING AND TESTING A CML QUALITY OF LIFE TRACKER, A DIGITAL SOLUTION TO FACILITATE EFFECTIVE AND CONSISTENT COMMUNICATION BETWEEN PATIENTS WITH CML AND HEALTHCARE PROVIDERS REGARDING THE IMPACT ON THEIR DAILY LIFE

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Background: Maintaining good quality of life (QOL) is an important treatment goal for patients with chronic myeloid leukemia (CML). Patients who report better QOL tend to have better adherence and overall outcomes. Encouraging patients to routinely assess their day-to-day QOL may foster better communication between patients and healthcare providers, help tailor treatment plans to individual needs, and improve patient well-being and long-term outcomes. We describe the design of the CML Quality of Life Tracker that will empower patients to be actively involved in managing their life with CML.

Objectives: Develop the design for an intuitive digital QOL tracker tool using validated QOL questionnaires to enable patients with CML to track the impact on their daily life.

Methods: A multi-step methodology was employed. A literature search was conducted to identify available questionnaires (general, oncology-specific, CML-specific) assessing symptom burden and treatment-related adverse events (AEs). Questionnaires were streamlined based on consultation with physicians (n=4) and CML patient advocates (n=3), and frequency of citation in the past 1 and 5 years. Questions from each were categorized into themes; these informed the tool development.

Results: Thirty-two standard patient-reported outcomes measures QOL questionnaires were identified as potential sources for the tracker and evaluated for their ability to assess disease symptom burden; 6 were excluded based on specific criteria (measuring side effects of treatments/ treatment pathways, measuring AEs [using CTCAE] in clinical trials, measuring adherence to pain medication, or focusing on conditions other than CML). A total of 767 questions consolidated from 26 questionnaires were categorized into themes and reduced to 402 after removing duplicates and questions related to safety, therapy, and AE-reporting. Questions were categorized into overarching themes, in consultation with the team of physicians and patient advocates. The 7 themes were: day-to-day routine, family/social life, hobbies/leisure activities, work, cognitive functions , sexual health/activity, and mental well-being.

Three main steps were proposed for the tracker. In step 1, patients will be asked if each of the 7 leading themes has had a positive, neutral, or negative impact on their day-to-day life and if they would like to focus on this in their next doctor's appointment. In step 2, they will be prompted to select a card that correlates to where they have experienced symptoms which have had the greatest negative impact on their QOL. In step 3, based on their selection in step 2, they will be presented with a tailored list of relevant symptoms derived from EORTC QLQ-C30 and EORTC QLQ-CML24 (and additional cardiovascular and sexual health symptoms) and asked to select all that they have experienced (**Table**). Patients may opt out after step 1 or 2. Upon

completion, patients can access summary reports of their responses to review with clinicians and collaboratively explore potential solutions.

Conclusions:
The CML Quality of Life Tracker is a digital solution that patients living with CML can quickly complete on a regular basis to record the impact on their day-to-day QOL. This tracker may empower patients to discuss daily QOL challenges with healthcare providers, supporting informed decisions and better outcomes.

Table: Body part/impacted QOL and the list of associated symptoms for each

Head	Stomach and digestion	Muscles and bones	Mental well-being[a]	Sexual health and activity	Whole body
Dry mouth	Lack of appetite	Bone pain	Felt tense	Reduced libido	Weight changes
Hair loss	Nausea	Joints pain	Felt worried	Irregular menstrual periods	Skin problems
Headaches	Vomiting	Muscles pain	Felt irritable	Difficulty getting an erection or ejaculating	Sweating
Eye problems	Abdominal pain/cramps	Muscles cramps	Felt depressed	Impact on fertility	Swelling in certain body parts
Pain	Stomach pain	Pain that affected your daily activity	Emotional ups and downs		Shortness of breath
Difficulty concentrating	Constipation		Worries about future health		Whole body pain
Difficulty remembering	Diarrhea		Worries about getting an infection		Weakness
	Frequent urination				Fatigue
					Drowsiness
					Need for rest
					Trouble sleeping
					Dissatisfaction with body due to disease or treatment
					Cardiovascular issues

[a] By capturing psychosocial challenges, this tool can facilitate timely referrals to supportive services such as psychiatry, social work, or peer support groups.

P35

EPCORITAMAB WITH RITUXIMAB + LENALIDOMIDE (R2) AND EPCORITAMAB MAINTENANCE DELIVER DEEP AND DURABLE REMISSIONS IN PREVIOUSLY UNTREATED (1L) FOLLICULAR LYMPHOMA (FL): 3-YEAR OUTCOMES FROM EPCORE NHL-2 ARMS 6 AND 7

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Introduction: Most patients (pts) with newly diagnosed FL receive chemotherapy (chemo)-containing regimens. While R² offers a chemo-free option in the 1L setting, it failed to improve results over chemoimmunotherapy. Thus, newer chemo-free options that provide more durable responses and improve long-term outcomes are needed. Epcoritamab (epcor) is a CD3×CD20 bispecific antibody approved as monotherapy for relapsed/refractory FL after ≥2 prior lines of treatment (tx) based on the results of the EPCORE® NHL-1 trial. In the phase 1b/2 EPCORE NHL-2 trial (NCT04663347), tx with fixed-duration epcor + R² in 1L FL (Arm 6) or epcor monotherapy maintenance post-standard of care (SOC) induction (Arm 7) resulted in sustained responses with manageable safety. We report updated results with median follow-up (mFU) of ~3 years in both arms.

Methods: In Arm 6, pts with CD20+ FL grade (Gr) 1–3A received SC epcor 48 mg (QW, cycles [C]1–2; Q4W thereafter for ≤2 years) with IV rituximab 375 mg/m² (QW, C1; Q4W, C2–6) and oral lenalidomide 20 mg (QD, days 1–21, C1–12). In Arm 7, pts in complete (CR) or partial (PR) response after 1–2 prior lines of SOC received epcor 48 mg maintenance monotherapy (QW, C1 [28 days]; Q8W, C2–13 [56-day cycles]) for up to 2 years. Primary endpoints were overall response rate (ORR) per Lugano criteria (Arm 6) and safety (Arm 7).

Results: At data cutoff (DCO; Apr 9, 2025), 41 pts received epcor+R² (Arm 6) and 19 epcor monotherapy maintenance (Arm 7). In Arm 6, median age was 57 years, 90% had Ann Arbor stage III–IV, 39% had FLIPI score 3–5, and 32% had bulky disease ≥7 cm. At DCO, 56% completed tx; most common reason for discontinuation (d/c) was AEs (n=14), generally after ≥6 tx Cs (8/14). With a mFU of 35.9 months, best ORR and CR rates were 95% and 88%. Median duration of response (mDOR), duration of CR (DOCR), PFS, and OS were not reached (NR). 33-month estimated DOR and DOCR were 89% and 93%; 36-month estimated PFS and OS were 86% and 88%. Of 36 pts in CR, 10 d/c tx for reasons other than progressive disease or death, had response measurement post d/c, and had CR at end of tx (EOT; median tx duration:

13.2 months). With a mFU for DOCR of 20.0 months post-tx, CR was maintained in 9 of these 10 pts (90%) at DCO. Of 23 pts who completed tx per protocol, 21 had CR at EOT and 20 maintained CR at DCO. With mFU for DOCR of 12.5 months post-tx, median DOCR was NR. All pts evaluable for minimal residual disease (MRD; n=26) were MRD-negative (10^{-6}) by clonoSEQ. With ~13 months additional FU, no new safety signals were reported (Falchi L, et al. *HemaSphere* 2024;8:P1146) and treatment-emergent AEs (TEAEs), including Gr≥3 TEAEs, declined over 2 years of tx. Two new TEAEs (both pneumonia) led to d/c. Of 13 pts (32%) with serious infections, 7 had events in the first 24 weeks of tx. Since the prior DCO, 1 patient experienced a new Gr3 TEAE of COVID-19. Rates of neutropenia were highest during the first 24 weeks of the combination tx. No febrile neutropenia was reported. One additional death, non-TEAE related, was reported. In Arm 7, median age was 56 years and most pts (84%) received epcor maintenance after 1 prior line of therapy; median time from last SOC dose to 1st epcor dose was 2.8 months. Eleven (58%) pts in CR and 8 (42%) in PR started epcor monotherapy; all 8 PR pts converted to CR during tx. Median DOR, DOCR, and OS were NR; 30-month estimated DOCR and OS were 88% and 84%. All 10 pts who completed tx per protocol had CR at EOT. With mFU of 12.2 months post-tx, all 10 remained in CR at DCO, and median PFS and OS were NR. Safety in Arm 7 was largely consistent with the prior report. Of 8 serious infection events, 6 occurred in the first year of epcor maintenance and none were reported after completion of epcor maintenance (week 96). After the last DCO, 1 patient experienced a new Gr3 TEAE of COVID-19. Neutropenias primarily occurred in the first 48 weeks of tx and rates decreased thereafter; no febrile neutropenia was reported. One additional death, non-TEAE-related, was reported.

Conclusions: Fixed-duration epcor + R² induced deep, durable remissions in 1L FL with 88% CR rate and 33-month estimated DOCR of 93%. Epcor monotherapy as maintenance further deepened and consolidated tx response post SOC induction with no relapses and manageable safety. The favorable efficacy and manageable long-term safety of these regimens reinforces the versatility of fixed-duration epcor in FL.

P36

FIXED-DURATION EPCORITAMAB + R-CHOP IN PATIENTS WITH NEWLY DIAGNOSED DLBCL AND HIGH IPI SCORES (3–5) LED TO SUSTAINED REMISSIONS AND DISEASE-FREE SURVIVAL BEYOND 3 YEARS: RESULTS FROM THE EPCORE NHL-2 TRIAL

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Introduction: Rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) is a standard of care (SOC) for patients with untreated (1L) diffuse large B-cell lymphoma (DLBCL). While R-CHOP is considered

curative, approximately one-third of patients relapse, typically within the first 2 years after an initial response. Patients with International Prognostic Index (IPI) scores ≥3 have poorer outcomes, with complete response (CR) rates of ~50% and 5-year PFS rates of ~46%–58% (Ruppert AS, et al. *Blood* 2020;135:2041–2048). Epcoritamab, a CD3×CD20 bispecific antibody, has demonstrated high response rates as monotherapy or in combination with SOC for patients with 1L DLBCL, regardless of age and fitness. Here, we report efficacy and safety from a 3-year follow-up of patients who received epcoritamab + R-CHOP in the EPCORE® NHL-2 trial (NCT04663347).

Methods: Patients with 1L CD20+ DLBCL and IPI score 3–5 received subcutaneous epcoritamab (0.16 mg on cycle 1 [C1] day 1 [D1], 0.8 mg on C1D8, and 48 mg thereafter; QW in C1–4; Q3W in C5–6) + R-CHOP for 6 Cs (21 days each), followed by epcoritamab monotherapy Q4W in 28-day Cs for a total of 1 year of treatment. The primary endpoint was investigator-assessed overall response rate (ORR) per Lugano criteria. Key secondary endpoints included CR rate, duration of response (DOR), duration of CR, PFS, OS, and safety/tolerability. Minimal residual disease (MRD) negativity was also assessed as a secondary endpoint using the exploratory AVENIO Oncology circulating tumor DNA (ctDNA) method (cutoff of <1 mutant molecule per mL).

Results: As of data cutoff (April 9, 2025), 47 patients had received epcoritamab + R-CHOP. Median age was 64 years (range, 19–82), 81% of patients had de novo DLBCL, 34% had bulky disease (≥10 cm), and 6/31 assessed patients had double-/triple-hit DLBCL per central lab. At screening, 57% of patients had IPI score 3 and 38% had IPI score 4–5. After a median follow-up of 38.8 months (range, 0.8–44.3), the ORR was 98% and CR rate was 85%. An estimated 67% of all responses and 75% of CRs were ongoing at 33 months. High CR rates were observed regardless of IPI score (IPI 3, 86% vs IPI 4–5, 83%). At 33 months, an estimated 80% of patients remained progression-free and 87% were alive; survival outcomes were consistent regardless of IPI score (3 vs 4–5). Efficacy outcomes were also similar across subgroups based on age (≤60 vs >60 years), tumor size (<10 vs ≥10 cm), or cell of origin (germinal center B cell [GCB] vs non-GCB). By C3D1, 86% (25/29) of MRD-evaluable patients were MRD negative. In patients with longitudinal paired samples, the reduction in ctDNA levels was sustained through C6D1, with a subset of patients showing further decreases. Rapid and sustained reductions in ctDNA were observed regardless of high-risk clinical features, including tumor size (<10 vs ≥10 cm) and IPI score (3 vs 4–5). Additional longitudinal MRD data will be presented. The majority (94%) of patients completed 6 Cs of R-CHOP, and median duration of epcoritamab was 11.5 months (range, 0.6–13.2). Most (32/47; 68%) patients completed treatment as planned. Reasons that patients did not complete treatment included AEs (11%), progressive disease (9%), withdrawal by patient (6%), COVID-19 control measures (2%), and other (4%; physician decision, incomplete response). At the end of treatment, 30/32 patients who completed treatment as planned had a CR and 1 had a partial response. With a median follow-up for DOR of 25.3 months after treatment, 87% (27/31) of those patients maintained their response. Safety was consistent with prior reports (Falchi L, et al. *Blood* 2024;144[Suppl 1]:581). Serious and grade ≥3 infections primarily occurred in the first 6 months of treatment and then decreased. No new serious infections were reported in the post-treatment period. No new grade 5 AEs were reported.

Conclusions: Fixed-duration epcoritamab + R-CHOP resulted in deep and durable remissions lasting >3 years in most patients with 1L DLBCL and high IPI scores. These findings suggest a long-term survival benefit and potential for high cure rates in this high-risk population. The long-term safety profile was consistent with previous data. Compared with R-CHOP alone, these results are favorable and support further investigation in the ongoing phase 3 EPCORE DLBCL-2 study (NCT05578976) of epcoritamab + R-CHOP versus R-CHOP in newly diagnosed patients with DLBCL.

P37

EPCORITAMAB + R-MINI-CHOP RESULTS IN 2-YEAR REMISSIONS AND HIGH MRD NEGATIVITY RATES IN ELDERLY PATIENTS WITH NEWLY DIAGNOSED DLBCL: RESULTS FROM THE EPCORE NHL-2 TRIAL

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Introduction: Dose-attenuated rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (R-mini-CHOP) is a standard of care (SOC) for patients with newly diagnosed (1L) diffuse large B-cell lymphoma (DLBCL) unable to receive full-dose R-CHOP due to age or comorbidities. However, outcomes following R-mini-CHOP remain suboptimal, with overall response rates (ORR) of 60%–80%, complete response (CR) rates of 40%–60%, 2-year PFS of 51%, and 2-year OS of 60% (Al-Sarayfi D, et al. *Am J Hematol* 2024;99:216–222). In the EPCORE® NHL-2 trial (phase 1b/2; NCT04663347), epcoritamab, a CD3×CD20 bispecific antibody, combined with R-mini-CHOP demonstrated promising efficacy and manageable safety in elderly patients with 1L DLBCL ineligible for full-dose R-CHOP (Leslie LA, et al. *Blood* 2024;144[Suppl 1]:3106). Here, we present results from this cohort after 2 years of follow-up.

Methods: Adults with newly diagnosed CD20+ DLBCL who were ineligible for full-dose R-CHOP due to age ≥75 years or age ≥65 years with comorbidities, received fixed-duration subcutaneous epcoritamab 48 mg (QW, cycle(s) [C] 1–2 [21 d each]; Q3W, C3–6 [21 d each]; Q4W, C7–8 [28 d each]; with step-up dosing in C1 [0.16 mg on C1 day (D) 1 and 0.8 mg on C1D8]) + R-mini-CHOP (Q3W, C1–6). The primary endpoint was ORR per Lugano criteria. Minimal residual disease (MRD) negativity was assessed as a secondary endpoint using the exploratory AVENIO Oncology circulating tumor DNA (ctDNA) method; MRD negativity was defined as <1 mutant molecule per mL.

Results: As of April 9, 2025, 28 patients had received epcoritamab + R-mini-CHOP. Median age was 81 years (range, 74–90), 89% of patients had de novo DLBCL, 46% had germinal center B-cell (GCB) and 39% had

non-GCB, 68% had International Prognostic Index (IPI) scores 3–5, 64% had elevated LDH, and 39% had bulky disease (≥7 cm). Most patients (22/28; 79%) completed treatment as planned, and median relative dose intensity of R-mini-CHOP was ≥94%. With a median follow-up of 28.0 months (range, 2.8–34.5), the ORR was 89% and the CR rate was 86%. Median duration of response and duration of CR were not reached (NR) at data cutoff. At 2 years, an estimated 78% of all responses and 78% of CRs were maintained. Median PFS and median OS were NR. The estimated 2-year PFS and OS rates were 76% and 82%, respectively, at data cutoff. Twenty of 22 patients who completed treatment had a CR at end of treatment; with a median follow-up for duration of CR of 22.6 months after treatment end, 18/20 (90%) remained in CR at data cutoff. MRD negativity was reported for 20 (95%) of the 21 patients evaluable for MRD at any time; 16/20 were MRD negative by C3D1. Among patients who were MRD negative at C3D1 and had paired longitudinal samples, 86% (12/14) sustained their MRD negativity through C6D1. High rates of MRD negativity were observed among high-risk subgroups, such as patients with bulky disease (8/9, 89%) and IPI score 3–5 (14/15, 93%). Safety was consistent with prior reports (Leslie LA, et al. *Blood* 2024;144 [Suppl 1]:3106). Eight (29%) patients experienced a grade ≥3 infection, with the majority (in 6/8 patients) occurring within the first 6 cycles of treatment. Treatment-emergent AEs led to epcoritamab discontinuation in 3 (11%) patients, including grade 2 rhinitis, grade 2 cytokine release syndrome, and grade 5 confusional state and cytomegalovirus infection reactivation in a patient aged 90 years also diagnosed with acute cerebrovascular accident.

Conclusions: Fixed-duration epcoritamab + R-mini-CHOP led to high ORR and CR rates, rapid and sustained MRD negativity, and durable remissions in elderly patients with newly diagnosed DLBCL ineligible for full-dose R-CHOP. Despite the elderly population, outcomes compare favorably with historical outcomes of R-mini-CHOP alone. These results, along with those from other arms of the trial, support combinations of epcoritamab with SOC across a range of disease settings and patient populations.

P38

FIXED-DURATION EPCORITAMAB MONOTHERAPY INDUCES HIGH RESPONSE AND MRD-NEGATIVITY RATES IN ELDERLY PATIENTS WITH NEWLY DIAGNOSED LARGE B-CELL LYMPHOMA (LBCL) AND COMORBIDITIES: RESULTS FROM EPCORE DLBCL-3

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Introduction: A proportion of newly diagnosed (1L) patients (pts) with LBCL are ineligible for standard curative chemotherapy with full-dose R-CHOP due to age or comorbidities. Alternative regimens (eg, R-mini-CHOP) and other investigational single-agent bispecifics have shown limited efficacy in the 1L setting. EPCORE® DLBCL-3 (NCT05660967) is a 2-stage, phase 2 trial evaluating fixed-duration epcoritamab (epcor), a CD3×CD20 bispecific antibody, as monotherapy or combined with lenalidomide in elderly pts with 1L LBCL and comorbidities. Based on promising efficacy and manageable safety in stage 1, epcor monotherapy was selected for further enrollment in stage 2. Here we report efficacy and safety of epcor monotherapy, including first results from stage 2.

Methods: Pts with 1L, CD20+ LBCL ineligible for anthracycline-based chemotherapy due to age ≥80 y or ≥75 y with comorbidities received SC epcor monotherapy: step-up dosing (0.16 mg on cycle 1 day 1 [C1D1]; 0.8 mg on C1D8) followed by 48 mg QW in C1–3 and Q4W in C4–12, for up to 1 y. Cytokine release syndrome (CRS) prophylaxis was mandatory during C1 and continued as needed; adequate hydration was recommended for all Cs. Primary endpoint was complete response (CR) rate per investigator (Lugano criteria). Minimal residual disease (MRD) negativity was assessed as a secondary endpoint in responders by circulating tumor DNA using the exploratory AVENIO assay (cutoff, <1 mutant molecule per mL).

Results: As of May 20, 2025, 66 pts received epcor monotherapy across stage 1 (n=44) and stage 2 (n=22). Median age was 82.5 y (range, 76–95); 82% were ≥80 y. 23% of pts had ECOG PS 2, 64% had significant renal impairment, 62% had Ann Arbor stage IV, 64% had IPI score ≥3, and 33% had bulky disease (≥7 cm). All pts had ≥1 comorbidity, including vascular disorders (80%), cardiac disorders (41%), nervous system disorders (32%), psychiatric disorders (20%), and diabetes/glucose impairment (24%). Median follow-up was 14.9 mo (95% CI, 11.8–16.7). At data cutoff, 30% of pts had completed treatment (tx) per protocol. Tx was ongoing in 21%, and 48% had discontinued, mainly due to disease progression (23%) or AEs (14%). Overall response rate (ORR) was 70% in the response-evaluable population (n=53), and 58% of pts had a CR. Median time to response was 1.5 mo (range, 1.2–3.4), and median time to CR was 2.2 mo (range, 1.2–5.4); 8 pts with a partial response or stable disease at 1st assessment achieved a CR at subsequent assessments. Median duration of response and duration of CR were not reached (NR). An estimated 72% of all responses and 79% of CRs were ongoing at 12 mo. In the overall population (N=66), median PFS was 13.0 mo (95% CI, 5.4–NR) and median OS was NR (95% CI, 13.0–NR). An estimated 54% of pts were progression free and 65% were alive at 12 mo. A majority (88% [23/26]) of MRD-evaluable responders were MRD negative at any time point. MRD negativity was reported early by C3D1 in most pts who attained a response and was sustained through C12D1 in a majority of those with available longitudinal samples. Overall MRD negativity was associated with prolonged PFS. Tx-emergent AEs (TEAEs; any grade) occurred in 94% of pts, with CRS (70%), diarrhea (23%), and fatigue (21%) being most frequent (≥20%); 61% had a grade (G) ≥3 TEAE. CRS events were primarily low G (G1: 38%; G2: 27%; G3: 5%), with most (94%) occurring in C1; 97% of cases resolved by data cutoff. ICANS occurred in 18% of pts (G1: 8%; G2: 8%; G3: 3%); 10/12 cases resolved by data cutoff. Neutropenia was reported in 14%. There were no cases of febrile neutropenia or clinical tumor lysis syndrome. 59% of pts had an infection of any G, and 18% had a G≥3 infection. Two additional G5 TEAEs (pneumonia, death) occurred since the previous disclosure.

Conclusions: Fixed-duration epcor monotherapy demonstrated robust efficacy (ORR, 70%), with early, deep, and durable responses in elderly pts

with 1L LBCL and comorbidities, a population with significant unmet need and poor outcomes. Safety was manageable and consistent with previous reports of epcor monotherapy in this population. Epcor is a promising single-agent, chemotherapy-free tx for pts who are not candidates for anthracycline-based chemotherapy. These findings, together with those from other trials (EPCORE NHL-2; NCT04663347) show that epcor, as monotherapy or combined with other tx, can be a favorable option for pts with 1L DLBCL across a broad range of ages and fitness levels.

P39

EPCORITAMAB + GEMOX ACHIEVES DURABLE >2-YEAR REMISSIONS IN RELAPSED/REFRACTORY (R/R) 2L+ DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL): LONG-TERM DATA REINFORCE CLINICAL POTENTIAL OF THE REGIMEN ACROSS A DIVERSE PATIENT POPULATION

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Introduction: Patients (pts) with R/R DLBCL ineligible for chimeric antigen receptor T cell (CAR T) therapy or autologous stem cell transplant (ASCT) have limited treatment (tx) options and poor outcomes. In R/R DLBCL, standard salvage therapy with R-gemcitabine plus oxaliplatin (R-GemOx) yielded a complete response (CR) rate of 25%, overall response rate (ORR) of 41%, median progression-free survival (mPFS) of 3.6 mo, and median overall survival (mOS) of 12.9 mo (Abramson JS, et al. *Lancet* 2024;404:1940–1954). In the EPCORE® NHL-2 trial (NCT04663347), epcoritamab (epcor), a CD3×CD20 bispecific antibody, combined with GemOx led to high response rates in pts with R/R DLBCL ineligible for ASCT. Here, we report long-term durability data from this cohort after >2 y of follow-up.

Methods: Pts with CD20⁺ R/R LBCL ineligible for ASCT due to advanced age, performance status, or lack of response to prior ASCT received subcutaneous epcor (step-up doses: 0.16 mg, 0.8 mg; 48 mg thereafter) QW in cycles (C) 1–3, Q2W in C4–9, and Q4W in ≥C10. GemOx (gemcitabine 1000 mg/m², oxaliplatin 100 mg/m²) was given Q2W for 4 C (8 total doses). The primary endpoint was ORR by Lugano criteria. Minimal residual disease (MRD) negativity was assessed as a secondary endpoint, using the exploratory AVENIO Oncology circulating tumor DNA (ctDNA) method (cutoff: <1 mutant molecule per mL). Data cutoff was April 9, 2025; efficacy assessments were per investigator.

Results: In total, 103 pts were treated. Median age was 72 y (range, 20–87). The pt population was 76% White, 4% Black/African American, and 4% Asian; pts were enrolled across the United States (US; 27%), Europe (72%), and Australia (1%). Pts had a median of 2 prior lines of tx (pLOT; range, 1–6); 37% had 1 pLOT; 61% had Ann Arbor stage IV, 7/66 (11%) assessed pts had double-hit/triple-hit disease, 33% had bulky disease (≥7 cm), 52% had primary refractory disease, 70% were refractory to last therapy, 10% had prior ASCT, and 28% had prior CAR T therapy. ORR was 81%, and 60% of pts had a CR. After a median follow-up of 27.9 mo (range, 1.0+ to 47.9), epcor + GemOx led to mPFS of 16.3 mo (95% CI, 8.3–not reached [NR]), and mOS of 28.2 mo (95% CI, 11.7–NR). At 2 y, an estimated 48% of all pts were progression-free and 53% were alive. Responses were durable, with a median duration of response of 27.3 mo (95% CI, 11.7–NR), and a median duration of complete response (mDOCR) of 40.7 mo (95% CI, 40.7–NR). An estimated 67% of complete responders remained in CR at 2 y. Among pts with 1 pLOT, CR rate was 74% and mDOCR was NR. Pts with >1 pLOT (some received up to 6 pLOTs) experienced clinically meaningful responses (CR rate: 52%), with mDOCR of 40.7 mo (95% CI, 17.5–NR). When analyzed by region, outcomes were consistent in pts from the US and Europe. Overall MRD negativity by C7 day 1 (D1), was observed in 71% (44/62) of MRD-evaluable pts. Deep and sustained MRD negativity through C7D1 was observed across difficult-to-treat subgroups. At data cutoff, 24% (25/103) of pts remained on tx. There were 16 pts who discontinued tx while in CR for reasons other than progressive disease or death; 12 (75%) of these pts remained in CR (median time since end of tx, 8.8 mo [range, 0.5–26.8]) at data cutoff. Safety was consistent with previous reports (Brody JD, et al. *Blood* 2025;145:1621–1631). Incidence of serious infections was highest (23%) in the first 24 weeks of tx, as expected, given known toxicities of the individual components, then decreased and remained consistent over time. With 14.7 mo more follow-up since the last report, 4 additional pts had an infection, and 4 additional pts experienced grade 5 treatment-emergent adverse events (pancreatic adenocarcinoma, lung infection, subacute sclerosing panencephalitis, COVID-19).

Conclusions: Epcor + GemOx demonstrated sustained remissions of >2 y, prolonged PFS and OS, and deep MRD negativity in challenging-to-treat 2L+ R/R DLBCL; these results are widely applicable to a diverse pt population across the US and Europe. With longer follow-up, the safety profile remained consistent with previous reports. These findings support the combinability of epcor with standard-of-care chemotherapy and offer an effective option for ASCT-ineligible pts, a population with otherwise limited tx choices.

P40

AGE-STRATIFIED PROGNOSTIC PERFORMANCE OF PATIENT- VS DISEASE-RELATED IPI FACTORS IN LARGE B-CELL LYMPHOMA

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Introduction

The International Prognostic Index (IPI, *NEJM* , 1993) has guided risk stratification in large B-cell lymphoma (LBCL) for over three decades. Its simplicity and clinical utility have driven its widespread adoption, even in the era of precision medicine. However, the prognostic value of the IPI and its components may not be uniform across the age spectrum of LBCL patients. We evaluated the contemporary prognostic performance of the IPI in a large international cohort,

focusing on age-stratified performance and the relative contributions of its individual components, grouped as patient- and disease-related factors.

Methods

We harmonized and pooled data from 6,941 patients with newly diagnosed systemic LBCL treated with R-CHOP-like regimens (R-CHOP, R-miniCHOP, DA-EPOCH-R, R-CHOEP, R-CHOP+X) with complete IPI data from a national registry and two prospective cohorts: NiHiL (Czech Republic, n=4,590; 2010–2023), LEO (USA, n=1,789; 2015–2020), and MER (USA, n=562; 2010–2015). IPI components included age, ECOG performance status (PS) as patient-related, and Ann Arbor clinical stage, serum lactate dehydrogenase (LDH), and extranodal (EN) involvement as disease-related factors. Prognostic performance was evaluated using multivariable Cox regression models and C-statistics. Age-stratified analysis was performed for: ≤40 years (7%; n=492), 41–60 years (28%; n=1,909), 61–80 years (59%; n=4,080), and >80 years (7%; n=460). The primary endpoint was overall survival (OS).

Results

In the pooled cohort, the median age was 66 years (range 18–95). Compared to the original IPI cohort (n=3,273), our cohort was older (age >60 years: 66% vs 41%) but otherwise comparable: ECOG PS 2–4 (25% vs 24%), clinical stage III–IV (63% vs 66%), elevated LDH (60% vs 52%), and >1 EN involvement (32% vs 30%).

In multivariable analysis, age (≤60 vs >60 years) was the strongest predictor of OS (HR=2.78; vs HR=1.96 in original IPI report), followed by ECOG PS (HR=2.07; vs 1.80), clinical stage (HR=1.50; vs 1.47), LDH (HR=1.41; vs 1.85, all $P<0.01$); EN involvement was not significant ($P=0.50$, HR=1.03 vs 1.48). Risk group distribution shifted from the original IPI: 28% were low-risk (0–1 factor; vs 35%), 23% low-intermediate (2; vs 27%), 24% high-intermediate (3; vs 22%), and 25% high-risk (4–5; vs 16%). Corresponding 2-year OS rates were 95%, 86%, 78%, and 63%, superior to those of the original IPI cohort (87%, 67%, 55%, 44%).

Age was associated with an increasing risk of mortality, with a more pronounced rise beyond 60 years. In a piecewise Cox model, the HR per year was 1.04 for ages ≤60 (linear), 1.05 for 61–69 (accelerated), and 1.06 for ≥70 years (exponential, all $P<0.01$). ECOG PS retained prognostic significance across age groups: HRs were 1.87 ($P=0.06$) for ≤40 years, 1.63 ($P<0.01$) for 41–60, 2.22 ($P<0.01$) for 61–80, and 1.43 ($P<0.01$) for >80 years. The strength of other IPI components declined with age. Clinical stage showed decreasing impact: HR 2.08 ($P=0.09$), 1.97 ($P<0.01$), 1.49 ($P<0.01$), and 1.31 ($P=0.05$). LDH was significant only up to age 80 years: HRs were 2.07 ($P=0.04$), 1.96 ($P<0.01$), 1.32 ($P<0.01$), and 1.21 ($P=0.13$). EN involvement was non-significant across all groups: HRs were 1.37 ($P=0.34$), 1.26 ($P=0.05$), 0.98 ($P=0.74$), and 1.06 ($P=0.67$). The IPI C-statistics was 0.676 overall and declined with age: ≤40 years (0.708), 41–60 years (0.683), 61–80 years (0.641), and >80 years (0.598).

We next stratified the IPI factors into patient- (age, ECOG PS) and disease-related (clinical stage, LDH, EN involvement). In patients ≤40 years, disease-related factors outperformed patient-related (C-index 0.693 vs 0.602), as in the 41–60 group (C-index 0.677 vs 0.589). In patients aged 61–80, predictive values were comparable (0.613 vs 0.622). In >80-year-olds, both declined substantially (0.580 vs 0.568).

Conclusion

The IPI remains a valuable prognostic tool in LBCL, particularly among younger patients. However, its overall predictive accuracy declines with age. In patients ≤60 years old, prognosis is primarily driven by disease-related factors, suggesting a role for molecular classifiers to enhance risk stratification. In older patients, incorporation of additional patient-level factors, such as comorbidities and nutritional status could be considered. These findings underscore the need for more individualized prognostic tools for LBCL patients.

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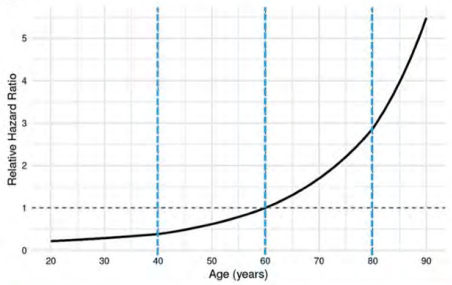
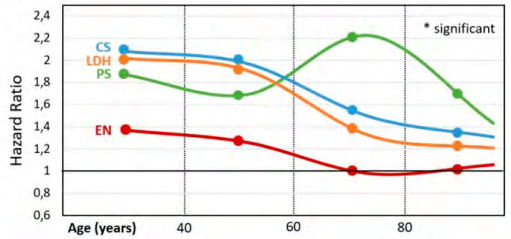
Figure. Cox proportional hazards model with piecewise linear splines for age predicting OS (standardized age 60 years; **A**), and age-stratified multivariate Cox proportional hazards models (**B**) predicting OS.

A.



Age	≤ 40 years	41–60 years	61–80 years	> 80 years
No.	492	1,909	4,080	460
HR	1.03	1.04	1.05	1.07
95% CI	0.99–1.07	1.03–1.06	1.05–1.06	1.04–1.10
P value	0.14	< 0.01	< 0.01	< 0.01

B.



Category	≤ 40 years	41–60 years	61–80 years	> 80 years
At risk	492	1,909	4,080	460
PS ≥2 vs <2	**65 (13%)** vs 427	**365 (19%)** vs 1,544	**1,144 (28%)** vs 2,936	**160 (35%)** vs 300
CS III-IV vs I-II	**286 (58%)** vs 206	**1,140 (60%)** vs 769	**2,661 (65%)** vs 1,419	**269 (58%)** vs 191
LDH el. vs n.	**265 (54%)** vs 227	**1,055 (55%)** vs 854	**2,547 (62%)** vs 1,533	**298 (65%)** vs 162
EN ≥2 vs <2	**152 (31%)** vs 340	**537 (28%)** vs 1,372	**1,374 (34%)** vs 2,706	**144 (31%)** vs 316
HR (95% CI)				
PS ≥2 vs <2	1.81 (0.97–3.57)	**1.63** (1.30–2.08)*	**2.22** (2.00–2.50)*	**1.43** (1.14–1.82)*
CS III-IV vs I-II	2.08 (0.90–4.76)	**1.97** (1.47–2.63)*	**1.49** (1.32–1.69)*	1.31 (1.00–1.72)
LDH el. vs n.	**2.07** (1.02–4.17)*	**1.96** (1.52–2.56)*	**1.32** (1.18–1.47)*	1.21 (0.94–1.54)
EN ≥2 vs <2	1.37 (0.71–2.63)	1.26 (0.95–1.59)	0.98 (0.88–1.10)	1.06 (0.81–1.39)

P41

TREATMENT PATTERNS IN PATIENTS AGED 80+ YEARS WITH LBCL: PROPENSITY SCORE ANALYSIS REVEALS COMPARABLE OUTCOMES BETWEEN R-CHOP AND R-MINICHOP

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Introduction

Although chemo-free regimens are increasingly used in elderly patients with large B-cell lymphoma (LBCL), most still receive conventional chemoimmunotherapy. Patients up to 75–80 years old are usually treated with full-dose R-CHOP, while 80+ patients receive the attenuated R-miniCHOP regimen as standard of care. Prior studies have shown that in patients aged 65+ years, R-CHOP was associated with improved survival based on propensity score matching (PSM) analysis (Al-Sarayfi, *Am J Hematol*, 2023), though this did not include comorbidity data. Furthermore, no formal studies have focused specifically on 80+ patients. We aimed to perform a PSM comparison of R-CHOP versus R-miniCHOP to better inform treatment decisions in this population.

Methods

We harmonized and pooled data from 237 LBCL patients receiving chemotherapy with rituximab (out of all 263 80+ patients) from the prospective LEO cohort (USA, n=147; 2015–2020) and NiHiL registry (Czech Republic, single center, n=116; 2010–2023). Of them, 205 (86%) receiving anthracycline(A)-based regimens were included. Baseline characteristics, comorbidities, treatments, and outcomes were collected. PSM (1:1, caliper 0.2) was performed to compare R-CHOP and R-miniCHOP using age, ECOG performance status (PS), clinical stage, lactate dehydrogenase (LDH), and major comorbidities (heart disease, history of stroke, and other recent cancer) as matching variables. The endpoints were overall survival (OS), event-free survival (EFS), and lymphoma-specific survival (LSS, defined as death attributed to lymphoma progression).

Results

Among 205 A-treated patients, median age was 83 years (range 80–99); 30% had ECOG PS 2–4, 67% clinical stage III–IV, 34% ≥2 involved extranodal sites, 59% elevated LDH, and 58% had International Prognostic Index (IPI) scores 3–5. Median follow-up was 5.5 years; median OS and EFS were 4.1 and 3.4 years, resp.

A-based regimens comprised full-dose R-CHOP-like (n=79; 39%; R-CHOP n=74, DA-EPOCH-R n=5) and R-miniCHOP (n=126; 61%). Patients receiving R-CHOP-like regimens had more favorable baseline characteristics (median age 82 vs 83 years, P=0.04; ECOG PS 2–4: 16% vs 39%, P<0.01; IPI 3–5: 46% vs 66%, P=0.03) and a lower, though not statistically significant, comorbidity burden (mean 1.14 vs 1.39; heart disease 35% vs 45%, P=0.22; history of stroke 4% vs 6%, P=0.64; other cancer 6% vs 5%, P=0.87). R-CHOP-like regimens vs R-miniCHOP had superior OS (median 6.3 vs 3.4 years; HR=0.61, 95% CI 0.42–0.90; P=0.01) and EFS (median 5.3 vs 2.1 years; HR=0.58, 95% CI 0.40–0.83; P<0.01), with no significant difference in LSS (2-year 89% vs 82%, HR=0.77, 95% CI 0.39–1.54, P=0.46).

To mitigate selection bias, we performed PSM on patients with available baseline characteristics treated with R-CHOP (n=69) and R-miniCHOP (n=121), resulting in 63 matched pairs. Matched cohorts were well balanced on age (median 82 years in both cohorts; P=0.68), ECOG PS 2–4 (17% vs 19%; P=0.82), IPI 3–5 (52% vs 59%; P=0.41), and comorbidity burden (mean 1.10 vs 1.29; P=0.30). The presence of heart disease (40% in both cohorts), history of stroke (5% vs 6%), and cancer (5% vs 6%) were similar. Survival modestly favored R-CHOP, but differences were not significant: median OS 5.3 vs 3.5 years (HR=0.73; 95% CI 0.46–1.15; P=0.17), median EFS 3.8 vs 2.8 years (HR=0.76; 95% CI 0.49–1.18; P=0.23), and 2-year LSS 88% vs 84% (HR=1.09; 95% CI 0.45–2.63; P=0.85). Treatment-related mortality was similar in both cohorts (16%). Among R-miniCHOP patients, survival outcomes did not differ between matched (n=63) and unmatched (n=63) groups (OS P=0.72; EFS P=0.23; LSS P=0.27). Unmatched (n=16) R-CHOP

patients had a trend toward improved survival compared to matched (n=63) individuals (OS *P*=0.10; EFS *P*=0.049; LSS *P*=0.24).

Conclusion

This is the first PSM-based analysis comparing R-CHOP and R-miniCHOP in LBCL patients 80+ years old. Despite inherent limitations of a non-randomized design and limited number of matched patients, our findings – derived from two prospective cohorts – suggest that R-miniCHOP may offer outcomes comparable to R-CHOP, particularly with respect to LSS. While these results support R-miniCHOP as an appropriate frontline option, treatment decisions remain highly individualized. These data may help inform clinical decision-making and provide a basis for designing future trials targeting this population, where R-miniCHOP could serve as a comparator arm.

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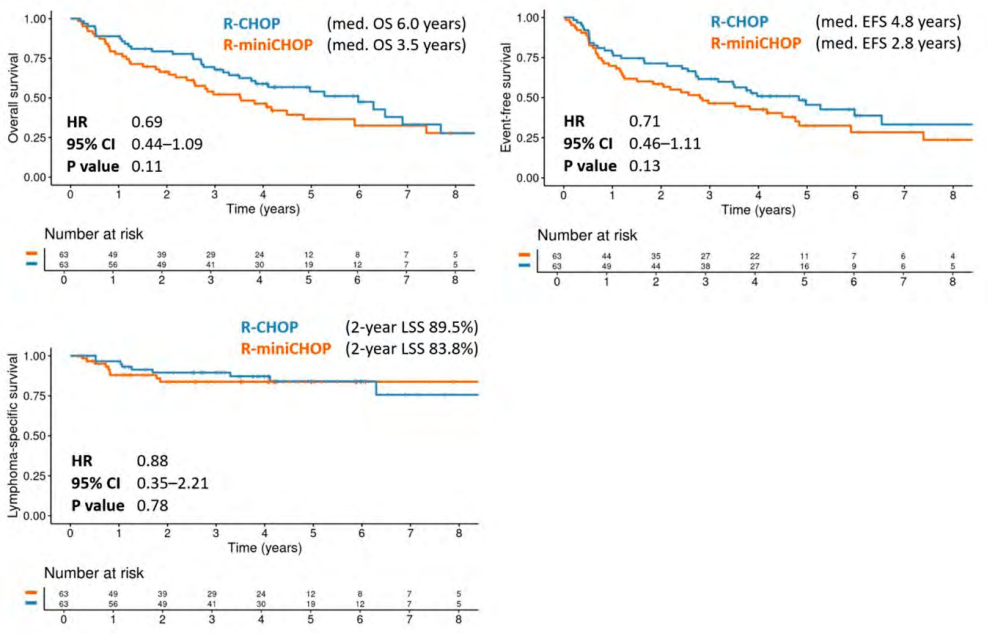
Figure. Survival curves of matched patients receiving R-CHOP vs R-miniCHOP.



P42

PREDICTIVE PERFORMANCE OF THE LEO COMORBIDITY INDEX IN PATIENTS AGED 80+ YEARS WITH LARGE B-CELL LYMPHOMA

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Introduction

The LEO Comorbidity Index (LCI), comprising ten equally weighted comorbidity categories (respiratory, cardiovascular, digestive, hepatic, renal, autoimmune, diabetes, cancer history, HIV, and stroke), has demonstrated prognostic utility in patients with non-Hodgkin lymphomas. However, its predictive accuracy may vary with increasing age, due to age-related rises in comorbidity burden and physiological vulnerability. To investigate these dynamics, we evaluated the age-stratified predictive performance of LCI in patients with large B-cell lymphoma (LBCL), with a focused analysis on individuals aged 80+ years. We also examined which specific comorbidities most strongly influenced overall survival (OS) and developed a simplified, clinically applicable index tailored to this population.

Methods

We first included adults ≥40 years with newly diagnosed LBCL and recorded comorbidities from the LEO registry (USA, 2015–2020; n=1,652) to assess age-stratified predictive performance of LCI using the C-index. Next, we focused on patients 80+ years (n=263), pooled from the LEO cohort (n=147) and NiHiL registry (restricted to General Hospital, Prague, Czech Republic, 2010–2023; n=116). Comorbidities were collected from patient self-reports (LEO) and/or medical records (LEO/NiHiL). Univariate and multivariate Cox models were used. The primary endpoint was OS.

Results

Among the total LBCL population (n=1,652), LCI predictive performance improved by age category with C-index for patients <65 of 0.584, 65–79 years 0.579, and ≥80 years 0.647, indicating stronger discrimination in the 80+ group.

SBORNÍK ABSTRAKTŮ

Subsequent analysis focused on patients aged 80+ years (n=263; median age 83). Within this cohort, 34% had ECOG performance status (PS) 2–4, 66% stage III–IV disease, 59% elevated lactate dehydrogenase, 35% >1 extranodal site involved, and 58% International Prognostic Index (IPI) score of 3–5. A total of 377 comorbidities were reported, averaging 1.43 per patient (1.22 in LEO; 1.61 in NiHiL). LCI distribution was: 0 points in 24% (n=62), 1 point in 33% (n=88), and 2–5 points in 43% (n=113). Higher LCI scores had worse ECOG PS 2–4: 19% (LCI=0), 33% (LCI=1), and 43% (LCI ≥2; *P*=0.01), and inferior OS (median 6.9 years LCI=0, 3.0 years LCI=1, and 2.8 years LCI≥2, resp.; *P*=0.02). Presence of ≥1 comorbidity was associated with shorter OS compared to no comorbidities (HR=1.67, *P*=0.01). The LCI was an independent predictor of OS after adjusting for age (HR=1.18, *P*=0.01, C-index 0.623), IPI (HR=1.16, *P*=0.03, C-index 0.628) as well as for recently proposed SENIOR-IPI (HR=1.14, *P*=0.049, C-index 0.665).

When evaluating individual comorbidities, heart disease (HR=1.54, *P*=0.01), stroke (HR=1.89, *P*=0.03), and recent malignancy (HR=1.99, *P*=0.02) were independent predictors of OS. Based on these findings, we developed a simplified LCI (sLCI) for use in 80+ patients incorporating only the three comorbidities (1 point each). Patients were categorized as sLCI: 0 (49%, n=129), 1 (44%, n=115), and 2–3 points (7%, n=19). sLCI scores 0, 1, 2–3 were associated with ECOG PS 2–4 27%, 37%, and 63%, respectively (*P*=0.01) and inferior OS: median 5.3 years (0), 2.5 years (1), and 1.4 years (2–3; *P*<0.01). sLCI remained an independent and strong predictor of OS after adjusting for age (HR=1.71, *P*<0.01, C-index 0.636), IPI (HR=1.66, *P*<0.01, C-index 0.635), and SENIOR-IPI (HR=1.60, *P*<0.04, C-index 0.659). The results were consistent in both NiHiL and LEO cohorts and remained significant among patients treated with anthracyclines (sLCI adjusted for age: HR=1.58, *P*<0.01, C-index 0.607; for IPI: HR=1.53, *P*=0.01, C-index 0.623; for SENIOR-IPI: HR=1.51, *P*=0.01, C-index 0.633).

Conclusion

Comorbidity burden becomes increasingly prognostic with higher age in LBCL patients. In the 80+ individuals, comorbidities – particularly heart disease, recent cancer, and stroke – have an independent impact on OS. While the original LCI captures the impact of broadly defined comorbidities, its prognostic utility attenuates in the 80+ patients, likely reflecting limited relevance of indolent chronic conditions in this population's constrained survival horizon. The sLCI, focused on the most prognostically relevant comorbidities, offers practical risk stratification. Incorporation of sLCI into clinical decision-making – alongside other prognostic indices – could better identify high-risk individuals and guide treatment strategies.

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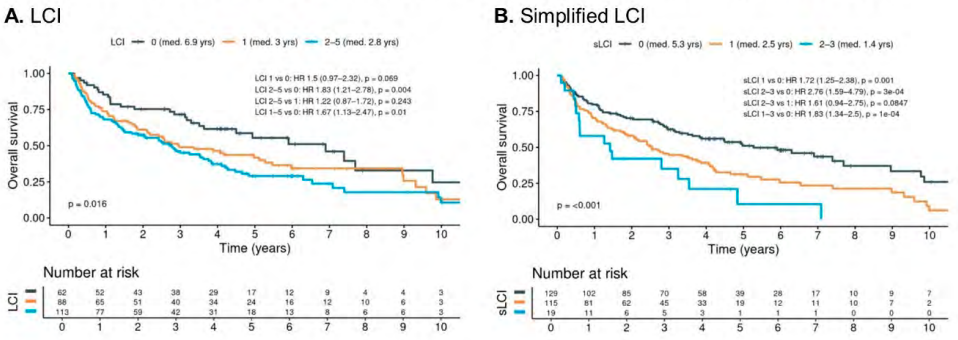
Figure. Survival curves by LEO Comorbidity Index (LCI, **A.**) and simplified LCI (**B.**).



P43

IMPROVED SURVIVAL WITH THIRD-LINE CAR-T THERAPY VS ALTERNATIVE TREATMENT IN DLBCL: A PROPENSITY SCORE–MATCHING ANALYSIS

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Introduction

Chimeric antigen receptor T-cell (CAR-T) therapy has transformed the management of relapsed/refractory diffuse large B-cell lymphoma (r/r DLBCL). Initially approved for use in the third line (L3) setting and beyond, CAR-T has increasingly been adopted earlier in the second line (L2). However, many patients continue to receive CAR-T as L3 therapy due to prior treatment choices or L2 CAR-T ineligibility (e.g., late relapses). The aim of this analysis is to compare survival outcomes between CAR-T and alternative L3 therapies using propensity score matching (PSM) to adjust for key clinical differences.

Methods

We analyzed 240 consecutive r/r DLBCL patients prospectively enrolled in the NiHiL project (NCT03199066), all initially treated with R-CHOP-like regimens and receiving L3 therapy between 2020–2023. Baseline characteristics, diagnosis(dg)-to-L3 interval, clinical parameters at L3 (age, ECOG performance status [PS], and year of L3), L3 and L4 treatment patterns and outcomes were collected. Patients with ECOG PS 0–2 at L3 (n=163) were included; those with prior L2 CAR-T failure (n=6) were excluded, resulting in 157 eligible patients. PSM (1:1, caliper 0.2, nearest neighbor) was performed between patients with the intention of receiving CAR-T and those treated with alternative L3 therapies. The matching was based on age, ECOG PS, and dg-to-L3 interval. The endpoints were event-free survival (EFS) and overall survival (OS), both calculated from the L3 initiation (for CAR-T patients defined as the start of bridging [holding] therapy, or apheresis if no bridging).

Results

Of the 157 included patients, 75 were intended-to-CAR-T and 65 received CAR-T (axi-cel n=26, or tisa-cel n=39), while 82 received alternative L3 treatments (novel therapies n=27, salvage chemotherapy n=14, other therapy n=41). PSM yielded 104 matched patients (52 per group).

We first compared the outcomes of patients included in the PSM (i.e., matched; n=104) versus those excluded (n=53). Among patients intended

for CAR-T (n=75), individuals excluded from PSM (n=23) were younger (*P*<0.01), and had longer dg-to-L3 interval (*P*<0.01); these unmatched CAR-T patients had better EFS at L3 (*P*=0.05; OS *P*=0.29) compared to matched CAR-T patients (n=52). In alternatively treated patients (n=82), those who were not matched by PSM (n=30) were older (*P*<0.01), had longer dg-to-L3 interval (*P*<0.01), but did not show survival differences compared to matched patients (n=52; EFS *P*=0.48, OS *P*=0.59).

PSM (n=104) achieved good balance across key covariates at L3 initiation between CAR-T and alternatively treated cohorts (both n=52): median age 64 vs 66 years (SMD 0.180), ECOG PS 1–2 in 35 (67%) vs 37 (71%) cases (SMD 0.083), median dg-to-L3 interval of 19 vs 15 months (SMD 0.148). Disease characteristics at initial diagnosis were also comparable between groups: clinical stage III–IV (81% vs 83%, *ns*), elevated LDH (69% vs 71%, *ns*), extranodal involvement >1 site (38% vs 48%, *ns*), IPI score 3–5 (60% vs 60%, *ns*), and primary refractory or early relapsing disease (46% vs 57%, *ns*). Of alternatively treated patients included in the PSM (n=52), 20 (38%) received novel therapies (Pola-BR n=14, bispecific antibodies n=4, lenalidomide plus rituximab n=1, ibrutinib n=1), 12 (23%) salvage, and 20 (38%) received other treatments.

After median follow-up of 20.9 months (living patients), EFS was significantly improved in the matched CAR-T group (43.5% vs 24.7% at 18 months, HR=0.62, 95% CI 0.40–0.98, *P*=0.04) compared to matched alternatively treated patients, with a trend towards prolonged OS (66.9% vs 43.1% at 18 months, HR=0.72, 95% CI 0.43–1.21, *P*=0.22).

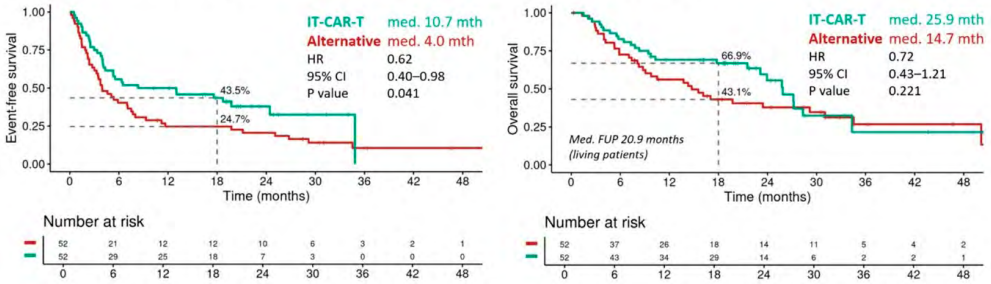
Among the matched patients who ultimately received CAR-T (n=48 out of 52), 18-month EFS was 47.2% and 18-month OS was 68.2%. Of these, 21 (44%) required subsequent L4 therapies (novel agents n=11, salvage n=1, other n=9). In the alternatively treated group (n=52), 31 (60%) required L4 therapy (CAR-T n=8, novel agents n=8, salvage n=4, other n=11).

Conclusion

After adjusting for key clinical covariates, L3 CAR-T therapy was associated with significantly improved EFS compared to alternative L3 treatments in r/r DLBCL. Despite its increasing use in earlier lines, CAR-T remains a valuable and effective treatment option in the L3 and beyond for eligible patients not previously exposed to this treatment modality.

Funding: NU21-03-00411, Charles University Haematology-Oncology Cooperatio Program.

Figure. Survival curves of patients selected by propensity score matching analysis.



P44

PROGNOSTIC IMPACT OF DIAGNOSIS-TO-TREATMENT INTERVAL IN FOLLICULAR LYMPHOMA PATIENTS TREATED WITH IMMUNOCHEMOTHERAPY: EVIDENCE FROM AN INTERNATIONAL COHORT WITH INDEPENDENT VALIDATION

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Introduction
Short diagnosis-to-treatment interval (DTI) is associated with aggressive disease biology and inferior outcomes in diffuse large B-cell lymphoma (DLBCL), and is recognized as a potential source of selection bias in clinical trials if not properly addressed. However, its prognostic significance in follicular lymphoma (FL) remains poorly characterized. We aimed to evaluate the prognostic significance of DTI in newly diagnosed FL patients who received frontline (1L) immunochemotherapy (IC), using a large international dataset and an independent external validation cohort.

Methods
For the discovery dataset, we evaluated patients diagnosed between 2002 and 2018 from ten observational cohorts as part of the FLIPI24 Consortium. External validation was conducted using data from patients enrolled in the Lymphoma Epidemiology of Outcomes (LEO) Cohort between 2010 and 2015. All patients had FL grade 1–3A and received 1L IC within 100 days of diagnosis. Patients with a concurrent DLBCL were excluded. Missing baseline laboratory values were addressed via multiple imputation. The primary endpoint was event-free survival at 24 months (EFS24); secondary endpoint was 5-year overall survival (OS), both calculated from treatment initiation. DTI was analyzed as continuous (via splines) and dichotomized variable (≤14 vs >14 days; as defined in DLBCL).

Results
In the discovery cohort (n=3,907), median age was 61 years (IQR 53–69), and 50% were male; 49% of patients were high-risk according to FLIPI, 26% according to PRIMA-PI, and 38% according to FLIPI24. Included patients received R-CHOP (54%), R-CVP (26%), or B-R (20%) as 1L treatment, followed by anti-CD20 maintenance in 60%. The median DTI was 30 days (IQR 17–48). Using the 14-day cut-off, 851 patients (22%) had short DTI. These patients had more adverse baseline characteristics, including B symptoms (42% vs 32%, P<0.01), ECOG performance status ≥2 (15% vs 8%, *P*<0.01), elevated LDH (51% vs 40%, *P*<0.01), hemoglobin <12 g/dL (30% vs 17%, *P*<0.01), and elevated β2-microglobulin (56% vs 44%, *P*<0.01). Prognostic indices (PIs) were also more frequently high-risk in the short-DTI group: FLIPI (56% vs 47%), PRIMA-PI (36% vs 24%), and FLIPI24 (51% vs 34%, all *P*<0.01).

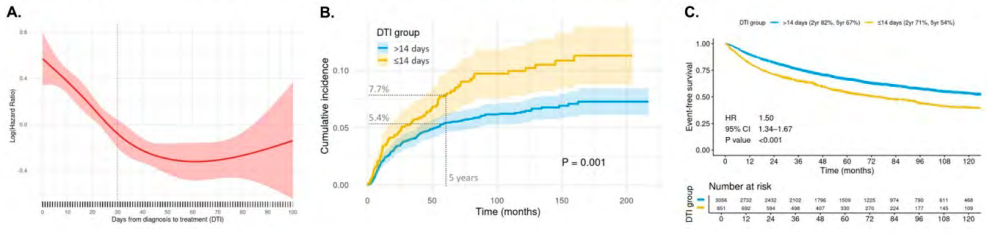
Spline modeling showed a decreasing EFS24 rate with increasing DTI, reaching a plateau at 30–40 days. In logistic regression model, each additional week of DTI improved EFS24 (OR 0.92, *P*<0.01). DTI attenuated but remained prognostic after adjusting for PIs (OR 0.93 for FLIPI, 0.95 for PRIMA-PI, 0.96 for FLIPI24, all *P*<0.01). When analyzed as a dichotomized variable, short DTI was associated with inferior EFS24: OR 1.74 adjusted for FLIPI, 1.63 for PRIMA-PI, and 1.53 for FLIPI24 (all *P*<0.01). The 2-year EFS of short vs long DTI groups was 71% vs 82% (HR 1.50, 95% CI 1.34–1.67, *P*<0.01), 5-year OS was 80% vs 86% (HR 1.38, 95% CI 1.21–1.56, *P*<0.01), and the 5-year cumulative risk of histologic transformation was 7.7% vs 5.4% (*P*<0.01).

In the validation cohort (n=516), median age was 61 years (IQR 52–69 years), 55% were male; 36% of patients were high-risk according to FLIPI; 29% according to PRIMA-PI, and 33% according to FLIPI24. Patients received B-R (59%) or R-CHOP (41%) as 1L treatment. The median DTI was 31 days (IQR 19–48), 84 patients (16%) had DTI ≤14 days. Similar patterns were observed; patients with short DTI had adverse baseline characteristics, PIs, and shorter 2-year EFS (72% vs 84%, HR 1.69, 95% CI 1.13–2.55, *P*=0.01). DTI remained associated with EFS24 when analyzed as a continuous variable (per week: OR 0.88 unadjusted, 0.90 with FLIPI, 0.89 with PRIMA-PI, and 0.89 with FLIPI24), and dichotomized (≤14 vs >14 days: OR 1.83 adjusted for FLIPI, 1.88 for PRIMA-PI, and 1.73 for FLIPI24).

Conclusion
Short DTI, defined as ≤14 days (observed in 22% of FL patients vs 46% in DLBCL), is a strong and independent predictor of inferior outcomes in newly diagnosed FL patients treated with IC. While DTI correlates with adverse baseline features and established PIs, it retains independent prognostic value. Although a 14-day threshold is reasonable for risk modeling, DTI contains

greater informational value when modeled as a continuous variable. Among PIs, FLIPI24 most strongly attenuated the DTI effect, underscoring its superior discriminative power over FLIPI and PRIMA-PI. Clinical trials designs should mitigate any potential barriers to enrollment of FL patients requiring urgent therapy.

Figure. Results of patients included in the discovery cohort (n=3907): spline curve of continuous DTI by EFS24 (**A**), survival curve (**B**) and cumulative incidence of histologic transformation (**C**) by DTI dichotomized (≤14 vs >14 days).



P45

FLIPI24 AS A PROGNOSTIC MODEL IN MARGINAL ZONE LYMPHOMA: RESULTS FROM AN INTERNATIONAL MULTI-COHORT ANALYSIS

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Introduction

Marginal zone lymphoma (MZL) is an indolent and heterogeneous disease. Several prognostic models have been proposed to assess outcomes in MZL, including FLIPI, IPI (primarily designed for overall survival [OS]), and MZL-specific scores such as MALT-IPI (event-free survival [EFS]) and MZL-IPI (progression-free survival). FLIPI24 is a recently developed prognostic index for patients with follicular lymphoma (FL) treated with first-line (1L) immunochemotherapy (IC), identifying a high-risk population with inferior survival and increased risk of lymphoma-related death. FLIPI24 has also demonstrated robust performance in the entire FL population, including patients under observation. This study aimed to evaluate the prognostic utility of FLIPI24 in patients with MZL and to compare its performance with other established indices (FLIPI, MZL-IPI, IPI, MALT-IPI).

Methods

We pooled harmonized data from newly diagnosed MZL patients with complete available FLIPI24 variables (age, white blood cell count [WBC], hemoglobin [HGB], serum lactate dehydrogenase [LDH], and beta-2-microglobulin [B2M] levels) treated with 1L IC. Data were collected from three prospective international cohorts: NiHiL (Czech Republic; n=897; enrolled 2005–2023), LEO (USA; n=62; 2015–2020), and MER (USA; n=32; 2010–2015), forming a pooled cohort of 991 1L IC-treated patients. The analysis was then extended to include all newly diagnosed MZL patients (n=2,116), regardless of treatment. The primary endpoint in the 1L IC-treated cohort was EFS at 24 months (EFS24), and for all MZL patients, it was OS. Secondary endpoints included OS24 for 1L IC-treated cohort, lymphoma-related mortality by FLIPI24 risk groups, and comparative prognostic performance of FLIPI, IPI, and MALT-IPI, by C-statistics; performance of MZL-IPI was assessed on a subgroup of NiHiL patients with available data (n=877 all MZL, n=485 1L IC-treated).

Results

Among the 991 1L IC-treated patients, the median age at diagnosis was 66 years (range 22–94); 47% were male; 11% had ECOG performance status ≥2, 68% clinical stage III–IV, 42% elevated LDH, 40% IPI 3–5, 42% FLIPI 3–5, and 62% MALT-IPI 2–3. The median B2M level was 2.8 mg/L, WBC 7.0 × 10^9/L, and HGB 13.1 g/dL. MZL subtypes were: 64% extranodal (EMZL), 22% nodal (NMZL), and 14% splenic (SMZL). Most common 1L IC regimens were R-CVP (46%), R-CHOP-like (32%), R-chlorambucil (9%), and B-R (9%).

SBORNÍK ABSTRAKTŮ

FLIPI24 stratified patients into low- (18%), intermediate- (37%), and high-risk (45%) groups. High-risk patients were older (median 54 vs 65 vs 71 years for low-, intermediate-, and high-risk groups, respectively; *P*<0.01), had more frequent adverse baseline features, and were more likely to have the SMZL subtype (2% vs 7% vs 25% for low-, intermediate-, and high-risk groups, respectively; *P*<0.01). After a median follow-up of 7.2 years, EFS24 was 89%, 85%, and 65% (*P*<0.01) across low-, intermediate-, and high-risk groups; OS at 2 years was 98%, 97%, and 79%; and OS at 5 years was 96%, 91%, and 62%, resp. (*P*<0.01). The 5-year cumulative incidence of lymphoma-related death was 13% in high-risk vs 6% in low-/intermediate-risk groups.

FLIPI24 showed better prognostic accuracy for EFS compared with FLIPI, MZL-IPI, IPI and MALT-IPI (C-index: 0.640 vs 0.630, 0.622, 0.620 and 0.603, resp.), as well as for OS (C-index: 0.710 vs 0.672, 0.641, 0.663, 0.652, resp.). Prognostic performance was consistent across MZL subtypes.

In the extended cohort of 2,116 newly diagnosed MZL patients (47% receiving IC, 17% rituximab monotherapy, 11% radiotherapy, 8% surgery, 2% antibiotics; 15% observed), FLIPI24 stratified patients into low- (24%), intermediate- (39%), and high-risk (37%) groups. OS at 2 years was 99%, 97%, and 83%, at 5 years it was 96%, 91%, and 62%, resp. (*P*<0.01). The 5-year cumulative incidence of lymphoma-related death was 12% in high-risk vs 4% in low-/intermediate-risk patients. FLIPI24 outperformed other indices (OS C-index: 0.754 vs MZL-IPI 0.654, FLIPI 0.700, MALT-IPI 0.694, IPI 0.699).

Conclusion

FLIPI24 demonstrates strong and consistent prognostic performance across all MZL subtypes, identifying ~40% of patients as high-risk with significantly worse survival and increased lymphoma-related mortality. The FLIPI24 outperforms FLIPI, MZL-IPI, IPI, and MALT-IPI in predicting outcomes and may serve as a practical tool to guide risk-adapted management and selection of patients for novel therapies in MZL.

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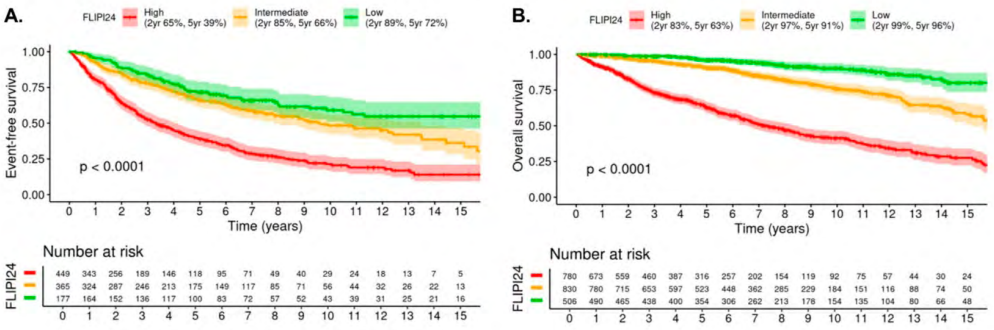
Figure. Survival curves of 1L-IC treated MZL patients (**A**) and of all included MZL patients (**B**).



P46

FIRST DISCLOSURE OF EPCORITAMAB + R-ICE IN PATIENTS WITH RELAPSED/REFRACTORY DIFFUSE LARGE B-CELL LYMPHOMA (R/R DLBCL) ELIGIBLE FOR AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT): EPCORE NHL-2

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Introduction: Approximately 50% of patients (pts) with LBCL are refractory to salvage chemoimmunotherapy (CIT), such as rituximab, ifosfamide, carboplatin, and etoposide (R-ICE). An unmet need remains to optimize salvage therapy (tx), increase complete response (CR) rates, and allow more pts to undergo ASCT. CAR-T is an emerging alternative to ASCT, particularly for pts who progress within 12 mo of 1L tx; however, logistics, cost, accessibility, and response durability remain as challenges. Epcoritamab (epcor), a subcutaneous CD3xCD20 bispecific antibody, has previously demonstrated high CR rates when combined with the salvage CIT rituximab, dexamethasone, cytarabine, and oxaliplatin or carboplatin (R-DHAX/C). Here, we present the first efficacy and safety results for epcor in combination with R-ICE in pts with R/R DLBCL eligible for ASCT from the NHL-2 trial (NCT04663347).

Methods: Pts had R/R DLBCL, were eligible for R-ICE and ASCT, and had received ≥1 prior line of tx (LOT). Pts received 48 mg epcor (0.16- and 0.8-mg step-up doses in cycle [C]1 and full doses once weekly [QW] C1–4; Q2W C5–9; Q4W C10+) and R-ICE in C1–3. C1–4 were 21 d and C5+ were 28 d. Tx was given until ASCT, progressive disease (PD), or unacceptable toxicity. The primary endpoint was overall response rate (ORR) per investigator assessment (Lugano criteria).

Results: At data cutoff (Dec 18, 2024), median follow-up was 11 mo (range, 6–15). Thirty-one pts had received epcor 48 mg + R-ICE. Median age was 62 y (range, 39–77), 55% were male, 61% were Ann Arbor stage III/IV, 42% had bulky disease ≥7 cm, 81% had 1 prior LOT (range, 1–3), and 65% had progressed within 12 mo of 1L tx. Among the 31 pts treated with epcor 48 mg + R-ICE, ORR was 87%; 20 pts (65%) had a CR and 7 (23%) a partial response (PR; **Table**). High ORRs and CR rates were seen across subgroups of interest (Table). Median number of initiated tx cycles was 4 (range, 2–14) for epcor and 3 (2–3) for R-ICE. The majority of pts (20/31; 65%) proceeded to ASCT. On the most recent scan prior to ASCT, 13/20 pts were in CR and 7/20 in PR. Five of the 7 with PR achieved CR following ASCT. Of the 11 pts

who did not proceed to ASCT, 3 (10%) remained on epcor (1 in CR and 2 in PR) and 8 (26%) discontinued tx due to PD (n=6) or other reasons (n=2). Of those, 6 proceeded to a subsequent tx: 3 to CAR-T, 1 to a subsequent trial, 1 to allogeneic SCT, and 1 to radiotherapy. At 6 mo, an estimated 81% of responses were ongoing, 74% of pts were progression free, and 100% of pts were alive. The most common tx-emergent adverse events (TEAEs) were neutropenia (74%), anemia (68%), and thrombocytopenia (68%). Cytokine release syndrome occurred in 52%; all were low grade (1/2) and resolved. One pt had immune effector cell-associated neurotoxicity syndrome (grade 1), which resolved. No clinical tumor lysis syndrome was observed. Infections occurred in 18 (58%) pts; 5 (16%) had serious infections. No pts discontinued epcor due to TEAEs. There were no grade 5 TEAEs.

Conclusions: Epcor + R-ICE demonstrated high CR rates in pts with R/R DLBCL eligible for ASCT, most of whom were high risk, having progressed <12 mo of 1L tx. Safety was manageable, with no discontinuations due to AEs. These results continue to demonstrate that epcor when combined with CIT may optimize salvage tx, increasing the proportion of pts proceeding to ASCT compared with historical rates, providing a greater chance of a cure.

Table. ORRs and CR rates with epcor + R-ICE in pts with R/R DLBCL

	ORR, n (%)	CR, n (%)
All pts (N=31)	27 (87)	20 (65)
Progressed within 12 mo of 1L tx (n=20)	17 (85)	11 (55)
Progressed >12 mo of 1L tx (n=11)	10 (91)	9 (82)
1 prior LOT (n=25)	22 (88)	17 (68)
>1 prior LOT (n=6)	5 (83)	3 (50)

1L, first line; CR, complete response; DLBCL, diffuse large B-cell lymphoma; epcor, epcoritamab; LOT, line of therapy; ORR, overall response rate; pt, patient; R-ICE, rituximab, ifosfamide, carboplatin, and etoposide; R/R, relapsed/refractory; tx, therapy.

P47

DIAGNOSTICKY OPOMÍJENÉ DEFEKTY NEGATIVNĚ OVLIVŇUJÍCÍ PROGNÓZU A PŘEŽITÍ PACIENTŮ S DIFÚZNÍM VELKOBUNĚČNÝM B-LYMFOMEM

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Difúzní velkobuněčný B-lymfom (DLBCL) je nejčastější ne-Hodgkinův lymfom s heterogenním klinickým průběhem. Zatímco přibližně 60 % pacientů dosáhne dlouhodobé remise po první linii imunochemoterapie, zbylých cca 40 % pacientů opakovaně relabuje či je refrakterních na léčbu. Přítomnost genetických aberací zodpovědných za agresivitu onemocnění u DLBCL je známa, není ale zatím rutinně testována na rozdíl od určení subtypu podle buněčného původu COO (cell-of-origin).

V naší práci jsme se zaměřili na detekci genetických změn při diagnóze DLBCL s cílem ověřit vliv aberací na prognózu a přežití pacientů. Vyšetřili jsme DNA izolovanou z uzlin 62 pacientů s DLBCL pomocí NGS panelu LYNX (PMID:40346678), cíleného na detekci mutací v 72 genech, chromozomových změn a translokací typických pro lymfomy.

U 16 z 62 pacientů (25,8 %) jsme detekovali bialelický defekt nádorového supresoru *TP53* a u dalších 10 pacientů (16,1 %) jsme detekovali samotnou del17p. Bialelický defekt nádorových supresorů *CDKN2A/CDKN2B* mělo 10 z 62 pacientů (16,1 %). Více než polovina pacientů (53,2 %) nesla aberaci v onkogenu *BCL2* (SNV/CNA/translokace) a 75,8 % pacientů mělo komplexní karyotyp (>5 chromozomových změn). Z testovaných aberací měly signifikantní vliv na přežití pacientů přítomnost bialelického defektu nádorových supresorů *CDKN2A/CDKN2B* (p=0,0221) a aberace v genu *BCL2* (p=0,0294).

Bialelické poškození genu *TP53* (p=0,0584) a komplexní karyotyp (p=0,0856) vykazovaly hraniční prognostický dopad na rozdíl od rozdělení pacientů podle COO (p=0,5521), které se neprojevilo v přežití pacientů vůbec.

Naše výsledky podtrhují potřebu revize klinických doporučení a zavedení genetického testování minimálního setu genů *BCL2, CDKN2A/CDKN2B* a *TP53* do rutinní praxe pro optimalizaci terapeutických strategií u pacientů s DLBCL. Přestože jsou tyto aberace opakovaně spojovány s progresí onemocnění, rezistencí na terapii a celkově horší prognózou DLBCL pacientů, není jejich stanovení součástí aktuálních doporučení. Naproti tomu rutinní stanovování COO subtypů ABC/GCB v současné době ztrácí svůj prognostický význam.

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P48

PRE-TREATMENT CIRCULATING TUMOR DNA PREDICTS SURVIVAL OF DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS AND ALLOWS GENETIC AND BIOLOGICAL EVALUATION

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SBORNÍK ABSTRAKTŮ

Introduction. Circulating tumor DNA (ctDNA) was identified as a powerful biomarker for treatment response evaluation and outcome prediction in malignant tumors, including diffuse large B-cell lymphoma (DLBCL). However, more studies are necessary to validate ctDNA utilization in general, and specifically in a real-world setting to facilitate standardization and transition into routine clinical practice. Therefore, we assessed pre-treatment ctDNA in 169 unselected previously untreated DLBCL patients consequently uniformly treated with R-CHOP chemoimmunotherapy at five academic hematology centers in the Czech Republic.

Methods. ctDNA was analyzed by CAPP-Seq (CAncer Personalized Profiling by deep Sequencing) and a custom panel of 521 genes. DNA alterations were identified by VarScan2. ctDNA concentration was calculated from average variant allele frequencies and reported as human haploid genome equivalents (hGE) per ml of plasma. Germinal center B-cell like (GCB) and non-GCB subtyping was done by immunohistochemistry. Genetic classes were analyzed by LymphGen 2.0, COSMIC (Catalogue Of Somatic Mutations In Cancer) signatures by SigProfiler.

Results. In all patients, the median age at diagnosis was 64 years (range 24-81), clinical stage III–IV in 68%, more than one extranodal involved sites in 40%, PS ECOG 2–4 in 24%, elevated LDH in 60%, International Prognostic Index (IPI) 3–5 in 50%, bulky disease over > 7.5 cm in 40%, and non-GCB DLBCL subtype in 47% of patients. Median follow-up was 2.73 years. The 2-year progression free and overall survivals (PFS and OS) were 73.6% and 84.3%, respectively.

Without any threshold for input DNA quality and quantity, ctDNA was detected in 136 out of 169 analyzed patients (80%). Clinical characteristics of patients with and without detected ctDNA did not show any differences. The median plasma ctDNA concentration was 995 hGE/ml. ctDNA concentration was significantly higher in patients with clinical stage III–IV (median 1416.9 vs. 542.7 hGE/ml, p = 2.62e-5), age > 60 years (median 1110.7 vs. 691.2, p = 0.04), PS ECOG 2–4 (median 2130.9 vs. 824.2 hGE/ml, p = 2.62e- 5), elevated LDH serum levels (median 1804.1 vs. 513.3 hGE/ml, p = 1.14e-9), bulky disease over > 7.5 cm (median 2234.9 vs. 624.8 hGE/ml, p = 4.58e-6), and in patients with IPI 3-5 (median 1646.7 vs. 631.7 hGE/ml, p = 8.33e-5).

To find a pre-treatment ctDNA plasma concentration that would identify high risk patients (based on PFS analysis), patients from the General University Hospital (n = 72) were used as a discovery cohort and patients from other centers (n = 64) as a validation cohort (clinically well-balanced). In the discovery cohort, the threshold was determined at 3000 hGE/ml using Harrell's C-index. High ctDNA (> 3000 hGE/ml, 32% of patients) was associated with inferior PFS (2-year PFS 46% vs. 88%, 5-year PFS 38% vs. 71%, HR 3.99, p = 0.001). In the validation cohort, ctDNA concentration > 3000 hGE/ml (19% of patients) was also associated with inferior PFS (2-year PFS 50% vs. 78%, 5-year PFS 40% vs. 67%, HR 2.9, p = 0.024). It confirmed identified threshold that was consequently used for further analyses.

In all patients, high ctDNA level (> 3000 hGE/ml, 26% of patients) was associated with inferior PFS (2-year PFS 48% vs. 83%, 5-year PFS 38% vs. 66%, HR 3.37, p < 0.001), and, importantly, also with inferior OS (2- year OS 74% vs. 88%, 5-year OS 54% vs. 75%, HR 2.56, p = 0.016). Moreover, ctDNA was an independent prognostic factor for PFS in multivariate analysis with IPI score (high ctDNA, HR 2.12, p = 0.029; IPI ordinal, HR 1.42, p = 0.01). High ctDNA patients had more advanced clinical stage, worse PS ECOG, higher LDH levels, higher IPI score (all with p < 0.001), and higher proportion of bulky disease (p = 0.005).

The LymphGen assigned a genetic subtype to 44% of cases, reflecting the capability of the LympGen model to classify 50-60% of tumors (if copy number and translocation information is available). COSMIC mutational signatures showed differences between genetic and cell of origin subtypes, e.g., activation induced cytidine deaminase activity specifically in non-GCB DLBCL.

Conclusion. Our study confirmed pre-treatment ctDNA concentration as a strong and independent risk factor associated with unfavorable DLBCL survival in a routine clinical setting, allowing parallel genetic and biological evaluation.

P49

FRONTLINE REGIMENS AND CONSOLIDATION IN PMBCL: A POOLED REAL-WORLD ANALYSIS FROM THE MAYO CLINIC AND CLSG

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Background

Primary mediastinal B-cell lymphoma (PMBCL) affects young adults; frontline immunochemotherapy aims for a rapid complete metabolic response. Contemporary real-world practice varies across centers and countries. We pooled two independent cohorts (Czech Lymphoma Study Group - CLSG and Mayo Clinic) to compare treatment patterns and effectiveness under an intention-to-treat framework.

Methods

Prospective registries from CLSG and Mayo were harmonized. Baseline characteristics, frontline regimen, radiotherapy (RT), and autologous stem-cell transplantation (ASCT) were abstracted. Best response and end-of-treatment (EOT) PET (negative = Deauville 1–3) were analyzed. Survival endpoints were progression-free survival (PFS) and overall survival (OS). Associations between regimen and RT/ASCT and between regimen and best response/EOT PET were tested by χ^2. Regimen-specific 5-year PFS/OS and hazard ratios (HRs) versus R-CHOP were summarized.

Results

We analyzed 574 patients (CLSG n=425, Mayo n=149) diagnosed between January 1, 2006, and December 31, 2023 who were treated with anthracycline-rituximab containing regimen. Age and sex distributions were similar (median 37–38 years; p=0.766; M/F comparable), LDH elevation was more frequent in CLSG (85.4% vs 75.8%; p<0.001). Harmonized IPI within each cohort among patients with available IPI: CLSG (n=416)—Low (0–1) 49.0%, Low-intermediate (2) 26.4%, High-intermediate (3) 16.3%, High (4–5) 8.2%; Mayo (n=133)—Low (0–1) 63.2%, Low-intermediate (2) 23.3%, High-intermediate (3) 11.4%, High (4–5) 0.8%. This confirms enrichment for lower-risk disease in Mayo and a broader spread including more high-risk cases in CLSG (p=0.0033).

Frontline regimen spectrum (pooled, denominators reflect available data). Among patients with regimen captured in the dataset (n=557): R-CHOP 313 (56.2%), EPOCH-R/DA-EPOCH-R 128 (23.0%), Intensive (R-SQ/R-MegaCHOP) 59 (10.6%), R-CHOEP 50 (9.0%), Others 7 (1.3%).

Use of RT and ASCT (pooled). RT was delivered in 183/557 (32.9%) overall; its use differed by regimen (row % RT: R-CHOP 41.2%, R-CHOEP 30.0%,

EPOCH-R 17.2%, Intensive 25.4%, Others 28.6%; χ^2 $p=3\times10^{-5}$). ASCT was performed in 60/559 (10.7%) overall with a strong regimen association (Intensive 67.8%, R-CHOP 4.8%, R-CHOEP 3.9%, EPOCH-R 0.8%, Others 28.6%; χ^2 $p<1\times10^{-6}$). Best response and EOT PET. Distribution of best response (CR/PR/SD/PD) did not differ by regimen (χ^2 p=0.584). EOT PET negativity also did not differ by regimen (χ^2 p=0.988).

Survival by Regimen (pooled summary)

Five-year PFS/OS and HRs vs R-CHOP: DA-EPOCH-R: 5-yr PFS 86.2% (95% CI 79.9–92.5), HR 0.60 (0.38–0.95; p=0.031); 5-yr OS 96.2% (92.4–100), HR 0.30 (0.15–0.57; p=0.0004). R-CHOEP: 5-yr PFS 83.7% (73.4–94.1), HR 0.67 (0.36–1.25; p=0.206); 5-yr OS 95.4% (89.2–100), HR 0.31 (0.13–0.73; p=0.007). Intensive (R-SQ/R-MegaCHOP): 5-yr PFS 81.4% (71.4–91.3), HR 0.71 (0.42–1.19; p=0.193); 5-yr OS 84.8% (75.6–93.9), HR 0.97 (0.51–1.87; p=0.922). R-CHOP (reference): 5-yr PFS 75.9% (71.0–80.8); OS 86.4% (82.4–90.4). Others: 5-yr PFS 71.4% (38.0–100), HR 0.96 (0.24–3.78; p=0.955); 5-yr OS 71.4% (38.0–100), HR 1.73 (0.28–10.75; p=0.559). Figure 1,2.

Regimen type was strongly associated with subsequent ASCT and RT utilization but not with best response category or EOT PET negativity. Notably, DA-EPOCH-R showed the lowest rates of RT and ASCT use, whereas Intensive (R-SQ/R-MegaCHOP) had a high ASCT rate—consistent with planned consolidation in that strategy. Survival signals favored DA-EPOCH-R (significantly better PFS and OS vs R-CHOP) and R-CHOEP (significantly better OS vs R-CHOP).

Conclusions

In this pooled real-world analysis from CLSG and Mayo, frontline regimen choice varied substantially and was linked to downstream treatment intensity (ASCT and RT). Dose-adjusted EPOCH-R achieved the best survival with reduced need for consolidative therapies, while R-CHOP had lower PFS/OS and higher RT use. These data support PET-adapted, dose-intensive strategies as effective real-world standards and underscore the importance of regimen selection on the overall treatment trajectory in PMBCL.

Acknowledgement

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SBORNÍK ABSTRAKTŮ

Figure 1 Overal survival by chemotherapy regimen

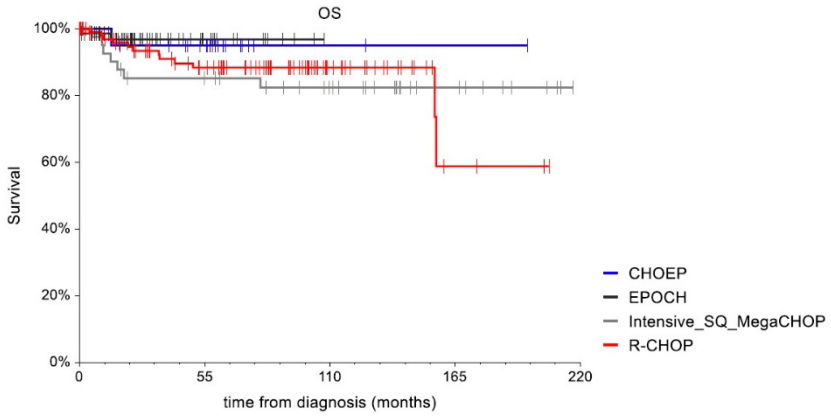
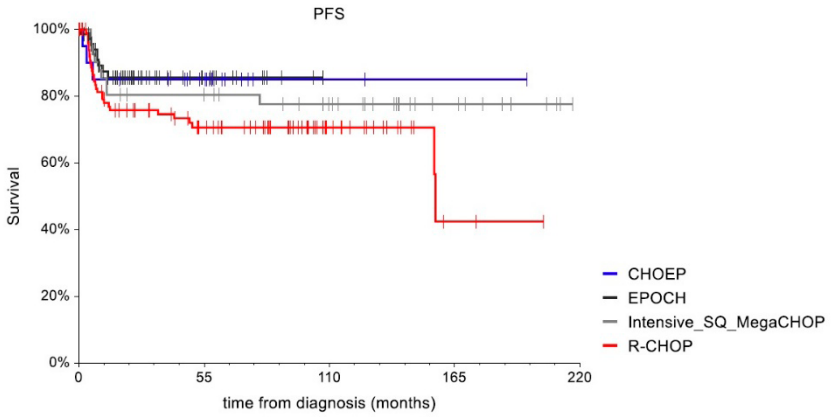


Figure 2 Progression-free survival by chemotherapy regimen



P50

FOUR CYCLES OF R-CHOP SHOW THE SAME EFFECTIVITY AS SIX CYCLES IN REAL-WORLD DLBCL PATIENTS WITH LOCALIZED, NON-BULKY DISEASE INDEPENDENTLY OF OTHER UNFAVOURABLE RISK FACTORS

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Background

The results of the FLYER study (Lancet Oncology, 2019) revealed that in young pts with limited clinical stage (CS) and favourable prognosis according to international prognostic index (IPI) 4 cycles of R-CHOP in diffuse large B-cell lymphoma (DLBCL) is non-inferior to 6 cycles of R-CHOP, with reduction of therapy-associated toxicity. The cycle-limited approach has been incorporated into daily practice, but so far, no real-world (RW) data have been published. Moreover, therapy de-escalation has also been used in RW patients who do not fulfil the FLYER inclusion criteria (IC).

Aims

1. To evaluate the results of the FLYER study in a RW population with the same IC in comparison to the original FLYER patient data set. 2. To analyse the outcome of pts with localized, non-bulky disease, who do not fulfil the FLYER study IC (age, LDH concentration, ECOG).

Methods

RW pts were identified in prospective lymphoma study (NiHiL GovTrial No. NCT03199066) with signed informed consent. Pts who achieved complete remission (CR) at the end of treatment (EoT) after 4 or 6 RCHOP cycles and did not receive any further therapy as consolidation were included. First, pts treated with 4 cycles of R-CHOP were identified and then matched in 1:2 ratio according to selected criteria (age, CS, ECOG and tumour size) with pts that received 6 cycles. In total 254 DLBCL pts with median follow-up (FU) 7.1 years were included. Primary endpoint was disease-free survival (DFS) defined for the purpose of this study as time from CR till progression or death and secondary endpoints were overall survival and toxicity profile. The results of subgroup of RW pts who would fulfil the FLYER study IC (RW-IC, N=83, median FU 7.3 years) were compared to selected 487 pts from FLYER (CR at EoT and no consolidation therapy), with median FU 5.4 years. CR was established by CT in FLYER, by CT or PET in RW. In addition, we compared the efficacy of 4 vs 6 cycles of R-CHOP in the subgroup of pts with localized, non-bulky disease who however do not fulfil the FLYER IC (RW-nonIC, N = 171; pts with higher IPI due to age and/or elevated serum LDH concentration and/or higher PS ECOG).

Results

The FLYER (N=487) and RW-IC (N=83) pts cohorts were well balanced. Median age at diagnosis was 47 (FLYER) and 46 (RW) years and most of pts had PS ECOG 0 (92% FLYER and 90% RW), no B-symptoms (94% and 93%) and the majority (83% in FLYER and 87% in RW) of pts had maximal tumour diameter less than 5cm. More pts in FLYER (41%) had CS II compared to RW (25%). There was no DFS difference among any subgroup with 3-year DFS probability 98% vs 96% (4 vs 6 cycles FLYER) and 96% vs 96% (4 and 6 cycles RW-IC), (NS) (Figure 1A). The RW-nonIC pts (N = 171) had median age of diagnosis 67 years (28 – 85), 36% elevated LDH, 4% PS ECOG 2-3, 72% of pts had IPI 1, 26% IPI 2 and 2% of pts IPI 3. The DFS did not differ among 4 and 6 cycles of R-CHOP (p=0.4332), with DFS probability 94% and 95% (Figure 1B). The analyses of subgroups of RW-nonIC (i.e. pts with elevated LDH, elderly, etc.) did not reveal any differences among 4 vs 6 cycles of received therapy.

Summary/Conclusion

Analysis of the combined sets of FLYER pts and RW pts confirmed the results of FLYER study in a RW population. In addition, our data suggest that DLBCL pts with localized, non-bulky disease, independently of FLYER IC could be considered for treatment de-escalation from 6 to 4 cycles of R-CHOP.

P51

CELL CYCLE DEPENDENT AND INDEPENDENT REGULATION OF AKT ACTIVITY IN GERMINAL CENTER B-CELL-LIKE DIFFUSE LARGE B-CELL LYMPHOMA – A DUAL ROLE OF CYCLIN DEPENDENT KINASE 2

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Introduction. The PI3K/AKT (phosphatidylinositol 3-kinase / protein kinase B) pathway is one of the most frequently mutated and deregulated pathways in cancer, including non-Hodgkin lymphomas. Particularly frequent in germinal center B-cell-like diffuse large B-cell lymphoma (GCB-DLBCL) is deletion of the PI3K/AKT pathway negative regulator PTEN (phosphatase and tensin homolog), a phosphatase acting on phosphoinositol-3,4,5-triphosphate. PI3K/AKT signaling regulates multiple critical cellular processes including cellular proliferation and metabolism. However, it is not known whether there is specific regulation of AKT activity during the cell cycle, and how it may be affected by oncogenic AKT hyperactivation.

SBORNÍK ABSTRAKTŮ

Methods. We used a spectrum of GCB-DLBCL lines, some modified to allow assessment of PTEN deletion. Knock in (KI) and knock out (KO) were done by CRISPR-Cas9 technology. Overexpression used the Sleeping Beauty transposon system. AKT activity was measured using a genetically encoded Förster resonance energy transfer (FRET)-based reporter by flow cytometry. Cell cycle was evaluated by Vibrant DyeCycle Ruby staining, FxCycle propidium iodide staining, or genetically encoded FastFUCCI biosensor. Cell cycle synchronization was done by double thymidine block. Cellular metabolism was evaluated by untargeted liquid chromatography mass spectrometry (LC-MS)-based metabolomics and by Seahorse instrument-based measurement of live cells.

Results. Simultaneous flow cytometry-based evaluation of AKT activity and cell cycle allowed us to identify an increase of AKT activity at the beginning of the S phase of the cell cycle in GCB-DLBCL cell lines. An S phase-specific increase of AKT activity was verified in cell cycle-synchronized cells over several cell cycles and was necessary for cell cycle progression. AKT inhibition blocked the cells in G1 phase. Additionally, after cell cycle synchronization and release, immunoblotting confirmed increased pAKT (Ser473, a surrogate marker for AKT activity) during S phase, as determined by cyclin A and E dynamics. Given the importance of cyclin dependent kinases (CDK) for cell cycle regulation, we next evaluated to what degree CDKs could drive AKT activity. Remarkably, AZD-5438 (CDK1/2/9 inhibitor) and JNJ-7706621 (pan-CDK inhibitor) fully eliminated AKT activity. In contrast, palbociclib (CDK4/6 inhibitor) did not have any significant AKT inhibitory effect. This prompted us to focus specifically on CDK2, a major kinase involved in G1-S transition. Induced expression of CDK2 resulted in a strong and specific increase of AKT activity. Importantly, we hypothesized that the CDK2 mediated increase of AKT activity is at least partially responsible for S phase specific metabolic increase necessary for synthesis of all cellular components before cell division. AKT activation by PTEN deletion, or expression of a constitutively active form of AKT, was associated with increased general metabolic activity (glycolysis and respiratory capacity, assessed by Seahorse) as well as in specific metabolic pathways (assessed by LC-MS).

The degree of AKT activity reduction after CDK2 inhibition and AKT activity increase in response to CDK2 overexpression prompted us to investigate additional potential functional interactions of CDK2 with AKT, apart from cell cycle-specific regulation. The PTEN inhibitor HOpic (PTENi) is able to increase AKT activity within minutes, but the PTENi-induced increase of AKT activity was completely eliminated by CDK2 inhibition (AZD-5438 - CDK1/2/9 inhibitor). This suggests that CDK2 acts on AKT within a feed-forward loop that is cell cycle-independent. Further details of interaction between CDK2 and AKT will be presented.

Lastly, oncogenic AKT activation by PTEN deletion not only increased AKT activity in general, but also eliminated cell cycle dependent dynamics of AKT activity.

Conclusion. We have shown cell cycle- and CDK2-dependent dynamics of AKT activity in GCB-DLBCL, responsible for stimulation of metabolism at the beginning of the S phase of the cell cycle. Additionally, CDK2 seems to be involved in a critical feed-forward AKT activation loop. PTEN deletion leads to easier G1-S transition on the background of increased AKT and metabolic activity.

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P52

PHOSPHOINOSITIDE DEPENDENT PROTEIN KINASE 1 IS A KEY METABOLIC REGULATOR AND POTENTIAL THERAPEUTIC TARGET IN LYMPHOMA

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Introduction. The PI3K/AKT signaling pathway is often overactive in various cancers, including non-Hodgkin lymphoma. For example, it could be activated by PTEN deletion (a key negative regulator of the PI3K/AKT pathway) or triggered by B-cell receptor (BCR) signaling. Numerous PI3K and AKT inhibitors have been explored, with several approved for clinical use. Despite this, these inhibitors faced challenges such as limited overall response rates, resistance development, and serious side effects. We focused on the phosphatidylinositol-dependent protein kinase 1 (PDPK1), a key kinase responsible for AKT activation, as a potentially universal and highly effective PI3K/AKT signaling target.

Methods. We have used 11 lymphoma cell lines (mainly of diffuse large B-cell lymphoma). Targeted knock-out (KO) was done by the CRISPR/Cas9 system and overexpression by the Sleeping Beauty transposon system. AKT kinase activity was measured by genetically encoded Förster resonance energy transfer-based reporter. FastFUCCI reporter was used for cell cycle analysis and Seahorse XFp analyzer for metabolic flux analysis. NOD scid gamma mice were used for *in vivo* cell line xenograft experiment. PDPK1 was pharmacologically inhibited by GSK2334470.

Results. In a panel of lymphoma lines, KO of BCR and PI3Kδ (the lymphocyte predominant PI3K isoform frequently targeted therapeutically) had a highly variable effect on cell growth, which may explain variable efficacy of PI3K and BCR inhibitors in the clinics. In contrast, PDPK1 KO was consistently and highly toxic to all tested lines, regardless of their PTEN status (**Figure 1**). PDPK1 is a regulatory kinase known to activate multiple downstream signaling pathways. Complementation experiments (with active forms of AKT, mTOR, MEK1, RSK1, and cMYC) showed partial PDPK1 KO toxicity rescue only with AKT and mTOR, suggesting that PDPK1 is primarily involved in AKT-mTOR pathway activation in lymphoma. Metabolism analysis also suggested activation of mTOR downstream of PDPK1. PDPK1 inhibition decreased and PDPK1 overexpression increased mitochondrial function and to a lesser degree glycolysis. PDPK1 inhibition was associated with cell cycle arrest in G1 phase of the cell cycle and was *in vitro* broadly synergistic with unspecific standard anti-lymphoma chemotherapeutics (doxorubicin, vincristine, and cisplatin) and highly synergistic with more targeted CDK4/6 inhibitor ribociclib. PDPK1 inhibition also reduced tumor growth in cell line xenograft model *in vivo*. Moreover, we have found that a kinase dead variant of PDPK1 could also partially rescue the PDPK1 KO, suggesting important PDPK1non kinase functions. Therefore, we are now evaluating PDPK1 protein targeted degradation as a better approach for complete functional PDPK1 (and PI3K/AKT activity) elimination.

Conclusion. PDPK1 seems to be critically involved in AKT pathway activation and metabolism regulation in lymphoma and may represent an universal and effective therapeutic target.

SBORNÍK ABSTRAKTŮ

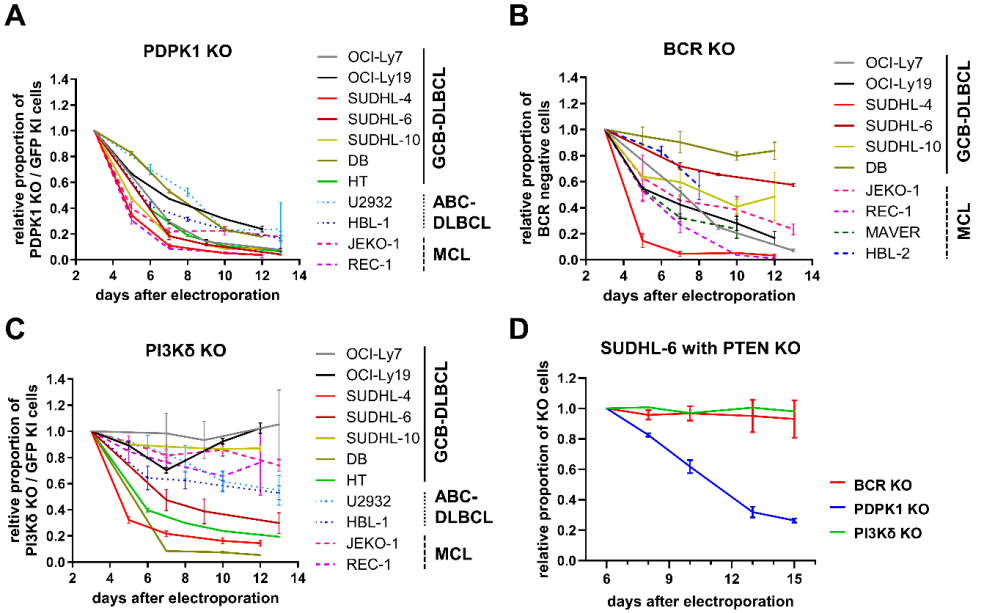

Figure 1. PDPK1 KO is uniformly toxic to all tested lymphoma lines **(A)** in comparison to variable toxicity of BCR KO **(B)** and PI3Kδ KO **(C)**. BCR and PI3Kδ KO, but not PDPK1 KO, are rescued by PTEN KO **(D)**. Proportion of KO cells over time normalized to the initial proportion determined 3 days after electroporation. Means with standard deviations of three replicates are displayed.
GCB-DLBCL - germinal center B-cell like diffuse large B-cell lymphoma, ABC-DLBCL - activated B-cell like diffuse large B-cell lymphoma, MCL- mantle cell lymphoma.

P53

TARGETED GENE EXPRESSION PROFILING FOR RAPID ABC/GCB STRATIFICATION OF DLBCL PATIENTS: EVALUATING THE PROGNOSTIC AND PREDICTIVE IMPACT OF COO SUBTYPING

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Introduction: Diffuse large B-cell lymphoma (DLBCL) is a highly heterogeneous malignancy, with distinct subtypes - activated B-cell-like (ABC) and germinal center B-cell-like (GCB) - defined by cell-of-origin (COO). These subtypes have diagnostic and therapeutic implications and are typically determined via immunohistochemistry (IHC), however, with variability in interpretation and limited accuracy. Gene expression profiling (GEP) offers a more reliable alternative but is not routinely available in diagnostic laboratories. Our aim was to establish a rapid and straightforward qPCR-based GEP test coupled with a statistical classifier to accurately stratify ABC/GCB subtypes and evaluate their clinical value.

Methods: We designed a qPCR assay targeting 28 representative genes and three housekeeping genes (*GAPDH, B2M, HPRT1*), with reagents lyophilized in triplicate on 96-well plates, enabling single-patient processing. Using frozen tissue samples from 89 DLBCL patients, we divided cohorts into training (26 ABC, 19 GCB) and validation (10 ABC, 10 GCB) sets, with an additional experimental cohort of 24 patients. Statistical analyses, including logistic/Cox regression and multigene scores, were used to develop and evaluate classification models.

Results: We established three models based on GEP results. The first model, based on IHC-determined subtypes, achieved 70% accuracy in differentiating ABC/GCB but was constrained by the inherent limitations of IHC. The second model, leveraging GEP-derived multigene scores, showed 78% concordance with IHC subtypes and identified double-expressor phenotypes (MYC and BCL2). However, COO subtyping by both IHC and GEP did not demonstrate prognostic significance for overall or progression-free survival in our cohort. Consequently, we developed a third model incorporating age and the expression of *LMO2, IL16, MYC, FN1,* and *ITPKB*, generating individualized risk scores for overall survival. The prognostic value was confirmed in the experimental cohort.

Conclusions: We developed a qPCR-based GEP test that provides reliable and cost-effective ABC/GCB stratification, suitable for routine diagnostic use. While COO subtyping may have limited prognostic value, its predictive utility is actual as novel targeted therapies for ABC DLBCL emerge. Furthermore, our prognostic model offers a robust tool for identifying high-risk patients, facilitating personalized treatment strategies.

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P54

THE EFFICACY AND TOXICITY OF CNS PROPHYLAXIS IN DIFFUSE LARGE B-CELL LYMPHOMA (CLSG-CNS-01): A RANDOMIZED, MULTICENTER, PROSPECTIVE PHASE 3 TRIAL

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The randomized, multicenter, prospective phase 3 trial (NCT02777736) compared central nervous system (CNS) prophylaxis with either intravenous (i.v.) or intrathecal (i.t.) methotrexate (MTX) in diffuse large B-cell lymphoma (DLBCL). Treatment consisted of 6 cycles of R-CHOP+2xR or DA-EPOCH-R+2xR. Patients with intermediate-or high-risk CNS-IPI were randomly assigned to CNS prophylaxis with either 2 doses of MTX 3 g/m^2 i.v. (arm A) or 6 doses of MTX 12 mg i.t. (arm B). Patients with low-risk CNS-IPI did not receive MTX prophylaxis (arm C). Primary objective was to compare cumulative incidence of CNS relapse between arm A and B. Secondary objectives included: ORR, PFS, OS, treatment safety. Overall 100 patients were enrolled between 7/2015 and 5/2024: 30 to arm A, 31 to arm B; 39

to arm C. ORR did not differ among arms (*P*=0.20). CNS relapses occurred in 3 patients after MTX prophylaxis (one in arm A, two in arm B) during median follow-up of 54.9 months. The 5-year cumulative incidence of CNS relapse between arms A and B was not significant (0% vs. 8.7%, HR 1.52, *P*=0.72). Median PFS was comparable in arms A and B (HR 0.66, *P*=0.20). CNS prophylaxis significantly increased neutropenia grade≥3 (*P*=0.0003) and infection grade≥3 (*P*=0.0063). Higher infection rate resulted in worse 5-year OS in arm A vs. B (47.2% vs. 72.4%, HR 0.46, *P*=0.04). Conclusion: cumulative incidence of CNS relapse did not differ significantly between i.v. and i.t. MTX prophylaxis. MTX i.v. delayed occurrence of CNS relapse, but it was associated with worse OS.

P55

COMPARABLE PFS IN ISOLATED PARENCHYMAL CNS VS SYSTEMIC RELAPSE OF DLBCL: RESULTS FROM A LARGE REAL-WORLD COHORT

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Introduction

Despite therapeutic advances, a significant proportion of patients with diffuse large B-cell lymphoma (DLBCL) experience disease relapse (R). Previously, we reported a 5-year cumulative incidence of systemic relapse (Sy-R) and central nervous system relapse (CNS-R) of 21.25% and 3.76%, respectively (Klanova et al., ASH 2023). Compared to Sy-R, CNS-R is generally associated with worse outcomes. Most CNS-Rs present with isolated CNS involvement (CNS-I-R), while a smaller subset develops concurrent CNS and systemic R (CNS+Sy-R). Based on localization, CNS-R can affect parenchymal (Par), leptomeningeal, or both sites (Lep/Comb). This study compared clinical outcomes of Sy-R, CNS-I-R (further divided into CNS-I- Par and CNS-I-Lep/Comb), and CNS+Sy-R in a large real-world DLBCL cohort.

Methods

Using the prospective NiHiL project (NCT03199066), we identified 1,364 patients with systemic DLBCL who experienced their first R between 2005 and 2023. Patients with CNS involvement at initial diagnosis or incomplete staging at R were excluded. Among the included patients (n=1,351), 1,186 (87%) had Sy-R, 127 (9%) had CNS-I-R (CNS-I-Par n=92; CNS-I-Lep/Comb n=34; unknown localization n=1), and 38 (3%) had CNS+Sy-R. In total, 1,153 patients received chemotherapy (CTx), including 901 treated with curative intent.

We first compared outcomes across Sy-R, CNS-I-R, and CNS+Sy-R. Subsequently, CNS-I-R cases were stratified by localization (CNS-I-Par vs CNS-I-Lep/Comb). The primary endpoint was progression-free survival (PFS); overall survival (OS) was the secondary endpoint, both measured from the date of R. Univariate and multivariate Cox regression analyses were performed.

Results

When comparing Sy-R, CNS-I-R, and CNS+Sy-R, the median time from diagnosis to relapse was 12.3, 14.2, and 9.3 months, respectively (P=0.01); median age at R was 68, 68, and 64.5 years, respectively (P=0.04). ECOG performance status (PS) 2–4 was more frequent in R involving the CNS (CNS-I-R 65%, CNS+Sy-R 63%) than in Sy-R (32%; P<0.01). In contrast, elevated LDH was more common in relapses with Sy involvement (Sy-R 67%, CNS+Sy-R 61%) compared to CNS-I-R (43%; P<0.01). With a median follow-up of 7.6 years, median PFS decreased across Sy-R, CNS-I-R, and CNS+Sy-R: 8 vs 6 vs 3 months (P<0.01), and median OS was 13, 6, and 3 months, respectively (P<0.01).

We then focused on CNS-I-R and compared outcomes by localization. Patients with CNS-I-Par (73%) were older (median age 70 vs 62.5 years; P=0.048) and had longer time from diagnosis to relapse (15.75 vs 8.15 months; P<0.01) than those with CNS-I-Lep/Comb (27%). Median PFS and OS for CNS-I-Par vs CNS-I- Lep/Comb were 7 vs 4 months (HR=0.49, P<0.01) and 8 vs 4 months (HR=0.48,P<0.01), respectively.

When comparing all four relapse types (Sy-R, CNS-I-Par, CNS-I-Lep/Comb, CNS+Sy-R), Sy-R and CNS-I-Par had the most favorable outcomes with similar PFS (median 8 vs 7 months; HR=0.98, P=0.87) and modestly better OS for Sy-R (median 13 vs 8 months; HR=0.77, P=0.04). CNS-I-Lep/Comb and CNS+Sy-R had similarly poor outcomes (median PFS 4 vs 3 months, HR=0.99, P=0.95; median OS 4 vs 3 months, HR=0.85, 95% CI 0.52–1.38, P=0.51).

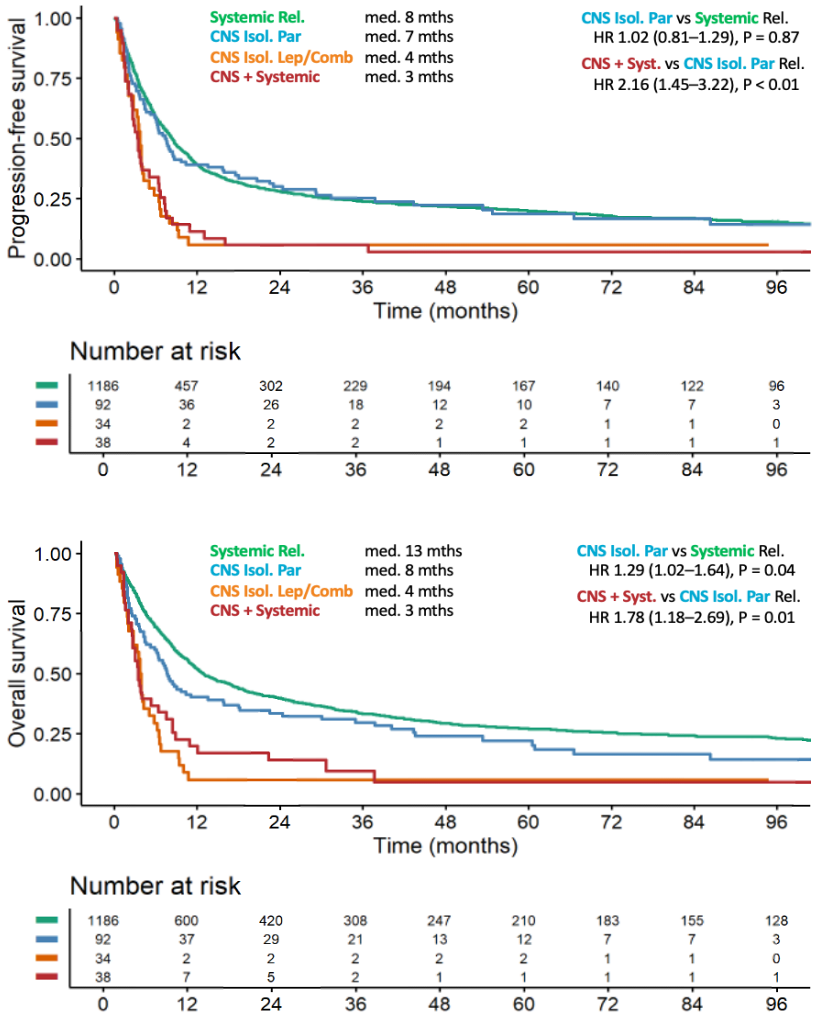
After adjusted for age, ECOG PS, LDH, and diagnosis-to-relapse interval, survival was similar between CNS-I-Par and Sy-R (PFS P=0.63; OS P=0.15). However, both CNS-I-Lep/Comb (PFS HR=1.82, P=0.01; OS HR=1.55, P=0.05) and CNS+Sy-R (PFS HR=1.60, P=0.01; OS HR=1.69, P=0.01) remained independently associated with worse survival compared to Sy-R. These findings were consistent in the curatively treated subgroup (n=901):

median PFS 10, 8, 4, and 3 months, median OS 19, 10, 4, and 4 months, resp., for Sy-R, CNS-I-Par, CNS-I-Lep/Comb, CNS+Sy-R resp. (all P<0.01).

Conclusion

While all DLBCL relapses are associated with poor prognosis, clinical outcomes vary by relapse pattern. Sy-R and CNS-I-Par showed comparable PFS, and better survival compared to other subtypes,. In contrast, CNS-I-Lep/Comb and CNS+Sy-R were associated with worst outcomes. The OS in CNS-R cases closely mirrors the PFS, suggesting that treatment remains challenging, and therapeutic failure often represents a terminal event. Novel approaches, including CNS-penetrating targeted agents and CAR-T, which show early promise in CNS-Rs, are needed for these patients.

Figure 2. Survival curves.



P56

IMPACT OF PRIMARY CNS PROPHYLAXIS IN HIGH CNS-IPI DLBCL: ANALYSIS FROM THE CZECH NATIONAL LYMPHOMA REGISTRY

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Background: Central nervous system (CNS) progression/relapse of diffuse large B-cell lymphoma (DLBCL) is a rare but fatal event. As conventional chemotherapeutics used in 1st line treatment do not effectively cross the blood-brain barrier, prophylactic methotrexate (MTX) or cytarabine (AraC) can be added intravenously or directly as intrathecal injection to selected patients (pts) based on risk factors.

However, the efficacy of this practice remains controversial. Here, we evaluate the effect of primary CNS prophylaxis on a large cohort of Czech pts with DLBCL treated with R-CHOP by comparing outcomes between pts who received CNS prophylaxis and those who did not, as well as among subgroups defined by different prophylactic drugs, doses, and timing of administration.

Methods: The studied cohort consisted of consecutively diagnosed DLBCL patients enrolled in the prospective Czech National Lymphoma Project (NiHiL, NCT03199066). Individual patient records were used to complete missing data and obtain additional information on CNS prophylaxis. In total, 1196 pts with DLBCL diagnosed between 2010 and 2021 and treated initially with R-CHOP, either at the Charles University General Hospital in Prague or the University Hospital Brno, were included, of which 338 had low (0-1), 528 intermediate (2-3), and 330 high (>3) CNS-IPI, respectively.

The endpoints were cumulative incidence (CI) of CNS progression/relapse, progression-free survival (PFS) and overall survival (OS). We focused mainly on the high CNS-IPI patient subgroup.

Results: Median follow-up was 6.2 years (yrs, range 0.06-14.51). The 5-year CI of CNS progression/relapse was 3.4% in the whole cohort and 8.8% in the high CNS-IPI subgroup. In this subgroup, 158 pts (47.9%) received CNS prophylaxis according to institutional guidelines: intravenous +/- intrathecal (IV+/-IT, n=100), only intrathecal (IT, n=58); 172 pts received no prophylaxis. IV prophylaxis was typically administered as 2-3 cycles of R-HD-MTX or R-HD-AraC, and patients were grouped according to if prophylaxis was either intercalated in/alternating with R-CHOP (I/A, n=43), or administered as separate cycles at the end of treatment with R-CHOP (EoT, n=57).

High CNS-IPI pts with IV+/-IT (n=100) and IT/no prophylaxis (n=230) were compared. Baseline parameters – median age (65.5 and 67.2 yrs resp., range 25-84), clinical stage, ECOG PS, diagnosis-to-treatment interval (DTI), cell-of-origin (COO) and double expressor (DE) status – were similar between groups.

There was no difference in the CI of CNS progression/relapse between IV+/-IT group and IT/no prophylaxis group with 9.5% and 10.1% at 5 yrs resp. We have observed better PFS (median 10.99 vs 2.99 yrs; p=0.002, HR=0.592, 95% CI 0.424-0.827) and OS (median 10.86 vs 5.59 yrs; p=0.005, HR 0.609, 95% CI 0.428-0.867) in favor of the IV+/-IT cohort. Because of inherent bias (pts without R-CHOP failure were more likely to have received IV prophylaxis at the end of treatment with R-CHOP) a subgroup of pts who reached complete remission (CR) at the end of 1st line treatment (n=203) was further analyzed – here, there was no significant effect of IV prophylaxis on either CI of CNS relapse (5.6% vs 7.3% at 5 yrs, HR=1.029, 95% CI 0.34 - 3.06), PFS

or OS. Furthermore, there was no significant difference in PFS, OS or CI of CNS progression/relapse between I/A and EoT IV groups.

In the whole patient cohort, diagnosis-to-prophylaxis interval and start-of-treatment-to-prophylaxis interval did not have significant impact on CNS progression. No difference in efficacy between MTX (n=131) and AraC prophylaxis (n=66) in preventing CNS progression/relapse, and no clear relationship between the dose of either IV MTX or AraC and risk of CNS progression was observed.

Conclusions: In our cohort, the addition of IV CNS prophylaxis to standard 1st line therapy of systemic DLBCL in high CNS-IPI patients did not seem to reduce the risk of secondary CNS progression. Moreover, we did not observe any impact of IV CNS prophylaxis dosage, timing, or choice of chemotherapeutic agent.

These data are consistent with a growing body of evidence suggesting that IV CNS prophylaxis may be an ineffective practice in 1st line DLBCL treatment, but prospective studies are needed to confirm this hypothesis.

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P57

VITAMIN D AND OTHER MICRONUTRIENTS INFLUENCE PROGNOSIS OF PATIENTS WITH NON-HODGKIN LYMPHOMA (NHL)

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Introduction: Lymphocytes, including lymphoma cells require many micronutrients to grow and differentiate properly. Vitamin D is one of the most studied micronutrients in this context. Its deficiency is often related to a worse prognosis, but clinical trials with vitamin D substitution produced controversial results. We hypothesized that vitamin D, but also other micronutrients may play a role in lymphoma prognosis. We analyzed a set of selected micronutrients (vitamin D, vitamin B12, folate, zinc, and selenium) in a robust cohort of lymphoma patients.

Methods: We present single-center retrospective analysis of prospectively collected data on consecutively included patients with newly diagnosed NHL. Blood samples for micronutrient analysis were taken once before the start of any therapy; basic clinical and demographic data were obtained from the Czech Lymphoma Study Group registry. First, we tested the relationship between each micronutrient and clinical parameters. Next, we analyzed if the deficit of one micronutrient is associated with others, and calculated their impact on survival parameters (progression-free survival, PFS; overall survival, OS). The project was approved by ethical board of University Hospital Brno, Czech Republic.

Results: In total, we recruited 1196 lymphoma patients newly diagnosed between 05/2011 and 06/2020. Median age at diagnosis was 65 years; 49.5% were males. The median follow-up of the cohort is 3.9 years (0.003-13.01), 334/1196 (27.9%) patients died (median follow-up of living patients is 4.6 years; range 0.05-11.88). There were 474 (39.6%) patients with diffuse large B-cell lymphoma (DLBCL), 220 (18.4%) with follicular lymphoma (FL), 93 (7.7%) with mantle cell lymphoma (MCL), 135 (11.3 %) with marginal zone lymphoma (MZL), 201 (16,8%) with other subtype of B-NHL, 73 (6.2%) with T-non-Hodgkin lymphoma (T-NHL). Clinical stage ≥3 was present in 724 (60,5%) pts, ECOG ≥2 was in 251 (21%) pts, and elevated LDH in 795 (66,5%) of cases. Vitamin D deficiency (≤50 nmol/L) was found in 59.9% of patients at diagnosis, including severe deficiency (<30 nmol/L) in 32% of cases. Selenium deficiency (<0.66 µmol/L) was present in 31.7%, zinc deficiency (<10 µmol/L) in 18.7%, and vitamin B12 deficiency (<145 pmol/L) in 6.6% of patients, whereas folate was normal in 99,6% of patients. According multivariate analysis, deficit of vitamin D was strongly associated with deficit of selenium, zinc, folate, hypoalbuminemia ($p<0.001$) and elevated CRP ($p<0.001$). Vitamin D, zinc and selenium levels were significantly lower in older patients, those with aggressive lymphoma subtype, higher LDH, and ECOG ≥2 (all $p<0.0001$).

Vitamin D deficiency was strongly associated with shorter OS (median 8,9 yrs vs. not reached (NR); $p<0.0001$) and PFS (median 5,06 yrs vs. NR; $p<0,0001$). Moreover, patients with blood zinc levels <10 µmol/L and selenium <0.66 µmol/L also had shorter median OS (4.4 vs. 8.9 yrs and 2.6 vs. 8.9 respectively) and PFS (2.4 vs. 13.1 yrs and 1.7 vs. 13.0 yrs; all $p< 0.001$). Vitamin B12 and folate levels showed no significant prognostic association.

Based on results above, we constructed a cumulative “micronutrient score”, which included vitamin D (cut-off 40 nmol/L), selenium (cut-off 0.66 µmol/L) and zinc (cut-off 10 µmol/L) in 659 pts with all three parameters known. Patients without any deficiency (n=227) had significantly better OS and PFS (medians NR) versus patients with lack of one (n=211), two (n=146) or all three nutrients (n=75), with respective median OS 8.9 yrs, 4.78 yrs and 1.23 yrs ($p<0.0001$), and PFS 13,01 yrs, 2,4 yrs, and 0,93 yrs ($p<0.0001$). We calculated the same score for patients with DLBCL (n=269) and FL (n=114) separately. For DLBCL pts with 0, 1, 2 or 3 micronutrient deficiencies, median OS were NR vs. 8.9 vs. 4.42 vs. 1.31 yrs ($p<0.0001$), and median PFS were NR vs. NR vs. 2.4 vs. 0.99 yrs ($p<0.0001$). For FL, the differences were not significant.

Conclusion: Deficiencies of vitamin D and immune micronutrients (especially selenium and zinc) are common in lymphoma patients at diagnosis and are associated with higher disease aggressivity and reduced survival. Routine assessment of these deficiencies may help to identify patients at high risk, and substitution could help improve treatment results by optimizing immune and regenerative functions. Prospective trials are needed to confirm our results.

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On behalf of Czech Lymphoma Study Group

P58

EPCORITAMAB COMBINATIONS DEMONSTRATE PROMISING EFFICACY IN PATIENTS (PTS) WITH RICHTER TRANSFORMATION (RT): FIRST RESULTS FROM ARMS 2B (EPCOR + LENALIDOMIDE [LEN]) AND 2C (EPCOR + R-CHOP) OF THE PHASE 1B/2 EPCORE CLL-1 TRIAL

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Introduction: RT is an aggressive transformation of chronic lymphocytic leukemia (CLL), most often into diffuse large B-cell lymphoma (DLBCL), and is associated with poor prognosis and limited treatment (tx) options. Epcor, a CD3×CD20 bispecific antibody, showed promising efficacy with a 52% complete response (CR) rate in first-line (1L) RT (Kater AP, iwCLL 2025). We report efficacy and safety of epcor+LEN or epcor+R-CHOP in 1L/2L RT from Arms 2B and 2C of the phase 1b/2 EPCORE® CLL-1 trial (NCT04623541).

Methods: Pts had biopsy-proven CD20⁺ DLBCL-RT and history of CLL or small lymphocytic lymphoma (SLL). In Arm 2B, chemotherapy-ineligible pts (1L/2L for RT) received SC fixed-duration epcor 48mg in 28-d cycles (C; QW, C1–3; Q4W, C4–26) with LEN (day [D]1–22, C1–C12). In Arm 2C, chemotherapy-eligible pts received fixed-duration epcor in 21-d Cs (QW, C1–4; Q3W, C5–6; Q4W, in 28-d Cs from C7+ up to 1y) with R-CHOP (Q3W, C1–6). Step-up dosing (0.16mg, 0.8mg) and corticosteroid prophylaxis were used to minimize cytokine release syndrome (CRS). Primary endpoint was ORR by PET-CT (Lugano criteria) per investigator.

Results: At data cutoff (DCO; June 2, 2025), 11 and 30 pts received tx in Arms 2B and 2C, respectively. In Arm 2B, median age was 74y, 6 (55%) pts were male, 9 (82%) had Ann Arbor stage III–IV, and 6 (55%) had *TP53* aberrations in screening DLBCL biopsy. Prior CLL/SLL tx was received by 6 (55%) pts (median prior lines of tx [pLOTs], 2.5) and RT-directed tx by 5 (45%) pts. Median time from CLL diagnosis to RT was 8.3y and from RT to first study dose was 1.2mo. At DCO, 5 pts remained on tx and 6 discontinued (d/c) tx (PD, 3; clinical progression, 1; AE, 1; ASCT, 1). With median follow-up (mFU) of 12.6mo (range, 0.3+ to 16.9), ORR was 82% (95% CI, 48–98) and CR rate was 73% (95% CI, 39–94). Estimated median duration of response (mDOR) and complete response (mDOCR) were 7.4mo (95% CI, 1.6–NR; 5/9 events)

and 7.4mo (95% CI, 1.4–NR; 4/8 events); 9-mo estimates were 50% each. Estimated median PFS and OS were 5.7mo (95% CI, 1.5–NR; 7/11 events) and NR (95% CI, 7.6–NR; 2/11 events); 9-mo estimates were 41% and 82%, respectively. Pharmacodynamic biomarkers will be presented. Common TEAEs were CRS (11 [100%]), neutropenia (9 [82%]), thrombocytopenia (8 [73%]), anemia, and hypokalemia (5 [45%] each). Grade (Gr) ≥3 TEAEs occurred in all pts, serious TEAEs in 10, and epcor-related d/c and fatal TEAEs in 1 pt each. CRS events were primarily low Gr (Gr1, 5; Gr2, 4; Gr3 and 4, 1 each) and resolved in 10 pts (median time to resolution [mTTR], 4.5d); 1 pt d/c due to CRS. ICANS occurred in 2 pts (Gr1/2 and resolved; mTTR, 2.5d). In Arm 2C, median age was 72y, 22 (73%) pts were male, 26 (87%) had Ann Arbor stage III–IV, and 9 (30%) had *TP53* aberrations in screening DLBCL biopsy. Prior CLL/SLL tx was received by 17 (57%) pts (median pLOTs, 1.0) and 3 (10%) had RT-directed tx. Median time from CLL diagnosis to RT was 4.4y and from RT to first study dose was 1.1mo. At DCO, 10 pts remained on tx, 5 completed tx, and 15 d/c tx (PD, 8; clinical progression, 1; AEs, 5; ASCT, 1). With mFU of 10.1mo (range, 0.5+ to 19.6), ORR was 73% (95% CI, 54–88) and CR rate was 60% (95% CI, 41–77). Estimated mDOR and mDOCR were 15.0mo (95% CI, 4.3–NR; 7/22 events) and 13.4mo (95% CI, 4.1–NR; 4/18 events); 9-mo estimates were 64% and 71%, respectively. Estimated median PFS and OS were 9.9mo (95% CI, 3.5–NR; 14/30 events) and 16.4mo (95% CI, 9.6–NR; 11/30 events); 9-mo estimates were 56% and 72%, respectively. Common TEAEs were CRS, anemia, and neutropenia (18 [60%] each), diarrhea (10 [33%]), and febrile neutropenia (9 [30%]). Gr ≥3 TEAEs occurred in 27 (90%) pts, serious TEAEs in 25 (83%), epcor-related d/c in 3 (10%), and fatal TEAEs in 3 pts (1 epcor related). CRS events were primarily low Gr (Gr1 and 2, 8 each; Gr3, 2; Gr ≥4, 0); all events resolved with mTTR of 3.0d and no pts d/c due to CRS. ICANS occurred in 4 pts (Gr1, 3; Gr3, 1); 3 cases resolved (mTTR, 1.0d) and 1 was ongoing at time of death. No CTLS was reported in either study arm.

Conclusions: Epcor+LEN or epcor+R-CHOP showed encouraging efficacy with ORRs of 82%/73% and CR rates of 73%/60% in RT. Epcor+R-CHOP showed promising durability, with most responses still ongoing. The safety profiles for both combinations were manageable and consistent with known profiles of each agent. These results support the combinability of epcor with established regimens and its potential as a core therapy in RT.

P59

CLONAL SHIFTS OF TP53 MUTATIONS IN PATIENTS TREATED WITH BTK AND BCL2 INHIBITORS: ANALYSIS OF ACCOMPANYING GENOMIC CHANGES

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Background:
The selective advantage of *TP53*-deficient clones is unambiguous in patients receiving chemoimmunotherapy (CIT), where minor *TP53*-mutated subclones frequently undergo expansion. In contrast, the diminishing of the *TP53*-deficient cell population is extremely rare in the CIT context. Drugs inhibiting BcR signaling or BCL2 are efficient even in patients with disrupted p53 pathway. Although *TP53* remains a marker of aggressive disease, its aberrations confer less selective advantage under these treatments. Still, clonal shifts involving *TP53* mutations have been occasionally observed. The extent to which additional genomic alterations contribute to such shifts, and which genomic lesions might outcompete *TP53*-dysfunctional clones, is not yet fully understood.

SBORNÍK ABSTRAKTŮ

Objectives:
We aimed to investigate mechanisms underlying pronounced clonal shifts of *TP53*-mutated clones during treatment with BTK (BTKi) or BCL2 inhibitors (BCL2i), with the focus on accompanying genomic changes that may drive or counteract *TP53* clonal dynamics.

Methods:
In a single-center cohort of 171 patients with CLL treated with targeted agents, *TP53* mutations were repeatedly tested using NGS on purified B-cells with a limit of detection of 0.3% variant allele frequency (VAF). Most patients previously received chemo(immuno)therapy (96%; range 1-10 prior lines, median 2). *TP53* mutations were detected in 108 patients (63%). Serial samples from selected cases with marked *TP53* clonal shifts underwent genomic profiling using the NGS panel LYNX (PMID: 34082072) covering 72 lymphoma-associated genes and genome-wide chromosomal aberrations, and/or whole-exome sequencing (WES) combined with genomic arrays. Single-cell DNA sequencing (Tapestri CLL panel) was carried out in one representative case.

Results:
Three main patterns of clonal evolution, i.e., diminishing, increase, and exchange of *TP53*-mut clone, were identified in 18 patients. The first group included four cases in which the TP53 mutated population diminished from a baseline VAF>5% (BTKi n=2; BCL2i n=2). In two out of three cases with available detailed analysis, the outcompeting wt-*TP53* population carried alterations previously associated with CLL progression and treatment resistance, i.e., highly complex karyotype with *CDKN2A/B* biallelic loss in one case, and *BCL2* mutation the other. The cause of *TP53*-mut clone regression in the third case (VAF decrease from 28% to 1%) remained unexplained.

The second group involved eight patients with a significant increase of *TP53* mutation (VAF_timepoint2 – VAF_timepoint1 >20%; BTKi: n=6; BCL2i: n=2). In three cases with detailed analysis, one showed an acquired *BTK* resistance mutation within the expanding *TP53*-mutant clone, while two others developed complex genomic alterations: chromothripsis of chromosome arm 1q and a highly complex karyotype with del(17p13), respectively.

The third group comprised six cases (BTKi: n=3; BCL2i: n=3) with inter-clonal *TP53* competition and replacement of the dominating *TP53*-mutant clone. The clonal exchange in three out of four patients analyzed in detail was accompanied by the emergence of two predominant *BTK* mutations, several subclonal mutations in *BCL2*, and a highly complex karyotype with del(17p13), respectively. No additional changes were identified in the fourth case. In a representative case from this group, single-cell DNA sequencing revealed convergent evolution of resistance driven by the emergence of four independent *BTK* Cys481Ser-mutated clones, collectively accounting for 99% of CLL cells (c.1442_1443delinsCT, and three clones carrying c.1442G>C). Each of these mutations was acquired within a clone carrying different *TP53* mutation. Further clonal evolution led to the acquisition of mutation in the *SPEN* gene, transcriptional repressor of NOTCH1 target genes, likely contributing to the dominance of one *TP53/BTK*-mutant clone representing 70% of CLL cells.

Conclusion:
Pronounced clonal shifts of *TP53* mutations during treatment with BTK or BCL2 inhibitors are infrequent, even in this heavily pretreated cohort. In contrast to CIT, a significant reduction of *TP53*-mutant population can occur under targeted therapy but is typically associated with expansion of aggressive *TP53* wild-type clones that acquired resistance-associated genomic alterations. In cases of *TP53*-mut clonal expansion or competition, we frequently observed the emergence of complex karyotype with del(17p13), and/or resistance mutations in *BTK* or *BCL2*. Single-cell analysis confirmed that targeted therapies exert strong selective pressure, promoting convergent evolution of resistance-associated mutations independently across multiple clones.

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P60

CASEIN KINASE 1Δ/E INHIBITION MEDIATES CHRONIC LYMPHOCYTIC LEUKEMIA SUPPRESSION BY A COMBINATION OF CELL CYCLE BLOCK AND DECREASED RESPONSE TO MICROENVIRONMENTAL STIMULI

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Chronic lymphocytic leukemia (CLL) is a haematological malignancy characterized by monoclonal B cell expansion and accumulation in peripheral blood and lymphoid organs. Despite large amount of effort invested in search of therapeutic options for CLL patients, CLL is still one of the most common health issues in the adult population. Due to stable 20% relapse rate, it is certainly a necessity to continue in development of more efficient therapeutic agents suitable for CLL treatment.

Wnt signalling is one of the drivers of CLL progression, mainly due to its positive impact on CLL cell motility and invasiveness. In attempt to suppress CLL, our research group has shown earlier that inhibition of casein kinase 1δ/ε (CK1δ/ε), the key Wnt pathway components, leads to improved overall survival in the Eμ-TCL1mouse model of CLL.

In this follow-up study, we were interested in mechanisms underlying the effect of CK1δ/ε inhibition on CLL suppression. Using the combination of *in vivo* experiments with Eμ-TCL1 mouse model and *in vitro* co-cultivation assays with CLL patient cells, we show that CK1δ/ε inhibition counteracts CLL progression on multiple levels. Firstly, CK1δ/ε inhibition causes slower cell cycle progression with the arrest in late S and G2 phase, which leads to decelerated CLL cell proliferation. Secondly, CK1δ/ε inhibition decreases CLL cell responsiveness to extracellular stimuli via inhibition of NFκB pathway. Finally, CK1δ/ε inhibition resulted in modulation of CLL microenvironment in the lymph nodes – the primary CLL expansion site in patients – where decrease of CD4+ and CD8+ T cells was observed.

P61

MEASURABLE RESIDUAL DISEASE IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) IN CLINICAL PRACTICE: A SINGLE-CENTER REPORT OVER THE YEARS 2018–2024

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Introduction

The European Medicines Agency has approved the assessment of measurable residual disease (MRD) as a surrogate marker of treatment response in patients (pts) with chronic lymphocytic leukemia (CLL) treated within clinical trials. In routine clinical practice, MRD monitoring is not systematically implemented. The clinical relevance of MRD-guided therapy, particularly in modern treatment regimens, remains uncertain.

SBORNÍK ABSTRAKTŮ

Methods
Newly diagnosed (ND) and relapsed/refractory (RR) CLL patients (pts) who achieved at least partial remission (PR) and had an absolute lymphocyte count (ALC) below 4×10^9/L underwent MRD assessment using 8-color flow cytometry CD81/CD43/CD79b/CD22/CD5/CD19/CD20/CD3) (Hallek et al., 2018; Rawstron et al., 2016), with a sensitivity of at least 10^{-4}. Patients were treated in a real-world (RWE) setting, and clinical outcomes were assessed according to the iwCLL 2018 response criteria. The initial MRD analysis in peripheral blood (PB) was performed no earlier than 3 months after the start of treatment, with follow-up monitoring every 3–6 months. More than 450 peripheral blood (PB) samples were assessed. Bone marrow aspirates from 38 selected pts have been analyzed so far to confirm MRD status in the BM compartment upon achievement of complete remission (CR). Progression-free survival (PFS) was calculated from the start of treatment.

Results
154 CLL pts (ND and RR) treated between 2018 and 2024 underwent MRD assessment in PB. In pts receiving fixed-duration therapy, MRD assessment at 6th or 12th month (CHT or Ven) after treatment initiation was used to evaluate MRD status. Patients on continuous BTKi therapy were evaluated for MRD after fulfilling defined criteria (achieving ≥ PR and ALC $<4\times10^9$/L) with a median time to MRD assessment of 24.5 months (range 3-72.8).

The most common regimens used in the MRD-negative (MRDneg) cohort were venetoclax-based regimens (Ven) in 65% of patients. The median limit of detection (LOD) for the flow cytometry (MFC) technique was 0.003%, and the median follow-up was 34.4 months. Overall, 80 (52%) of 154 pts were considered MRDneg, which was associated with an improved PFS (HR = 0.59; 95% CI 0.35–0.97; p = 0.035). Estimated median PFS was 62.2 months for MRDneg pts vs 42.0 months for MRDpos pts (Figure 1). Among high-risk patients with unmutated IgVH, longer PFS was observed in the MRDneg group. In the overall cohort, TP53 aberration was present in 29/154 cases (18.8%), making MRD-based analyses within this subgroup underpowered, and no statistically significant differences were observed.

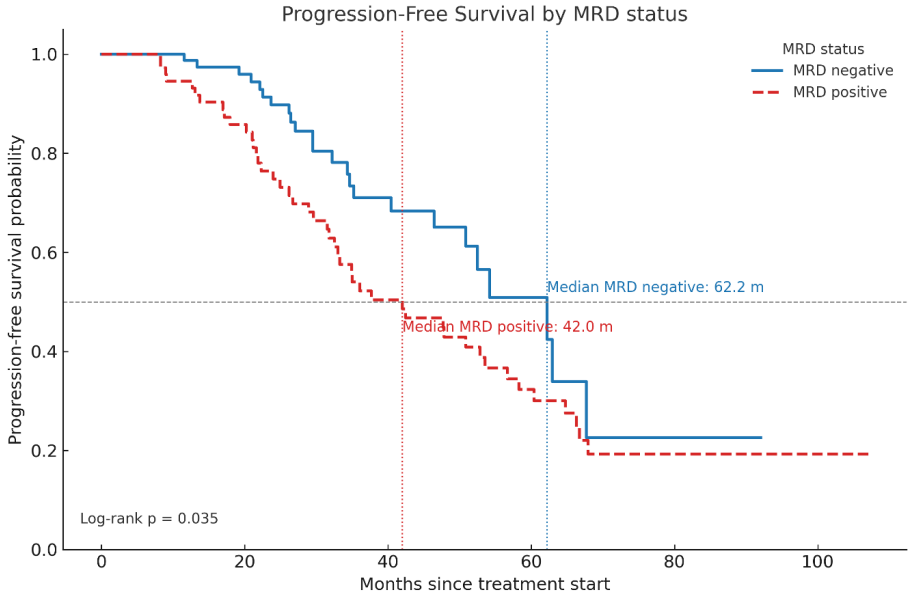
NDCLL (N=82; MRDneg 57.3%). Ven was used more often in MRDneg than MRDpos pts (51.3% vs 25.0), CHT predominated in MRDpos (68.2% vs 48.7%%; p=0.013). Median PFS was not reached (NR) with Ven in either MRD cohort and was 33.2 vs 46.4 months (p = 0.419) in CHT MRDpos vs CHT MRDneg, respectively.

RRCLL (N=72; MRDneg 45.8%): Ven was more common in MRDneg pts (73.5% vs 21%), BTKi predominated in MRDpos pts (60.5% vs 8.8%; p<0.001). In the MRDneg group, median PFS was not reached (NR) with Ven vs 30 months with CHT . Median PFS was also NR with BTKi; however, this subgroup included only 4 pts. Among MRDpos patients, the longest PFS was observed with BTKi (60 vs 33 vs 20 months for BTKi, Ven, and CHT).

MRD in BM and MRD kinetics: Among patients in CR who underwent BM MRD assessment, PB and BM MRD negativity were concordant in 24/35 cases (68.6%), with the highest MRD negativity rates observed in Ven. Sustained MRDneg was observed in 30/47 (63.8%) and 11/33 (50%) of ND and RRCLL pts, respectively.

Conclusion
In this RWE analysis, MRD negativity was associated with longer PFS, even in high-risk pts with unmutated IgVH. Ven-based regimens, achieved the highest MRDneg rates, followed by CHT , while nearly all pts on BTKi remained MRD positive. This is in line with clinical trials and RWE data. The main drawbacks were short follow-up and cohort heterogeneity, limiting statistical power. Relatively frequent MRDneg loss over time underscores the need for more sensitive thresholds (i.e. $<10^{-5}$) to improve patient stratification and guide treatment decisions in routine practice. Updated data will be presented at the conference.

SBORNÍK ABSTRAKTŮ



Group	0	12	24	36	48	60	72	84	96
MRD negative	80	77	56	30	19	7	2	1	0
MRD positive	74	67	47	30	22	14	7	6	4
Total	154	144	103	60	41	21	9	7	4

P62

CLONALITY ASSESSMENT IN MULTICLONAL CHRONIC LYMPHOCYTIC LEUKEMIA USING SANGER AND NEXT-GENERATION SEQUENCING APPROACHES

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Chronic lymphocytic leukemia (CLL) is typically characterized by the presence of a dominant B-cell clone with a unique immunoglobulin heavy-chain (IGH) gene rearrangement. However, a subset of patients exhibits multiple clonal IGH rearrangements, reflecting underlying multiclonality. Understanding the clonal architecture and its evolution over time is essential for insights into disease biology and progression.

In a cohort of 3,167 CLL patients, routine diagnostic assessment of IGHV mutational status was performed using cDNA and Sanger sequencing. Among these, 249 patients (8%) exhibited multiple clonal productive IGH gene rearrangements. Specifically, we identified 224 biclonal, 23 triclonal, and 2 tetraclonal CLL cases. In 173/249 cases, the co-detected clones were concordantly mutated (115) or unmutated (58). The remaining 76 cases showed discordant mutational status among the clones. IGHV mutational status was examined repeatedly over time in 100/249 multiclonal CLL patients, with a median interval of 3.4 years between the first and last examinations. Over time, one or more clones disappeared in 53 patients, while in 21 patients a new clone emerged later (17/21 patients were monoclonal at diagnosis). In 5 cases, the original dominant clone was replaced by another.

To gain a deeper insight into the genomic clonal composition of multiclonal CLL cases, we analyzed tumor DNA from a representative cohort of 87 such cases (35% of the multiclonal cohort) with a capture-based NGS panel LYNX. This panel allows for the detection of markers associated with the most common lymphoid malignancies, including antigen receptor rearrangements. In parallel, we compared detected clonotypes with results from amplicon IGH NGS. The median number of fragments covering complete IGH rearrangements in the LYNX panel was 248, while the median number of usable reads in IGH NGS was 1.7 million. The most abundant clonotype expected was detected in all 87 cases using both methods. Using LYNX, a second expected clone was confidently detected in 53 out of 87 (61%) cases, and in an additional 10 cases below the confident detection threshold (defined as ≥3 fragments and >1% of clonal productive IGH rearrangements). IGH NGS identified the expected second clone in 53/87 (61%) cases at ≥1% of productive IGH rearrangements, and in other 22 cases at levels below 1%. Using either method, we identified additional clonal rearrangements (>1%) that were missed by Sanger sequencing—11 cases using LYNX and 17 using IGH NGS. Notably, in cases where the Sanger-identified clones had concordant IGHV mutational status, all newly identified rearrangements also matched in mutational status: 11 in LYNX and 12 in IGH NGS.

We also performed amplicon IGH NGS analysis on a cohort of 62 consecutive CLL cases, seemingly monoclonal by Sanger IGH analysis. In 60 out of 62 cases, the expected rearrangement was the dominant clone. The remaining two cases exhibited a highly polyclonal pattern. In 6 of the 60 cases, an additional clone representing more than 1% of productive IGH rearrangements was detected. In 5 of these 6 cases, the newly identified clone had a concordant IGHV mutational status with the concurrent dominant clone. Notably, three of the additional clonal rearrangements had a relative size >25%, and all three belonged to the same IGHV family as the respective dominant rearrangements.

Our results provided a coherent clonality assessment in the analyzed multiclonal CLL cases, with notable prevalence of concordant IGHV mutational status among co-detected clones.

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P63

VALIDATION OF NOVEL PROGNOSTIC MODELS IN SYMPTOMATIC WALDENSTRÖM'S MACROGLOBULINEMIA BASED ON REAL-WORLD DATA

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Introduction

Waldenström's macroglobulinemia (WM) is a rare, indolent, IgM-producing lymphoplasmacytic lymphoma with diverse clinical outcomes. Prognostic models have evolved alongside treatment shifts from chemotherapy to chemoimmunotherapy and targeted therapies. In addition to the original International Prognostic Scoring System for WM (IPSSWM), newer models such as the revised IPSSWM (rIPSSWM) and the Modified Staging System for WM (MSSWM) have been developed, incorporating markers such as lactate dehydrogenase and albumin. However, their performance has not yet been thoroughly validated.

Aim: To evaluate the performance of prognostic models in a large, real-world patient cohort and to examine treatment patterns and survival outcomes of symptomatic WM (sWM) in the Czech Republic over the past ten years.

Methods

sWM patients who started first line (L1) treatment between January 2014, and January 2024 were included. The primary data source was the Czech Myeloma Registry and Czech Lymphoma Study Group Project (NCT03199066). The primary endpoint was overall survival (OS) (calculated from L1 initiation), secondary endpoints were preferred regimen, treatment responses and progression-free survival (PFS). Patients with complete data for scoring systems were included in multivariable analysis and validation of existing prognostic models. The Kaplan-Meier method was used for survival analysis, and groups were compared using pairwise log-rank tests with Benjamini-Hochberg correction.

Results

In total, 315 patients were included in the analysis (median age 69 years at L1 initiation, 58% male). Molecular genetic testing data for MYD88 and CXCR4 were available for 143 (45%) and 27 (11%) patients, with 137 (96%) and 11 (41%) positively tested for MYD88 and CXCR4 mutations, respectively. Cytopenia, IgM-associated complications, and B symptoms were the most frequent indications for treatment. In the front-line setting, 96% of patients received chemoimmunotherapy (76% cyclophosphamide, 22% bendamustine, and 2% bortezomib-based combinations), 2% rituximab monotherapy and 2% other combinations. With a median follow-up (FU) of 4.8 y (IQR 2.8; 6.8) the overall response rate (ORR) was 86% (257/296 patients with known response: CR 12%, VGPR 17%, PR 67%). Median PFS was 5.3 years (4.5-7.1 years, 95% CI). The 3-year and 5-year probability of OS were 87% (84–91%, 95% CI) and 80% (75–86%, 95% CI), respectively. Among 76 patient deaths 26 (34%) was directly related to WM (progression, histologic transformation, treatment related complications, secondary malignancies).

We were able to evaluate the IPSSWM risk score in 304 (97%) patients (low 16%, intermediate 36%, high 48%), rIPSSWM in 291 (92%) patients (very low 15%, low 23%, intermediate 29%, high 21%, very high 12%) and MSSWM in 302 (96%) patients (low 19%, low-intermediate 29%, intermediate 31%, high 21%). The 5-year OS according to the IPSSWM was 95%, 89% and 71% for low, intermediate and high risk respectively, for rIPSSWM was 95%, 95%, 75%, 70% and 64% for very low, low, intermediate, high and very high risk respectively, and for MSSWM 96%, 83% 74% and 70% for low, low-intermediate, intermediate and high risk respectively. All models significantly differentiated between high-risk and low-risk groups, while intermediate groups provided poor resolution. Additionally, in a direct comparison using a subset of 291 patients with all risk system available, the respective C-index and AIC values were: 0.72 and 455 for rIPSSWM; 0.68 and 464 for MSSWM; and 0.66 and 461 for IPSSWM. Interestingly, Beta-2microglobulin (B2M) was a significant prognostic factor, both as a continuous variable and as a binary variable (≥ 4 mg/L). In the binary form, B2M was significant in both univariable (HR: 2.79; $p < 0.001$) and multivariable Cox regression analyses adjusted for age, LDH, and albumin (HR: 2.2; $p = 0.004$).

Conclusion

Over the past decade, rituximab-based immunochemotherapy has been the most commonly used treatment for WM in the Czech Republic. In our real-world cohort, rIPSSWM was the best-performing model, achieving the highest concordance index and the lowest AIC. However, the MSSWM provided comparable results using fewer predictors, potentially simplifying its clinical application.

P64

LEUKÉMIE Z VELKÝCH GRANULÁRNÍCH LYMFOCYTŮ, ZKUŠENOSTI PRACOVIŠTĚ

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Úvod:
Leukemie z velkých granulárních lymfocytů (LGLL) představuje vzácné chronické lymfoproliferativní onemocnění charakterizované perzistující lymfocytózou, cytopeniemi a častými přidruženými autoimunitními onemocněními. Cílem této retrospektivní analýzy bylo zhodnotit klinické charakteristiky, průběh a léčebné výsledky pacientů s LGLL sledovaných na Hemato-onkologické klinice FN Olomouc v letech 2005–2025.

Metody:
Soubor tvořilo 17 pacientů (9 žen, 8 mužů) s diagnózou LGLL. Medián věku při stanovení diagnózy byl 66 let (minimum-maximum: 30–81 let), medián délky sledování 53,5 měsíců (3–194 měsíců). Hodnoceny byly základní klinické a laboratorní charakteristiky, přítomnost autoimunitních onemocnění, výskyt cytopenií a odpověď na podanou léčbu.

Výsledky:
Počet lymfocytů v periferní krvi byl v mediánu $6{,}7\times10^9$/l (rozmezí $1{,}7–16\times10^9$/l). Revmatoidní artritida byla přítomna u 7 pacientů (41 %), další nádor u 4 (24 %). TCR klonalita byla detekována nejčastěji v β-řetězci (8 případů). Neutropenie byla zjištěna u 76 %, anémie u 41 % a trombocytopenie u 18 % pacientů; infekce se vyskytly u 71 %. Léčbu vyžadovalo 10 pacientů (58,8%), přičemž kompletní remise bylo dosaženo u 2 pacientů, parciální remise u 5 pacientů, stabilní/progresivní choroba u 1 pacienta. Nejčastěji používanými léčebnými režimy byly kortikoidy, metotrexát, alemtuzumab a cladribin, resp. filgrastim.

Závěr:
Naše data potvrzují velkou heterogenitu klinických a laboratorních charakteristik u pacientů s LGLL s častým výskytem přidružených autoimunitních a nádorových onemocnění a infekcí. Kortikosteroidy s metotrexátem představují účinnou první linii léčby s dosažením odpovědi u většiny pacientů. Dlouhodobé sledování je nezbytné vzhledem k chronickému průběhu a riziku relapsů.

Dedikace: MZ ČR – RVO (FNOL, 00098892) and IGA_LF_2025_005

P65

CIRCULATING TUMOR CELLS FOR THE STAGING OF MULTIPLE MYELOMA: A EUROPEAN POOLED ANALYSIS OF 2446 NEWLY DIAGNOSED PATIENTS

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Background

Current risk models in multiple myeloma (MM) are prognostic, but improvement is needed for individualized treatment decisions. There is growing evidence that levels of circulating tumor cells (CTCs) may be more prognostic than bone marrow plasmacytosis and independent of other well-established risk factors (e.g. cytogenetics).

Aims

The optimal use of CTCs as a biomarker for risk assessment in clinical practice has not yet been defined. In this pooled analysis of prospectively collected data we aim to assess the independent prognostic value of CTC, and to determine how to use CTCs in clinical practice.

Methods

Patient-level data was collected from 2446 newly diagnosed MM (NDMM) patients from five collaborative groups (Czech Republic, Greece, Italy, HOVON/Netherlands-Belgium, PETHEMA/Spain). All patients had CTC enumeration performed prior treatment. Approximately half the patients were enrolled in clinical trials (59%; GEM-CLARIDEX, CASSIOPEIA, HOVON-143, FORTE, GEM2012MENOS65) and the other half was treated in routine practice (Czech Republic, Greece). Median age was 63 (IQR 57-74); 52.5% of patients were transplant-eligible and 47.5% ineligible. Induction treatment included doublet (2.2%), triplet (86.8%) and quadruplet (11%) regimens. Assessment of CTCs was centralized and performed with EuroFlow (64%) or other flow cytometry methods (36%).

Results

Median CTC levels were 0.017% (IQR 0.014%-0.119%) and were comparable between centers (p=0.3). Logarithmic increments of CTC levels identified five subgroups with significantly different median PFS: CTC≤0.001%, 77 months; CTC 0.001-0.01%, 52 months; CTC 0.01-0.1%, 41 months; CTC 0.1-1%, 31 months; CTC ≥1%, 16 months (p<0.0001, Figure panel A). The subgroup with ≥1% CTCs displayed dismal PFS resembling that of patients with primary plasma cell leukemia. As a continuous parameter, high CTC levels were strongly correlated to inferior PFS (HR 1.18, 95% CI 1.12-1.24, p<0.001) independent of the Revised International Staging System, 1q gain/amp and induction regimen adjusting for transplant eligibility. Subgroup analysis confirmed the prognostic impact of CTCs in various groups of patients (Figure panel B). Cut-offs within a range of 0.01-0.1% CTCs had a similar impact in dichotomizing patients with low vs high-risk of progression in both clinical trials and routine practice, as well as in transplant-eligible and ineligible patients. Of note, patients with high CTC levels treated with anti-CD38 containing quadruplets displayed similar PFS to those with low CTC levels treated with triplets (medians of 48 and 51 months, respectively, p=0.986). Similarly, patients with standard-risk cytogenetics having high CTC levels showed PFS similar to those with high-risk cytogenetics [i.e., t(4;14), t(14;16) and/or del(17p)] having low CTC levels (medians of 38 and 37 months, respectively, p=0.770).

Summary/Conclusion

This large European pooled analysis of 2446 patients treated in clinical trials and routine practice confirms CTCs as an independent risk factor in NDMM. While patients' categorization according to logarithmic levels of CTCs provides the most relevant prognostic information, a cut-off in the range of 0.01-0.1% can be used to dichotomize patients with different risk of progression across all clinically relevant subgroups. Additionally, the use of the 1% cutoff enables the identification of 9% of patients with ultra-high-risk MM.

P66

HIGH-DIMENSIONAL PROFILING REVEALS PREDOMINANT DEPLETION OF NK CELL EFFECTOR PHENOTYPES IN RELAPSED/REFRACTORY MULTIPLE MYELOMA PATIENTS TREATED WITH ANTI-CD38 THERAPY

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Background: Anti-CD38 monoclonal antibodies (mAbs) have become a standard part of multiple myeloma (MM) therapy. Their activity is mostly mediated by NK cells through antibody-dependent cellular cytotoxicity (ADCC). Although the association of increased CD38+ NK cells with worse outcomes was demonstrated, the underlying mechanisms of resistance to anti-CD38 mAbs remain poorly understood.

Aims: To identify immune effector cell subsets predictive of treatment efficacy and driving resistance to anti-CD38 mAbs.

Methods: Bone marrow (BM) samples (N=111) of relapsed/refractory MM (RRMM) treated with anti-CD38-based regimens (CD38-IMID [N=64]; CD38-PI [N=33]; CD38-mono [N=14] were assessed at baseline (BASE; N=110) and progression (PD; N=23), using conventional EuroFlow 8-color MM panel,

followed by high-dimensional spectral cytometryesigned for deep profiling of lymphoid subsets and their activation/exhaustion status. Subset proportions were expressed as percentage of lymphocytes, unless stated otherwise.

Results: Patients were treated predominantly with daratumumab over isatuximab (92%, 82%, and 100% of CD38-IMID, CD38-PI, and CD38-mono regimens). Treatment groups differed by line of therapy (p <0.001), with CD38-IMID mainly used in line 2 (56%), CD38-PI in lines 2-3 (55%, 30%), and CD38-mono in line ≥4 (86%). Median progression-free survival (PFS) for the CD38-IMID group was 37 months, compared to CD38-PI (16 months; p = 0.012) and CD38-mono (3 months; p <0.001).

First, RRMM BM (N=110) collected at BASE were assessed by the EuroFlow MM panel. Lymphocyte pool consisted of median 13.9% B (CD19+CD56-), 19.7% NK (CD19-CD56+), and 61.6% T cell (CD19-CD56-) lineage. 79.7% NK cells were CD38+, while only 29.2% T cells were CD38+. Elevated total NK cells were observed in the PD group (p=0.07). This difference was driven by increased CD38+ NK cell proportion (p <0.028). Lasso-penalized multivariable Cox regression adjusted for therapy group and all immune subsets identified higher percentages of CD38+ NK cells and mature B cells (CD19+CD38low/−CD81low) as significant predictors of PFS at BASE. Increased CD38+ NK cells were associated with worse PFS (HR 1.65, p=0.003), while higher levels of mature B cells correlated with improved PFS (HR 0.79, p=0.002). These findings remained significant also in uniform CD38-IMID group (p <0.007).

To better characterize the immune landscape driving these associations, detailed subset distribution was explored using spectral cytometry at BASE (N=14). CD16-, CD16+CD57-, CD16+CD57+ cells represented median 1.68%, 4.26%, and 6.17% of lymphocytes. Comparing CD38+/- NK compartments, CD16+CD57− subset was significantly enriched in the CD38+ NK pool (p=0.006). Gating on 8 other key NK markers revealed that KLRB1+ (p=0.063) cells were also enriched in the CD38+ pool, whereas NKG2C+ cells were more frequent in the CD38- pool (p <0.001).

To further investigate the impact of anti-CD38 mAbs on immune cells, paired BASE and PD samples were analyzed using conventional (N=23) and spectral (N=14) panels. Basic exploratory analysis using both methods showed that proportion of total T cells increased (p <0.017), while total B cells (p <0.012) and total NK cells (p=0.023) dropped at PD. Furthermore, both CD38+ NK and CD38+ T cells decreased (p <0.004). Finally, spectral cytometry was utilized in both BASE and PD to identify NK subsets most affected by anti-CD38 therapy. As expected, most reduced NK cells in lymphocytes were CD38+ (median drop, log2-fold change: 6.84%, 1.72; p=0.008), followed by CD16+CD57+ (5.85%; 1.31; p=0.023), KIR2DL+ (5.11%; 1.52; p=0.008), KLRB1+ (4.58%, 1.46; p=0.008), and CD16+CD57- cells (2.42%, 1.04; p=0.023).

Conclusion: This study provides in-depth analysis of immune cells in anti-CD38 mAb-treated patients using conventional and spectral cytometry. Higher BASE proportion of CD38+ NK cells was associated with worse PFS, while higher levels of mature B cells predicted better outcomes. Post-therapy, CD38+ NK and CD38+ T cells, total NK cells, and total B cells decreased, while total T cells increased. Importantly, deeper profiling revealed CD16+CD57- and KLRB1+ cells among those enriched in the CD38+ NK pool at BASE. Post-treatment, CD16+CD57+, KIR2DL+, and KLRB1+ NK cells showed the greatest reduction. These results indicate a complex interplay of fratricide, resulting in preferential depletion of mature effector NK cell phenotypes by anti-CD38 therapy.

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P67

BIOLOGY OF CIRCULATING TUMOR CELLS (CTCS) IN MULTIPLE MYELOMA (MM): COMPARATIVE TRANSCRIPTOMIC PROFILING REVEALS EGRESSION-ASSOCIATED CHANGES RELATED TO CTC BURDEN

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Background:
CTCs have emerged as a key prognostic factor in newly diagnosed (ND) MM. However, mechanisms of plasma cell (PC) egression from the bone marrow (BM) to peripheral blood (PB) remain poorly understood. To date, differential expression comparisons between BM plasma cells (BMPCs) and CTCs have been hampered by technical limitations driven by low CTC levels precluding successful isolation of sufficient number of PCs. In this work, we address this gap in understanding of CTC biology.

Aim:
To characterize transcriptomic differences between CTCs and paired BMPCs in NDMM using a wide range of CTC levels and to identify mechanisms underlying PC egression from BM and their survival in the PB.

Methods:
PB samples from 29 NDMM/primary plasma cell leukemia (pPCL) patients were analyzed by next-generation flow cytometry (EuroFlow protocol) and stratified into logarithmic groups: 8 patients (10–100%), 6 (1–10%), 8 (0.1–1%), 4 (0.01–0.1%), and 3 (0.001–0.01%). Paired CTCs and BMPCs were either sorted by FACS (N=21) or by combining MACS and FACS (N=8) to obtain sufficient number of cells in low-CTC samples. Differential gene expression was analyzed using DESeq2 (paired design) with apeglm shrinkage, and pathway enrichment with clusterProfiler. Associations of gene expression with progression-free (PFS) and overall survival (OS), were assessed by log-rank tests in 635 CoMMpass NDMM patients with transcriptomic and genomic data. To derive the CTC-like score, genes upregulated in CTCs were weighted by $\log_2$FC and $-\log_{10}$(padj). For each CoMMpass sample, the score was computed as the weighted sum of expression values. All reported p-values were adjusted using Benjamini-Hochberg method.

Results:
We identified 1,142 differentially expressed genes, including 66 upregulated and 28 downregulated (absolute $\log_2$FC>1) in CTCs.

Genes and transcriptional signatures related to proliferation (e.g., PR score; Zhan et al., 2006) were significantly lower in CTCs than BMPCs ($p<0.001$), consistent with reduced proliferative capacity in absence of BM niche. Notably, key BM homing molecules CXCR4 and CD138 had lower expression in CTCs.

Conversely, transcriptomic classifier for pPCL (PCL-like score, Bruinink et al., 2022) was higher in CTCs than in BMPCs ($p<0.001$). Upregulated genes such as FLNA, TAGLN2, CD44, or EMP3 suggest altered cytoskeletal dynamics and microenvironment interactions, implying greater mechanical resilience and BM egression capacity. Notably, all 3 genes of the ANXA2-S100A10-AHNAK complex were upregulated, linked to extracellular matrix (ECM) remodeling and cytoskeletal dynamics promoting cell spreading.

Importantly, differences between CTCs and paired BMPCs were more pronounced in cases with lower CTC burden. Gene expression variability in CTC samples was low across logarithmic groups, unlike BMPCs where variability was higher, especially between high- and low-CTC cases. This trend was particularly strong in 24/66 (36.4%, $p<0.05$) CTC-upregulated genes. The majority of these genes were associated with worse prognosis in CoMMpass, suggesting a role in disease aggressiveness.

To investigate genomic alterations linked to CTC burden, we computed CTC-like transcriptional signature score for CoMMpass samples. Higher scores were associated with cytogenetic features, including 1q gain, t(14;16), and 13q deletion, and inversely with hyperdiploidy, alterations previously linked to elevated CTC numbers in CoMMpass and other datasets.

Conclusion:
The combination of MACS and FACS techniques enabled us to isolate sufficient numbers of CTCs for transcriptomic profiling. In the majority of NDMM cases, CTCs exhibited reduced PR and increased PCL-like profile compared to their BM counterparts. While high expression of proliferation genes is typically linked to poor prognosis, their lower levels in CTCs might reflect the shift into a less supportive environment, rather than reduced aggressiveness. Transcriptional differences between BMPCs and CTCs increased with decreasing CTC burden, while BMPCs resembled CTCs more as CTC burden rises. Upregulation of ANXA2, S100A10, AHNAK, CD44, FLNA, and TAGLN2, alongside downregulation of CXCR4 and CD138, suggest that the underlying mechanisms of PC egression from BM to PB include altered cytoskeletal dynamics, ECM remodeling, and reduced BM homing. The derived CTC transcriptional score was associated with cytogenetic features, including t(14;16), 1q gain, and 13q deletion using CoMMpass database of 635 patients.

P68

SELECTIVE DEPLETION OF B-CELL LINEAGE SUBSETS DURING TREATMENT WITH ANTI-BCMA VS ANTI-GPRC5D BISPECIFIC ANTIBODIES (BSABS) UNDERLIES DIFFERENT RISK OF INFECTIONS IN PATIENTS WITH MULTIPLE MYELOMA (MM)

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Background:
Anti-BCMA and –GPRC5D bsAbs are effective and approved therapies in relapsed/refractory MM. However, infections pose a significant challenge to their use, particularly in combination with other drugs. Increasing evidence suggests that BCMA bsAbs are associated with higher risk of infections compared to GPRC5D. One of the reasons may be on-target off-tumor toxicity corresponding with different expression pattern of these antigens throughout the B cell lineage.

Aim:
To investigate underlying mechanisms responsible for higher infection rates with anti-BCMA bsAbs.

Methods:
This multi-center study included 75 RRMM patients treated with BCMA (teclistamab, elranatamab; n=28) or GPRC5D (talquetamab; n=47) bsAbs at 4 European centers (Athens, Pamplona, Hradec Kralove and Ostrava). The bone marrow (BM) immune composition was analyzed before (n=70) and during treatment (n=40) using next generation flow cytometry. Additional immunophenotyping was performed to characterize in greater depth, pro-B, large and small pre-B and immature subsets within the B cell compartment (n=26). Surface BCMA expression on B cell subsets was evaluated using spectral flow cytometry (n=7). Bulk and single cell RNA sequencing (scRNA-seq) data was used to investigate expression of *BCMA* and *GPRC5D* in B cell subsets (n=11). The effect of anti-BCMA bsAbs was further investigated in MIcγ1 immunocompetent mice that express BCMA in MM plasma cells (PCs) but not in mature B cells or B cell precursors.

Results:
Anti-BCMA bsAbs were associated with higher infection rate (82% vs. 53%; p=0.012), more profound hypogammaglobulinemia and more frequent use of IVIG replacement (74% vs. 32%; p=0.001) compared to GPRC5D. BM immune profiling revealed no significant differences between both groups at baseline. By contrast, during treatment, significant depletion of mature B cells and normal PCs was observed in BCMA group (both p<0.001).

Bulk and scRNA-seq uncovered distinct patterns of *BCMA* and *GPRC5D* expression throughout the B-cell lineage. *BCMA* was expressed on mature B cells and, unexpectedly, on B cell precursors. Furthermore, the highest BCMA expression (after PCs) was found in small pre-B cells, which was confirmed with multidimensional flow cytometry. By contrast, *GPRC5D* expression was limited to PCs and, unlike *BCMA*, significantly lower levels were observed in normal PCs vs tumor cells.

Deep and longitudinal immunophenotyping unveiled that anti-BCMA bsAbs depleted immature B cells and small pre-B cells, which resulted in the accumulation of pro-B cells. In addition, depletion of mature B cells and normal PCs persisted throughout treatment and only the interruption of anti-BCMA bsAbs therapy allowed the regeneration of B cell compartment. Using the MIcγ1 experimental model as a negative control since scRNA-seq data displayed negative *BCMA* expression in both mature and precursor B cells, we confirmed no depletion of any B-cell subset after treatment with anti-BCMA bsAbs.

Conclusion:
Anti-BCMA bsAbs are associated with higher risk of infections and more profound and persistent hypogammaglobulinemia. Distinct patterns of BCMA and GPRC5D expression throughout the B cell lineage explain different on-target off-tumor effects, which leads to the depletion of mature B cells, normal PCs and subset of B cell precursors with anti-BCMA bsAbs. These findings may help the management of bsAbs treated patients such as individualized use of IVIG prophylaxis.

P69

GENOMIC LANDSCAPE OF PARA-SKELETAL AND EXTRAMEDULLARY PLASMACYTOMAS IN MULTIPLE MYELOMA PATIENTS

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Background

Extraosseous disease in multiple myeloma (EMM) is associated with poor prognosis. EMM manifests in two forms: para-skeletal plasmacytomas (PS), which originate from osteolytic bone lesions, and extramedullary plasmacytomas (EMD), which develop in soft tissues without direct bone involvement. EMM occurs in approximately 15% of newly diagnosed multiple myeloma cases, while in relapsed or refractory multiple myeloma secondary EMM is observed in up to 40% of patients, representing a treatment-resistant subtype linked with adverse outcomes and early mortality. Despite its clinical relevance, molecular insights into the distinct characteristics of EMD and PS remain limited.

Aims

Our primary objective was to conduct a comprehensive molecular analysis of a large cohort of EMM tissue samples to distinguish the differences between PS and EMD subtypes.

Methods

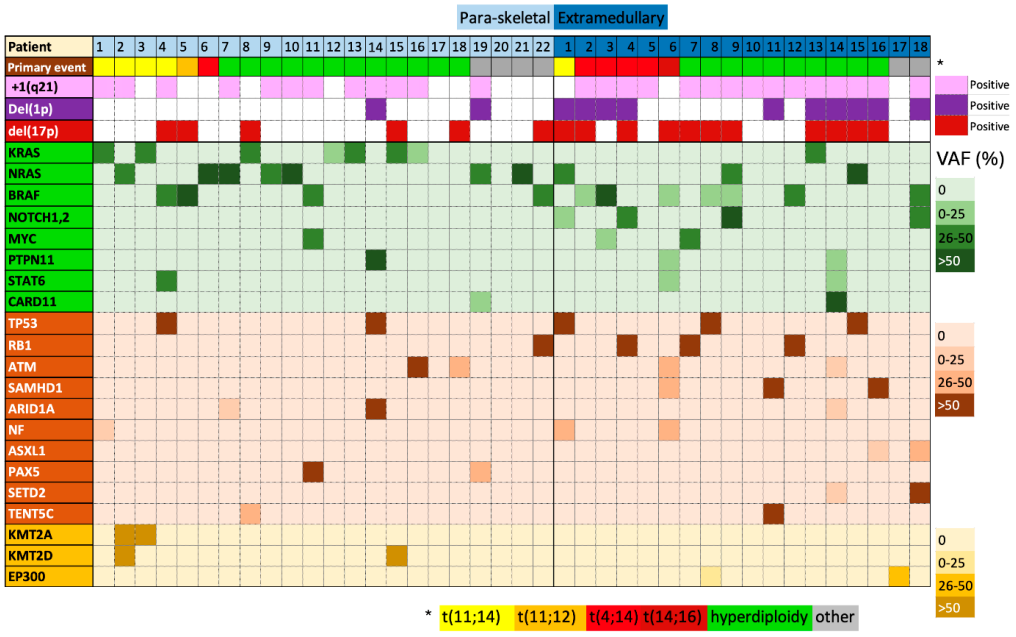
Patients enrolled into the study were treated with EMM at University Hospital Brno between 2022 and 2025. All patients signed informed consent with the study. We analyzed 40 native tumor tissues obtained from EMM biopsies via surgical sampling or CT-guided procedures. These tissues underwent targeted next-generation sequencing (NGS) using our in-house LYNX panel, while tissue imprints were examined by interphase fluorescent in situ hybridization (I-FISH). Additionally, flow cytometry was performed on available samples (EMM tumor cells 72.5% [29/40]; bone marrow plasma cell (BMPCs) 75.0% [30/40]. We also analyzed 15 parallel CD138-sorted cryopreserved BMPCs collected at the time of EMM development. Comparative analyses between PS and EMD cases were conducted using Fisher's exact test.

Results

Seventeen EMM samples were collected from NDMM patients and 23 from RRMM patients, comprising 18 EMD plasmacytomas and 22 PS plasmacytomas. EMD plasmacytomas exhibited a significantly higher frequency of deletions in the 1p region (55.6% [10/18] vs. 9.1% [2/22]; p = 0.002) compared to PS plasmacytomas. In addition, rare *NOTCH1/2* gene mutations were exclusively observed in EMD plasmacytomas (21.2% [4/18] vs. 0.0%; p = 0.034), whereas *KRAS* mutations were more prevalent in PS plasmacytomas (31.8% [7/22] vs. 5.6% [1/18]; p = 0.053) (Figure 1). In paired comparisons of EMM and BM plasma cells, new mutations in the MAPK pathway (*KRAS, BRAF*) were identified in 33.3% (5/15) of EMM plasma cells, and new biallelic inactivation of tumor suppressor genes (*TP53, RB1, SAMHD1*) was observed in 20.0% (3/15) of cases. Moreover, tumor cells in EMD plasmacytomas demonstrated significantly lower expression of NCAM (CD56) compared to those in PS plasmacytomas (35.7% [5/14] vs. 86.7% [13/15]; p = 0.008).

Conclusions

Our study identifies distinct molecular differences between EMD and PS plasmacytomas in multiple myeloma. EMD plasmacytomas show frequent 1p deletions, rare *NOTCH1/2* mutations, and demonstrate lower NCAM expression. In contrast, PS plasmacytomas show a higher prevalence of *KRAS* mutations. Additionally, significant differences were observed between EMM tumor cells and BMPCs. These findings highlight the genomic heterogeneity of EMM, which may have implications for future targeted therapeutic strategies.



P70

A MULTICENTER PHASE 2 STUDY DESIGNED TO OPTIMIZE THE SCHEDULE OF BELANTAMAB MAFODOTIN PLUS BORTEZOMIB AND DEXAMETHASONE IN RELAPSED REFRACTORY MULTIPLE MYELOMA

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Background: The results of two phase 3 studies for patients (pts) with relapsed refractory multiple myeloma (RRMM) have been published recently. Belantamab mafodotin in combination with bortezomib and dexamethasone (Vd) and belantamab mafodotin with pomalidomide and dexamethasone (Pd) confirmed significant benefit in PFS over daratumumab Vd and bortezomib Pd, respectively (Hungria et al., NEJM 2024; Dimopoulos et al., NEJM 2024).

However, ocular findings are still considerable adverse events, so the dosing needs to be adjusted to further optimize the use of the antibody-drug conjugate. Herein, we present updated results of an ongoing single-arm study evaluating the combination of belantamab mafodotin plus Vd in deescalated dose and frequency.

Methods: The Czech multicenter phase 2 study CMG012022 (EU CT 2024-516250-22-00) enrolled RRMM pts after ≥1 line of therapy. The patient entry criteria mirror the DREAMM-7 study, however, the dosing is reduced. Patients receive eight 28-day cycles of bortezomib: 1,3 mg/m^2 SC days 1, 8, 15, 22 and dexamethasone: 20 mg PO days 1, 2, 8, 9, 15, 16, 22, 23 plus belantamab mafodotin: 1,9 mg/kg IV every 8 weeks until progression or unacceptable toxicity. The data cut-off for this evaluation focused on ocular findings was January 31, 2025.

Results: Fourty-five pts after a median of 3 (range 1-7) prior lines of therapy started the treatment at seven university hematooncology centers. Characteristics of the cohort of patients have been previously published (Popkova et al., ASH 2024). After a median follow-up of 247 (range 7-631) days one third (15/45) of patients were still on treatment by the data cut-off. Median number of belantamab Vd cycles administered was 7 (range 1-14) among the whole cohort whereas the median number of days on treatment was 171 (range 7-499) for 30 patients who terminated the treatment. Reasons for the end of treatment included progression 64% (19/30), death not related to treatment 13% (4/30), patient's decision 7% (2/30), adverse event 7% (2/30), medical decision 3% (1/30), belantamab discontinuation 3% (1/30) or lost to follow-up 3% (1/30). The response was evaluable in 41 patients (4 patients discontinued treatment before a second cycle). Overall response rate has reached 68,3% (28/41) so far with CR in 14,6% (6/41), VGPR in 31,7% (13/41) and PR in 22% (9/41) of pts. Moreover, patients after ³3 applications of belantamab mafodotin reached ORR of 84,6% (22/26). Out of 15 pts evaluated for minimal residual disease (MRD), four were MRD negative. In total, 38 patients were examined for ocular finding (7 patients discontinued treatment before a second dose of belantamab mafodotin), of whom 65,8% (25/38) developed an ocular adverse event. A total number of 237 eye examination was performed. Nineteen percent (45/237), 27,8% (66/237) and 1,3% (3/237) of the ocular assessments revealed keratopathy visual acuity (KVA) grade 1, 2 and 3, respectively. The maximum severity of ocular finding was KVA grade 1, 2 and 3 at 18,4% (7/38); 39,5% (15/38) and 7,9% (3/38) of patients, respectively.

Conclusion: The ocular findings seem to be limited (7,9% of KVA grade 3) due to the significantly de-escalated belantamab mafodotin dosing. Despite a reduced dose and frequency of belantamab mafodotin (1.9 mg/kg Q8W) the ORR reaches 68,3% (28/41) with MRD negativity rate of 9,8% (4/41). The clinical trial is ongoing, the results will have to be validated by the final analysis.

P71

DE-ESCALATED TECLISTAMAB DOSING IN RELAPSED/REFRACTORY MULTIPLE MYELOMA: CZECH MYELOMA GROUP REAL-WORLD EVIDENCE STUDY

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Background

Teclistamab is a bispecific antibody engineered to target multiple myeloma (MM) cells by simultaneously binding to B-cell maturation antigen (BCMA) on MM cells and CD3 on T cells, inducting a T-cell-mediated anti-tumor immune response. Teclistamab is standardly administered on a weekly basis for at least six months; in patients who achieve a complete response, the dosing frequency may subsequently be reduced to every other week. In real-world settings, however, dosing schedules are often modified at the physician's discretion, in contrast to the strict protocols of clinical trials. This study presents a real-world evidence analysis of teclistamab monotherapy in heavily pretreated relapsed/refractory MM patients (RRMM), focusing on the efficacy and safety of a reduced dosing frequency schedule.

Patients and Methods

A total of 73 RRMM patients were treated with teclistamab between 2023 and 2024 outside of clinical trials at all major hematology centers in the Czech Republic. Teclistamab was administered either on a standard weekly schedule or on a non-weekly schedule, as determined by the treating physician. Responses were evaluated in accordance with the International Myeloma Working Group (IMWG) Response Criteria. Patient data were collected from the Czech Registry of Monoclonal Gammopathies (RMG) and subsequently analyzed.

Results

The median age at the first teclistamab administration was 67.0 years (range, 41–83). The median number of prior lines of therapy was 5 (range, 3–13), with 68.5% (50/73) of patients being penta-refractory (i.e., refractory to two proteasome inhibitors, two IMiDs, and an anti-CD38 antibody). The median follow-up was 4 months (range, 1–17).

Teclistamab dosing was reduced in 24.7% (18/73) of patients. Among these, 72.2% (13/18) received dosing twice per month, and 27.8% (5/18) received dosing once per month. The median time from treatment beginning to frequency reduction was 1 month (range, 0-3). Dosing frequency was reduced after an initial response in 55.6% (10/18) of patients, due to adverse events in 33.3% (6/18), and because of frailty in 11.1% (2/18). The median age in the non-weekly dosing group was 69 years (range, 49–83) compared to 66 years (range, 41–85) in the weekly dosing group (p = 0.065). The median performance status (ECOG) was 1 (range, 0–3) in both groups (p = 0.37).

Median progression-free survival (PFS) was comparable between the weekly and non-weekly dosing groups [9.1 months (95% CI: 2.7–NA) vs. 11.3 months (95% CI: 6.4–NA); p = 0.141]. In estimated 12-month overall survival rates, there was a clinical meaningful trend towards to weekly dosing (weekly: 62.3% [95% CI: 47.6–81.5] vs. non-weekly: 36.6% [95% CI: 14.0–95.9]; p = 0.667) but not reached level of statistical significance.

In terms of infectious complications, the median number of all infection episodes was 2 (range, 0–12) in the weekly dosing group versus 1 (range, 0–11) in the non-weekly group (p = 0.73). However, the median number of severe (grade 3–4) infection episodes was 0 (range, 0–4) in the weekly group versus 1 (range, 0–3) in the non-weekly group, a difference that reached statistical significance (p = 0.033). Among patients with non-weekly dosing, severe infections occurred in 23.5% (4/17) of cases before de-escalation and in 52.9% (9/17) after de-escalation (p = 0.157). The median cumulative dose of administered intravenous immunoglobulin (IVIG) was 60 g per patient in both groups (p = 0.90).

Conclusion

Our real-world evidence analysis indicates that non-weekly dosing of teclistamab yields PFS comparable to the standard weekly protocol. Data for overall survival are not mature yet. No benefit in reducing the frequency or severity of infections was observed. These findings underscore the importance of supportive care, including appropriate polyclonal immunoglobulin substitution, particularly in older or frail patients.

P72

CHARACTERIZATION OF OPHTHALMIC EXAMINATION FINDINGS (OEFS) AND IMPACT ON READING AND DRIVING IN PATIENTS WITH RELAPSED/ REFRACTORY MULTIPLE MYELOMA (RRMM) TREATED WITH BELANTAMAB MAFODOTIN (BELAMAF)

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Background:
In the phase 3, randomized DREAMM-7 and DREAMM-8 trials, belamaf plus standard backbone therapies demonstrated significant progression-free survival benefit in patients (pts) with RRMM after ≥1 line of therapy; significant overall survival benefit was observed in DREAMM-7. Ocular events were common in pts treated with belamaf-based regimens but were generally reversible and effectively managed with dose modifications. Efficacy was maintained in these pts despite extended dose delays.

Aims:
To provide a post hoc characterization of OEFs during treatment in pts from DREAMM-7 (data cutoff: October 2, 2023) and DREAMM-8 (January 29, 2024).

Methods:
DREAMM-7 (NCT04246047) compared belamaf + bortezomib + dexamethasone (BVd) vs daratumumab + bortezomib + dexamethasone, and DREAMM-8 (NCT04484623) compared belamaf + pomalidomide + dexamethasone (BPd) vs pomalidomide + bortezomib + dexamethasone. OEFs in pts receiving belamaf-containing regimens were graded per the Keratopathy and Visual Acuity (KVA) scale, which included corneal examination findings and changes in best-corrected visual acuity (BCVA). While grade ≥2 KVA events drove protocol-recommended belamaf dose modifications, a pt with a grade ≥2 OEF may have only had corneal examination findings or, if normal baseline BCVA (20/25 or better in ≥1 eye), a mild bilateral BCVA change to <20/50 (ie, no clinically meaningful change in vision). The first 5 occurrences of grade ≥2 OEFs, as applicable, were assessed in pts with normal baseline BCVA, using the overall KVA grade. Impact of ocular events on reading and driving was also evaluated by eye care professionals.

Results:
Of the 211/242 (87%) pts treated with BVd in DREAMM-7 who had normal BCVA at baseline, almost two-thirds did not experience a bilateral BCVA change to ≥20/50 (ie, no clinically meaningful change in vision) despite most having ≥1 grade ≥2 OEF. In those with a bilateral BCVA change to ≥20/50 at some point on treatment, this change was experienced for ≈11% of time on treatment. The majority of pts evaluated for reading and driving at the time of their ocular examination did not report having to stop these activities; of those who stopped at some point, >90% returned to baseline reading and driving in a median of ≈3 weeks. Pts with normal baseline BCVA were stratified into 2 groups according to bilateral BCVA change: no change to ≥20/50 (ie, no clinically meaningful change in vision) and change to ≥20/50 to <20/200. Recurrences of OEFs were common, with or without clinically meaningful vision changes. In pts with multiple occurrences, the duration of each occurrence was consistent and predictable across groups, ranging from 12 to 13 weeks (<20/50) and 11 to 16 weeks (≥20/50 to <20/200). BCVA changes to ≥20/200 were rare (2%) and improved in all pts with adequate follow-up. Similar results were observed with BPd in DREAMM-8.

Summary/Conclusion:
Of pts with normal BCVA at baseline who were receiving belamaf-containing regimens, almost two-thirds did not experience a clinically meaningful change in vision; in pts who did, this was experienced for ≈11% of time on treatment. Overall, the majority of pts evaluated were able to continue reading and

driving throughout treatment; any impacts on reading and driving were short-lived. Ocular monitoring frequency can be guided by the resolution data.

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P73

EVOLUTION OF MYELOMA BONE DISEASE OVER TIME: COMPARISON OF SELECTED BIOMARKERS IN MGUS, SMM AND ACTIVE MM

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Background:
Myeloma bone disease (MBD) is a hallmark of multiple myeloma (MM) and is present in up to 80% of newly diagnosed patients. The extent of lytic bone involvement varies and generally correlates with more advanced disease stages. MBD arises from an imbalance in bone homeostasis caused by increased bone resorption factors combined with suppression of bone formation factors. As MM progresses from its precursor conditions—monoclonal gammopathy of undetermined significance (MGUS) and smoldering myeloma (SMM)—we aimed to compare selected cytokines associated with MBD across these three conditions.

Patients and Methods:
We assessed 121 patients with monoclonal gammopathies: 77 patients with active MM (at diagnosis or relapse), 14 individuals with SMM, and 30 individuals with MGUS.

We measured the following serum cytokines and bone marrow microenvironment parameters known to be associated with MBD: procollagen type I propeptides (P1NP), Crosslaps (CTX-1), cross-linked telopeptide of type I collagen (ICTP), osteoprotegerin (OPG), nuclear factor kappa B (NF-kB), receptor activator of nuclear factor κB (RANK), receptor activator of nuclear factor-kB ligand (RANKL), MIP-1 alpha, Dickkopf-1 (DKK-1), osteopontin, hepatocyte growth factor (HGF), secreted Frizzled-related protein-1 (sFRP-1), and syndecan-1.

Patients with MM who started therapy were re-assessed after 4 and 12 months of treatment. They were grouped based on therapeutic outcome into: those achieving very good partial response (VGPR) or complete response (CR) (n = 27), partial response (PR) (n = 23), and non-responders (n = 21). Changes in cytokine levels over time were compared within these cohorts.

Results:
No statistically significant differences were found between MGUS and SMM in any analyzed parameter, although there was a trend toward increased osteoresorptive factors in SMM. Similarly, most parameters did not differ significantly between SMM and MM, except for CTX-1 (median 0.329 vs. 0.672 ng/ml, p = 0.02), RANK (median 0.4 vs. 0.65 ng/ml, p = 0.044), RANKL (median 30,472 vs. 7,354 pg/ml, p = 0.01), and syndecan-1 (median 33.1 vs. 77.7 ng/ml, p = 0.028).

More significant differences were observed between MGUS and MM: P1NP (median 39.15 vs. 51.4 µg/l, p = 0.045), CTX-1 (median 0.323 vs. 0.672 ng/ml, p = 0.0004), MIP-1 alpha (median 22.1 vs. 26.1 pg/ml, p = 0.039), HGF (median 1,702 vs. 2,837 pg/ml, p < 0.001), DKK-1 (median

2,381 vs. 3,419 pg/ml, p = 0.002), syndecan-1 (median 21.2 vs. 77.7 ng/ml, p < 0.001), and NF-kB (median 0.078 vs. 0.6 ng/ml, p = 0.007).

In MM patients undergoing therapy, there was a trend toward decreased osteoresorptive cytokines, more pronounced in patients achieving CR, VGPR, and PR compared to non-responders. Significant changes over time were found in syndecan-1 (p = 0.003), HGF (p = 0.03), Activin A (p = 0.02), and NF-kB (p = 0.001).

Conclusions:
Our data suggest that MBD evolution is a continuous process, more pronounced in active myeloma but with potential origins and slow progression even in premalignant conditions, leading to an imbalance between osteoresorption and osteoprotection. Therapy administration prevents further bone destruction and decreases osteoresorptive cytokines in correlation with the depth of therapeutic response.

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P74

PRIME EDITING-MEDIATED CORRECTION OF FANCA MUTATION IN PRIMARY CELLS FROM FANCONI ANEMIA PATIENTS

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Fanconi anemia (FA) is a rare genetic disorder caused by mutations in one of the 23 genes involved in the FA DNA repair pathway. The hallmark manifestation of FA is bone marrow failure (BMF), which most FA patients develop already in childhood. Currently, the only curative option for BMF is allogeneic transplantation of hematopoietic stem and progenitor cells (HSPCs) which requires a suitable donor. Gene therapies introducing a healthy copy of the causal gene into patient's HPSCs are in clinical trials. However, genome-editing strategies that correct causal mutations could represent a more attractive therapeutic strategy for FA patients, particularly techniques such as prime editing, which can introduce precise correction with high specificity. Nevertheless, its potential for the correction of FA mutations needs to be evaluated.

Using prime editing, we aimed to correct a causal mutation in the *FANCA* gene. We designed pegRNAs and nicking sgRNAs for the *FANCA* c.1 A>G mutation shared by two FA patients treated at our department, and tested the designed prime-editing tools in fibroblasts derived from the patients. Overall, we tested the PE2, PE3, PE5 and PE5max prime-editor systems and epeg, speg and apeg pegRNA modifications. We achieved up to 26% of precise editing at day 5 after delivery of PE tools by nucleofection, which was

accompanied by up to 20% of imperfect editing. Nevertheless, in contrast to imperfect edits, the proportion of precisely edited DNA increased over time in culture up to 44%, indicating proliferative advantage of cells with precisely corrected FANCA gene. In agreement with this observation, we detected the restored, full-length FANCA protein in the edited samples. Furthermore, the edited samples showed restoration of the FA pathway, as evidenced by monoubiquitinylation of FANCD2 upon exposure to Mitomycin C. Altogether, our results demonstrate that prime editing is capable of precise correction of the FANCA gene in the cells derived from FA patients.

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P75

HEPATITIS-ASSOCIATED VS IDIOPATHIC PAEDIATRIC APLASTIC ANAEMIA: CLONAL ARCHITECTURE AND DISEASE TRAJECTORY

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Background and aims

Between 2004 and 2025, 72 paediatric patients were treated for aplastic anaemia (AA) in the Czech Republic, comprising 43 idiopathic and 29 hepatitis-associated (HA-AA). Among HA-AA patients, histology classified 19 as AA, 8 as refractory cytopenia of childhood (RCC), and 2 as non-evaluable. 20 of 29 HA-AA patients were male. Of 43 idiopathic AA patients, 12 received upfront HSCT and 31 IST (13 later transplanted); in HA-AA, 7 received upfront HSCT and 22 IST (9 later transplanted). Two patients required liver transplantation. This study compares clonal architecture and disease trajectory in idiopathic AA and HA-AA.

Methods
All samples were evaluated using flow cytometry. HA-AA samples, including longitudinal, underwent T-cell receptor beta (TRB) sequencing (EuroClonality-NGS protocol) and whole exome sequencing (WES). In idiopathic AA patients with suspected germline predisposition, WES or panel sequencing was performed.

Results
HA-AA is characterised by a predominance of activated (HLA-DR$^+$) CD8$^+$ T cells, unlike idiopathic AA, which typically lacks increased HLA-DR expression and CD8$^+$ dominance, suggesting a distinct pathogenesis. No differences were found between RCC and AA histological subtypes. WES revealed no monogenic cause in HA-AA but detected variants of unknown significance, including CXCR4, ASXL1, WRAP53, and ACD. No variants of interest were detected in the AA group. TRB sequencing in HA-AA showed variable clonal expansion at diagnosis, with diversification following successful treatment in most—but not all—patients. Persistence of expanded clones did not necessarily portend poor response. CDR3 amino acid sequences were largely private, with occasional matches to viral antigen–associated sequences, such as CMV.

Conclusions
HA-AA is associated with a distinct immunophenotypic and genotypic pattern irrespective of histology. HA-AA is marked by elevated activated CD8$^+$ cells, detectable in peripheral blood during preceding hepatitis. TRB repertoire is restricted at the diagnosis of AA.

P76

FOSTAMATINIB PŘINÁŠÍ NOVÉ MOŽNOSTI KOMBINAČNÍ LÉČBY PACIENTŮ S IMUNITNÍMI CYTOPENIEMI

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Léčba pacientů s imunitními cytopeniemi vyžaduje individuální přístup.

Většina doporučených léčebných postupů i nově vyvíjených léků pro imunitní trombocytopenii (ITP) je založena na studiích zaměřených výhradně na pacienty s primární imunitní cytopenií, zatímco jedinci s kombinovanou poruchou imunity bývají z těchto studií vylučováni.

U pacientů se sekundární imunitní trombocytopenií či s kombinací imunitní trombocytopenie a autoimunitní hemolytické anémie nebo autoimunitní neutropenie je vhodné zvažovat kombinaci léčebných přípravků zasahujících různé patofyziologické mechanismy onemocnění.

U primární ITP je standardem druhé linie léčby podávání agonistů trombopoetinových receptorů (TPO-RA). Pokud je pacient na tuto léčbu alergický nebo má jiné nežádoucí účinky, je vhodné zvolit alternativní lék z jiné terapeutické skupiny. Při nedostatečném efektu agonistů TPO receptoru či při nutnosti zohlednit jiné přidružené onemocnění, například hemolytickou anémii, lze využít kombinovanou léčbu nebo monoterapii fostamatinibem.

V předkládaném souboru prezentujeme data deseti pacientů s kombinovanou poruchou imunity léčených fostamatinibem v monoterapii nebo v kombinaci s agonistou trombopoetinového receptoru. Naše zkušenosti ukazují, že tato léčba může být bezpečnou a účinnou alternativou u pacientů s komplexními poruchami imunity, u nichž standardní postupy selhávají nebo nejsou vhodné.

P77

METABOLIC CONSEQUENCES OF DISTINCT SF3B1 MUTATIONS IN MYELODYSPLASTIC NEOPLASMS

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SF3B1 spliceosome mutations are among the most frequent genetic lesions in myelodysplastic neoplasms (MDS), with the K700E variant typically linked to favorable prognosis. However, the consequences of other *SF3B1* variants remain incompletely understood. We aimed to characterize the clinical, transcriptomic, and mitochondrial bioenergetic effects of distinct *SF3B1* variants in MDS patients and isogenic cell models. Clinical and molecular data from 121 *SF3B1*-mutated MDS patients were analyzed. RNA sequencing was performed on CD34⁺ cells and CRISPR-edited NALM-6 lines harboring individual *SF3B1* variants. Splicing, gene expression, and mitochondrial bioenergetic parameters were profiled. The K666N variant was associated with significantly shorter progression-free survival compared to K700E. Transcriptomic analyses revealed variant-specific splicing abnormalities, with greater similarity between wild-type and K700E, and between K666N and H662Q. Retained introns were more frequent in K666N and H662Q. Mitochondrial-related genes were frequently mis-spliced and differentially expressed in K666N-mutant cells. In isogenic lines, K666N induced reduced complex IV activity and marked OXPHOS impairment. In conclusion, not all *SF3B1* variants confer the same biological or clinical effects. K666N, in particular, is associated with adverse prognosis, widespread splicing dysregulation, and profound mitochondrial dysfunction. These findings highlight molecular heterogeneity among *SF3B1* variants and support integrating variant-specific information into diagnostic and therapeutic strategies in MDS.

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P78

ALTERED T CELL BIOENERGETICS IN HYPOPLASTIC MYELODYSPLASTIC NEOPLASMS AND IDIOPATHIC APLASTIC ANEMIA

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Background and Aims

Bone marrow failure (BMF) in hypoplastic myelodysplastic neoplasms (MDS-h) and idiopathic aplastic anemia (iAA) results from the destruction of hematopoietic progenitors by autoreactive T cells; however, the molecular mechanisms underlying the aberrant T cell response remain unclear. Therefore, we performed transcriptome profiling of T cells from MDS-h and iAA patients to uncover dysregulated cellular pathways relevant to BMF.

Methods

Transcriptome profiles of peripheral blood CD3+ cells from 21 MDS-h/iAA (hypo-BM) patients and 10 age-matched controls were determined by RNA-seq. Mitochondrial respiration was measured in CD3+ cells using Seahorse assay.

Results

Principal component analysis (PCA) of the RNA-seq data clearly segregated the hypo-BM patients and controls into separate groups, demonstrating disease-specific expression patterns. Differential expression analysis identified 142 up- and 178 down-regulated protein-coding genes (PCGs) (|logFC|>0.5, FDR<0.05) in hypo-BM patients compared to controls. Functional annotation of the deregulated PCGs revealed that patient T cells carry signatures of increased inflammation, apoptosis, hypoxia response, and decreased oxidative stress-induced apoptosis. Gene set enrichment analysis revealed a significant enrichment of the Oxidative Phosphorylation (OXPHOS) and DNA Repair gene sets in the control phenotype compared to the patient phenotype ($p<0.05$). Metabolic assay showed significantly reduced basal and maximal respiration rates in patient T cells ($p<0.05$), confirming the suppression of OXPHOS.

Conclusions

Germline variants predisposing to immunodeficiency are overrepresented in MDS-h/AA patients and may increase susceptibility to immune-mediated BMF. They may affect the “immune fitness” of patients and represent potential risk factors for progressive cytopenia and/or treatment failure.

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P79

ETIOLOGIE A LÉČBA SPLANCHNICKÉ TROMBÓZY VE VÝCHODNÍCH ČECHÁCH

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Úvod: Trombóza ve splanchnické oblasti (SVT) nemá u nás tak jednotnou léčbu jako žilní trombóza v žilách dolních končetin. Příčiny a průběh jsou variabilní a nemocní jsou léčeni na různých odděleních.

Cíl práce: Zhodnocení managementu se SVT a na základě analýzy pak formulovat doporučení k sjednocení léčebných postupů v péči o tyto pacienty.

Metodika: Retrospektivní a prospektivní zhodnocení údajů 80 nemocných (n.) s SVT z Pardubického a Královéhradeckého kraje v období 2007-2024.

Výsledky:
1) Všichni n. byli symptomatičtí s variabilními potížemi. Všichni n. měli různě intenzivní bolesti břicha. Dále 18 n. bylo subfebrilních, 20 n. mělo nauzeu, 4 krvácení do GIT.
2) U 20 n. byla diagnóza stanovena týž den a u ostatních n. vždy do 72 hodin.
3) Dopplerovský ultrazvuk byl prvním vyšetřením u 45 n. a u 35 n. bylo ihned provedeno angioCT vyšetření břicha
4) 60 n. bylo primárně přijato na interní oddělení (z toho 3 na hematologii) a 20 n. na chirurgii.
5) Antikoagulační terapií bylo léčeno 76 n., u 8 dalších n. s Budd Chiariho syndromem byla zavedena i transjugulární intrahepatální portosystémová spojka. 4 n. byli léčeni primárně chirurgicky.
6) Stran etiologie převládá nález mutace JAK-2 kinázy jako první příznak myeloproliferativního onemocnění.
7) Doporučena byla vždy následná dispenzarizace n., 66 n. bylo dispenzarizováno na interní a hematologické ambulanci a 14 n. na příslušné chirurgické ambulanci.
8) Doba dispenzarizace byla různá. Všichni n. měli alespoň 6 měsíců antikoagulační terapii warfarinem, z toho u 72 n. byla tato terapie jednoroční, 5 n. bylo léčeno nízkomolekulárním heparinem a 3 n. byli léčeni přímými orálními antikoagulancii.

Závěr: Spokojenost může být s rychlostí stanovení diagnózy a spektrem vyšetření k objasnění etiologie SVT. Nejednotná zůstává stran antikoagulační terapie ve smyslu typu a její délky a také načasování provedení kontrolního zobrazovacího vyšetření

P80

CHRONIC INFLAMMATION DRIVES HSC AGING, CLONAL EXPANSION, AND ALTERED INFECTION RESPONSE IN MICE

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Hematopoietic stem cells (HSCs) maintain a lifelong production of mature blood cells. We and others have previously shown that HSCs directly respond to stress conditions, such as chronic inflammation, which compromise their functionality. Chronic inflammation is a hallmark of autoimmune and autoinflammatory disorders such as rheumatoid arthritis (RA) and interestingly, patients suffering from chronic inflammation are more susceptible to infections. Additionally, low grade inflammation may occur during aging, leading to “inflamm-aging” and clonal hematopoiesis of indeterminate potential (CHIP). Several studies investigated how inflammation affects HSCs, however the impact of autoinflammatory disorders on HSC aging, the onset of clonal hematopoiesis and HSC’s response to infections remain poorly understood. To address this, we employed *Pstpip2*^{cmo} mice that exhibit a progressive autoinflammatory disease called chronic multifocal osteomyelitis (CMO). Our results showed that CMO HSCs have significantly decreased glucose uptake ability and a significant loss of H4K16ac polarity, both of which are hallmarks of stem cell aging. Accordingly, our data indicates that the CMO environment gives Tet2$^{-/-}$ cells a mild growth advantage, in comparison to the WT environment, thus promoting clonal hematopoiesis. Our RNAseq data revealed that Tet2$^{-/-}$ LSKs exposed to the CMO environment have upregulation of interleukin-1 and interferon signaling pathways, as well

as pathways related to enhanced neutrophil degranulation, and negative regulation of T-cell activation. Further, we observed that, when challenged with *C. albicans*, CMO mice display a greater increase in peripheral blood and BM granulocytes, but a mitigated activation of myeloid multipotent progenitors, in comparison to WT. Surprisingly, CMO mice were able to survive lethal dose of *C. albicans* significantly longer than WT mice. In conclusion, our data indicates that CMO HSCs display signs of accelerated aging, CMO environment imprints inflammatory signatures onto Tet2$^{-/-}$ cells, and that CMO mice have an advantage when faced with *C. albicans* infections.

P81

POLATUZUMAB VEDOTIN INDUCED ACTIVATION OF B-CELL RECEPTOR SIGNALING CREATES A SYNTHETIC LETHALITY WITH BTK AND AKT INHIBITION IN MANTLE CELL LYMPHOMA

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Introduction. Polatuzumab Vedotin (PV) is an antibody-drug conjugate of anti-CD79B monoclonal antibody linked with monomethyl auristatin E (MMAE), a microtubule inhibitor. PV was FDA approved in the first line treatment of large B-cell lymphomas in 2023. It has shown a great clinical promise also in other non-Hodgkin lymphomas, including within salvage therapy of mantle cell lymphoma (MCL). Binding of PV to CD79B is followed by its internalization and release of MMAE. CD79B is not only a B-cell specific marker, but also a signaling subunit of the B-cell receptor (BCR) complex. CD79B mediates signal transmission from BCR towards proximal mediators (e.g., BTK, Bruton's tyrosine kinase) and second signaling messengers (e.g., Ca2+ and AKT, protein kinase B pathways). Therefore, we hypothesized that PV might affect BCR signaling itself with unknown consequences of BCR signal activation for lymphoma.

Methods. We have used 6 MCL cell line models. AKT activity was measured using genetically encoded Förster resonance energy transfer (FRET)-based AKT activity reporter with FRET detection by flow cytometry, including kinetics measurement. Ca2+ response was measured using Indo-1 ratiometric dye. Cell cycle and apoptosis were measured using Click-iT EdU and CellEvent Caspase-3/7 fluorescent dyes, respectively. Cell growth was evaluated using CellTitr-Glo assay or live cell imaging by IncuCyte. Drug synergism in cross-titration experiments was calculated using Synergyfinder.

Results. Treatment of model MCL cell lines with PV induced strong and fast activation of the BCR signaling. PV induced immediate, large and dose dependent increase of AKT activity with maximum reached within two minutes. AKT activation was similar following PV treatment as response to unconjugated anti-CD79B and anti-IgH (immunoglobulin heavy chain) antibodies. Similarly, PV induced strong Ca2+ signaling response comparable to anti-IgH based BCR stimulation. Next, we evaluated whether this anti-CD79B antibody-mediated activation of BCR signaling contributes to PV toxicity by mechanism of activation induced cell death. In contrast to anti-IgH based BCR-stimulation (which was toxic to all tested MCL cell lines), unconjugated anti-CD79B antibody stimulated cell growth and induced corresponding changes in the cell cycle. As expected, PV treatment resulted in accumulation of cells in the G2/M cell cycle phase with apoptosis induction. To verify that BCR signal activation does not contribute to the PV toxicity, we concurrently inhibited BCR signaling. Combined PV treatment accompanied by BTK inhibition, AKT inhibition, or inhibition of Ca2+ response did not reduce PV toxicity. On the contrary, antibody based CD79B stimulation had an opposite effect. Unconjugated anti-CD79B antibody strongly protected MCL

cell lines against unconjugated MMAE toxicity. Next, we used live cell imaging to continuously evaluate treatment time dependent changes of half inhibitory concentrations (IC50). As expected, unconjugated MMAE showed a steady and time dependent decrease of IC50 (the same concentration of MMAE was more effective at later timepoints). In contrast, PV IC50 showed an increase within the 24 to 48 hours interval before starting to decrease (the same PV concentration was less effective at later timepoints between 36 to 48 hours in comparison to 24 hours). This observation is consistent with a combined effect of MMAE toxicity and anti-CD79B mediated growth stimulation in PV conjugate. If our conclusion is correct and PV induced BCR signaling stimulate cell growth, it should be highly therapeutically effective to combine PV with BCR signaling inhibitors. Indeed, using a cross-titration-based drug synergy evaluation, we detected a strong and wide synergism between 1) PV and BTK inhibitor ibrutinib, and 2) PV pan AKT inhibitor capivasertib, both pending in vivo mice tumor xenograft model-based confirmation.

Conclusion. We have identified a very important cellular side effect of PV - stimulation of BCR signaling and associated stimulation of cell growth. Importantly, this anti-CD79B mediated cell growth stimulation could be eliminated by combining PV with BCR signaling inhibition. Our data thus provide a strong foundation for future research and preclinical testing of these therapeutic combinations.

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P82

C/EBPΔ PRESERVES HEMATOPOIETIC STEM HOMEOSTASIS AND REGULATES MYELOID COMMITMENT IN A CONTEXT-DEPENDENT MANNER

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Background: CCAAT enhancer-binding proteins (C/EBPs) are key transcription factors that regulate lineage-specific gene expression, cell proliferation, self-renewal, and differentiation. In the hematopoietic system, distinct C/EBPs play a critical role in maintaining hematopoietic stem and progenitor cell (HSPC) function, promoting granulocytic production. C/EBPd has been described as a myeloid transcription factor that amplifies the inflammatory response in granulocytes. However, its role at the HSPC level remains elusive.

Aims: to elucidate the role and molecular mechanism of C/EBPd in regulating HSPC function under steady-state and emergency granulopoiesis conditions.

Methods: A whole-body C/EBPδ knockout (dKO) mouse model was generated. We used murine *in vivo* lipopolysaccharide (LPS) administration, single-cell RNA sequencing (scRNA-seq) of HSCs, flow cytometry, DNA fiber spreading assays, transplantation assays, and HSPC culture assays.

Results: dKO mice showed an expanded HSPC pool compared to WT controls, with increased numbers of hematopoietic stem cells (HSCs) and myeloid-biased multipotent progenitor cells (MPP3). *In vitro* assays using liquid culture and semi-solid medium demonstrated an expansion of both dKO HSCs and MPP3 cells in comparison to WT controls with increased myeloid output. Upon transplantation, dKO MPP3 cells showed increased myeloid reconstitution in the bone marrow of recipient mice compared to

WT controls, suggesting that C/EBPd might act as a negative regulator of myelopoiesis. Transcriptomic profile of δKO HSCs reveal a signature consistent with increased myeloid bias, enhanced quiescence and activation of stress-response programs. Cycle analysis of dKO HSCs confirmed an increased proportion of cells in G0 compared to WT. Moreover, dKO HSCs MPP3 displayed hallmarks of replication stress when cycling, reflected in an abnormal increase of DNA replication speed in steady state conditions. Next, we assessed the role of C/EBPd in emergency granulopoiesis. Sc-RNA-seq of HSCs from WT mice challenged with LPS or PBS for 4 hours revealed that C/EBPd is specifically upregulated in myeloid-biased HSCs during emergency granulopoiesis, suggesting its crucial role at early stages of this process. Thus, we challenged dKO and WT mice with LPS and analyzed the hematopoietic response 4 hours post-challenge. While WT mice expanded the MPP3 compartment upon LPS administration, dKO mice failed to do so. In addition, granulocytic production was compromised in dKO cells both *in vitro* and *in vivo* after LPS injection. In addition, δKO HSCs failed to efficiently upregulate key components of the myeloid transcriptional program in response to LPS, exhibiting an inflammatory to myeloid transcriptional arrest.

Conclusions: In steady-state conditions, C/EBPd is an essential transcription C/EBPδ plays a role in preventing an uncontrolled expansion of the HSPC pool and premature myeloid commitment. During emergency granulopoiesis C/EBPδ is essential for initiating the transcriptional program that drives myeloid differentiation and is required for efficient myeloid expansion to meet the increased granulocytic demands.

P83

NOVEL EUROFLOW QUALITY ASSESSMENT ON B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA MINIMAL RESIDUAL DISEASE. PROGRAM DEVELOPMENT AND FOUR ROUNDS OF IMPLEMENTATION

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SBORNÍK ABSTRAKTŮ

Background
Minimal residual disease (MRD) is one of the strongest prognostic indicators in both pediatric and adult patients with B-cell precursor acute lymphoblastic leukemia (BCP-ALL). The EuroFlow consortium developed a BCP-ALL MRD panel for flow cytometry, enabling highly sensitive MRD measurement in virtually all BCP-ALL patients (Theunissen et al., 2017). This panel is used worldwide and supported by EuroFlow educational activities.

Aims
Our objective was to develop a quality assessment (QA) scheme for the measurement and interpretation of the EuroFlow BCP-ALL MRD panel.

Methods
Building upon previous experience with other EuroFlow QA programs (Kalina et al., 2015, Neirinck et al., 2024), we developed a scheme with two components: I) a wet part to assess the performance of the locally performed BCP-ALL MRD panel staining on healthy peripheral blood (PB) samples and II) a dry part to evaluate the analysis and data interpretation of centrally provided BCP-ALL MRD files. An advantage of this setup is that it does not require sending around samples. As both tubes 1 and 2 of the panel are identical apart from one parameter, we included only tube 1 in both parts of the program. To establish quality parameters for the wet part, 60 healthy PB samples of tube 1 of the BCP-ALL MRD panel were collected in 11 EuroFlow laboratories. For each parameter (fluorescent n=8, scatter characteristics n=2), we defined 1-2 target populations (in total n=5) for evaluation. For the dry part, we gathered 15 BCP-ALL MRD files of BCP-ALL patients on treatment. Each file was analyzed independently by three experts from EuroFlow laboratories to assess technical quality and to determine the consensus MRD value.

Results
We established target populations for each parameter and defined the QA reference range based on collected healthy PB samples in two beta rounds. The median CV of the 11 evaluated parameters was 38% (range 17-278%). The highest CV was found for side scatter and CD38 median fluorescent intensity (MedFI) on dendritic cells. For the dry part, a novel analysis method was introduced, including merging the locally measured healthy PB fcs file with the centrally provided BCP-ALL patient file. This analysis strategy was tested and optimized during two beta rounds performed solely in EuroFlow labs. Out of 15 collected BCP-ALL patient files, the analysis of 8 files showed concordant MRD results with no technical errors. These files were included in the library for the QA 2024 rounds. After two beta rounds, we opened the program to all participants and have run four rounds (2023-2024). The number of participants increased up to 30 in 2024. Participants are asked to provide data on MRD analysis (number of events), MRD calculation (%), limit of detection and quantification and BCP-ALL immunophenotype. Results of wet part (1 value per center) and MRD events identification (0-3 values per center) are shown in Figure 1. All parameters are evaluated and discussed in the Summary of the QA round.

Summary/Conclusion
We successfully developed and launched a novel QA program for EuroFlow BCP-ALL MRD panel measurement. To date, four rounds were completed. We provide participants with feedback on EuroFlow BCP-ALL MRD panel measurement, data analysis and interpretation, aiming to standardize and improve MRD diagnostics.

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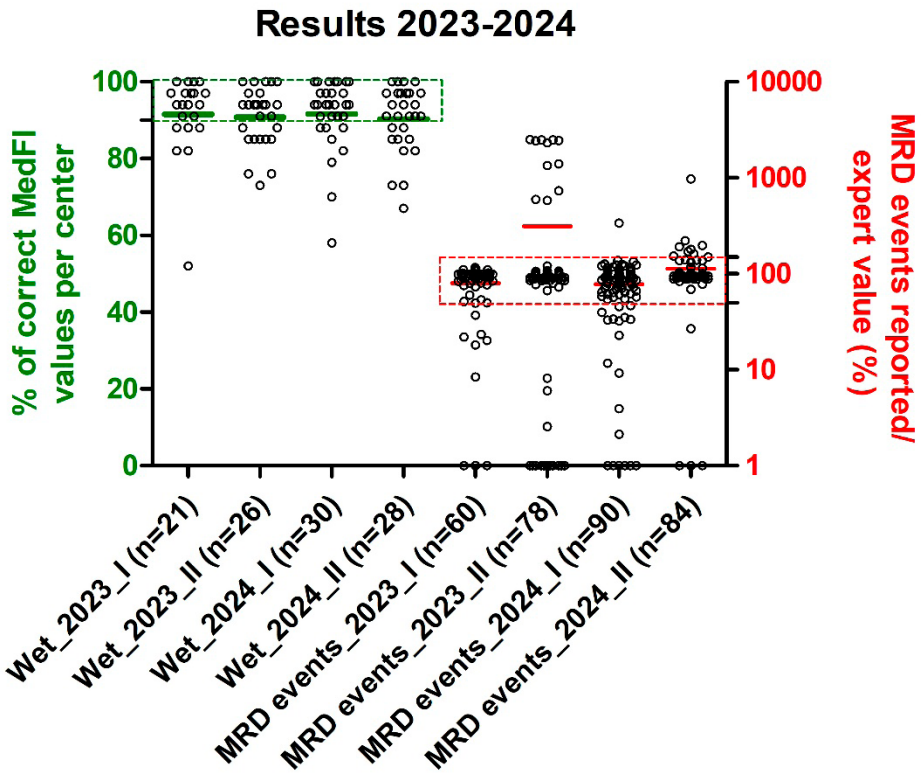
SBORNÍK ABSTRAKTŮ



P84

CHIMERISM ANALYSIS AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION: CLINICAL RELEVANCE AND METHODOLOGY

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Monitoring cell chimerism after allogeneic hematopoietic stem cell transplantation has a broad spectrum of clinical applications—from monitoring engraftment or graft failure early after transplantation to tracking the effectiveness of immunotherapy or detecting malignant disease relapse. Today, a wide range of methods are utilized for chimerism analysis, and every laboratory has its methodology for evaluating and interpreting results. The most commonly used method still remain the analysis of short tandem repeats. Here, we focused on identifying the most common sources of variability in the results, which were determined during the international proficiency testing program for quantitative analysis of cell chimerism organized by our laboratory since 2005. In the Czech Republic, there are no guidelines for chimerism testing, and each laboratory belonging to the transplant center introduces new methods, such as HLA-loss relapse testing and chimerism analysis in cell subsets, based on the requirements of clinicians.

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SBORNÍK ABSTRAKTŮ

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SBORNÍK ABSTRAKTŮ

BP01

Detection of clinically relevant variants in the TP53 gene below 10% allelic frequency: A multicenter study by ERIC, the European Research Initiative on CLL

Pavlová Š. (Brno)

http://dx.doi.org/10.1002/hem3.70065

BP02

NGS-MRD negativity in post-HSCT ALL spares unnecessary therapeutic interventions triggered by borderline qPCR results without an increase in relapse risk

Šeferna K. (Praha)

http://dx.doi.org/10.1002/hem3.70124

SBORNÍK ABSTRAKTŮ

OŠETŘOVATELSKÝ PROGRAM

SBORNÍK ABSTRAKTŮ

OŠETŘOVATELSKÝ PROGRAM I.

„INFEKCE POD KONTROLOU“

ES01

Standardní a cílená protiepidemická opatření na pracovištích Kliniky dětské hematologie a onkologie 2. LF UK a FN Motol z pohledu Oddělení nemocniční hygieny a epidemiologie

Jana Hrončeková
Oddělení nemocniční hygieny a epidemiologie, Fakultní nemocnice v Motole, Praha 5, Česko

Z hlediska prevence infekcí včetně infekcí spojených se zdravotní péčí je nutné správně definovat a nastavit tzv. protiepidemická opatření, která zohledňují všechna rizika pro jejich přenos a akviraci. Nastavená protiepidemická opatření ve FN Motol zohledňují legislativní požadavky, evidence based doporučení a výstupy risk assessmentu místních podmínek. Protiepidemická opatření musí být obzvláště důsledně nastavována a dodržována na hematoonkologických pracovištích. Ve FN Motol se na jejich nastavování významně podílí i vedení Kliniky dětské hematologie a onkologie 2.LF UK a FN Motol. Péče o bezpečné prostředí včetně aeroskopického monitoringu vzduchu na přítomnost mikroskopických vláknitých hub a opatření k jejich eliminaci jsou nedílnou součástí standardních protiepidemických opatření. Cílená protiepidemická opatření při výskytu onemocnění vyvolaných různými mikroorganismy (obzvláště rezistentními kmeny) slouží k zamezení přenosu mezi jednotlivými pacienty a jsou zaváděna neprodleně po jejich nahlášení. Při jejich realizaci jsou využívané standardizované pokyny, které mají personálu usnadnit jejich zavedení.

ES02

Sepse u dětských hematoonkologických pacientů – závažná komplikace léčby

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[2)]*Ústav lékařské mikrobiologie 2. LF UK a FN v Motole, Fakultní nemocnice v Motole, Praha 5, Česko*

Sepse je závažnou komplikací u imunokompromitovaných pacientů léčených pro hematologickou malignitu či solidní nádory a u pacientů po transplantaci krvetvorných buněk a je spojena se signifikantní morbiditou i mortalitou. Gram-negativní bakterie jsou nejobávanějším původcem sepsí u onkologických pacientů. Celosvětový nárůst antimikrobiální rezistence zvyšuje pozornost k adekvátní a včasné kontrole infekce a k optimalizovanému použití antibiotické léčby.

Na Klinice dětské hematologie a onkologie FN v Motole je dlouhodobě (od roku 2000) monitorována problematika sepsí a kolonizací, zejména u nejrizikovější skupiny pacientů léčených pro hematologické malignity. Vzácněji ale zaznamenáváme život ohrožující septické stavy s nutností resuscitační péče i u pacientů léčených pro solidní nádory.

V letech 2000 – 2025 27 – 65 % pacientů léčených pro hematologickou malignitu mělo alespoň 1 sepsi, kolonizace rezistentními kmeny bakterií u onkologických pacientů se dlouhodobě pohybuje mezi 25 – 55%. Původcem sepsí v posledních letech nadále zůstávají G+ kmeny ve 2/3 případů (zejména *koaguláza negativní stafylok*oky), nejčastějšími původci G - sepsí jsou klinicky významnější Enterobakterie (kmeny *E.coli, Klebsiella, Enterobacter),* 38 – 52% gram negativních bakterií v hemokulturách je způsobená rezistentními kmeny (obvykle s produkcí širokospektrých betalaktamáz=ESBL+). Sepse způsobená kolonizačním kmenem byla zaznamenána v 15 – 26% případů.

Pro management sepse je nezbytná pečlivá monitorace klinického stavu pacientů včetně vyhodnocování nespecifických příznaků (bolesti břicha, zvracení, nevůle, rychlá změna stavu…). Podstatné je včasné zahájení empirické ATB léčby horečky v průběhu neutropenie či při klinických septických projevech s případnou úpravou cílené léčby.

ES03

Hygienické režimy a zkušenosti s jejich aplikací v ÚHKT

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ÚHKT je terciární zdravotnické zařízení specializované na onkologická i neonkologická onemocnění krvetvorby dospělých. Součástí péče je transplantační program a buněčná terapie přípravky pokročilé terapie. Typická je dlouhá doba hospitalizace a opakované hospitalizace kvůli navazujícím cyklům chemoterapií.

Tomu je přizpůsobeno spektrum ošetřovacích bariérových infekčních i neinfekčních. Po aktualizaci dle vyhlášky č. 306/2012 Sb. uplatňujeme režimy: bariérový obecný, kontaktní, kontaktní – Clostridia, respirační, respirační zvýšený, transplantační A, transplantační B (po enfraftmentu).

Probíhá pravidelný mikrobiologický screening hospitalizovaných. Data o kolonizaci pacientů patogeny s nepříznivým profilem citlivosti k antibiotikům jsou sledována manuálně Oddělením nemocniční hygieny i přes software HAIDi. Doba do finálního výsledku mikrobiologického screeningového vyšetření u pozitivních záchytů dosahuje 5 dnů.

V příspěvku budou prezentovány praktické zkušenosti s aplikací jednotlivých režimů i analýza výzev při zavádění izolačních režimů, včetně personálních, prostorových a materiálních nároků.

ES04

Jak chránit planetu a neohrozit pacienta?

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Klinika anesteziologie a resuscitace, Fakultní nemocnice Královské Vinohrady, Praha, Česko

Zdravotnictví je jedním z největších veřejných sektorů a zároveň významným producentem emisí, odpadu a spotřeby zdrojů. Ať už si to uvědomujeme či nikoli, udržitelnost je s naší každodenní klinickou praxí úzce propojena – od volby léčebných postupů, přes používání jednorázových pomůcek až po organizaci péče a provoz zdravotnických zařízení. V době klimatické krize proto stojíme před otázkou: jak chránit planetu, aniž bychom ohrozili pacienta?

Přednáška představí důvody, proč by se zdravotníci měli tématu udržitelnosti věnovat, a nabídne přehled současných přístupů k jejímu „měření“. Shrne výsledky vybraných studií a projektů, které se snaží kvantifikovat ekologickou stopu zdravotnictví, a ukáže, jaké poznatky z nich lze převést do našeho prostředí.

Zvláštní pozornost bude věnována jednoduchým, praktickým krokům, které mohou zdravotníci podniknout i bez systémových změn.

SBORNÍK ABSTRAKTŮ

OŠETŘOVATELSKÝ PROGRAM II.

WORKSHOP PSYCHOLOGIE

Jak se dělá duševní zdraví: co si lze z psychoterapie odnést jako univerzální poznání pro duševní zdraví

Miroslav Světlák
Ústav lékařské psychologie a psychosomatiky Masarykovy univerzity v Brně

Anotace: Psychoterapie není pouze léčbou symptomů, ale také zdrojem hlubšího porozumění tomu, jak vzniká duševní zdraví. Tato přednáška ukazuje, jaké klíčové principy a poznatky o lidské psychice se v terapii odhalují a jak mohou být srozumitelně předány i těm, kteří terapii sami nepodstupují. Může jít o inspiraci pro vlastní sebepéči, ale i o užitečný rámec pro zdravotníky, kteří chtějí psychoterapii lépe chápat, smysluplně ji indikovat nebo o ní srozumitelně mluvit s pacienty.

OŠETŘOVATELSKÝ PROGRAM III.

WORKSHOP KOMUNIKACE

Komunikační dovednosti s mediačním nástrojem

Petra Absolonová
Gastroenterologické oddělení, MOU Brno

Vztah mezi pacientem a zdravotní sestrou je klíčovým prvkem kvalitní zdravotní péče. Efektivní komunikace nejen zvyšuje důvěru pacienta, ale významně přispívá k prevenci nedorozumění a konfliktních situací. Tato prezentace se zaměřuje na posílení komunikačních dovedností zdravotních sester prostřednictvím využití principů mediace – nástroje, který umožňuje aktivní naslouchání, identifikaci potřeb a podporu konstruktivního řešení napětí.

Účastníci se seznámí s praktickými technikami, jak zaujmout neutrální postoj, vést dialog bez obviňování a stát se prostředníkem mezi pacientem a zdravotnickým týmem. Představíme konkrétní příklady z praxe, kde mediace pomohla předejít eskalaci problémů, a nabídneme návody, jak tyto nástroje využívat v každodenní péči.

Cíle prezentace:

- Pochopit význam a roli sestry v kontextu komunikace s pacientem.
- Seznámit se se základy mediačních technik a jejich využitím ve zdravotnictví.
- Rozvíjet schopnost aktivního naslouchání, empatie a zvládání emočně náročných situací.
- Posílit profesní roli sestry jako facilitátora bezpečné a respektující komunikace.

Přínos pro praxi:
Zdravotní sestry získají konkrétní nástroje a strategie, jak zlepšit vzájemnou komunikaci s pacienty i kolegy, snížit výskyt konfliktních situací a zvýšit svou profesní jistotu v náročných mezilidských interakcích.

OŠETŘOVATELSKÝ PROGRAM IV.

VARIA

S01

Fytoterapie a koření v hematoonkologii – vliv na účinnost léčby

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Anotace:
U hematoonkologických pacientů je běžné užívání fytoterapie a přírodních doplňků, často bez vědomí zdravotníků. Mnohé z nich však mohou významně ovlivnit farmakokinetiku a farmakodynamiku onkologické léčby.

Klinicky významné interakce:
- Třezalka tečkovaná: snižuje účinnost léčiv (např. venetoklax, ibrutinib, glukokortikoidy).
- Kurkuma: riziko interakcí s cílenou terapií a azoly.
- Zázvor, česnek, ginkgo: zvyšují riziko krvácení (při antikoagulaci, trombocytopenii).
- Grapefruitová šťáva: riziko toxických hladin léčiv (např. BTK inhibitory).
- Skořice (cassia): riziko hepatotoxicity a interakcí s warfarinem.

Role sestry:

- Aktivně se dotazovat na užívání bylinných přípravků a „superpotravin".
- Edukovat pacienty: „přírodní" neznamená bezpečné.
- Při podezření na interakci konzultovat lékaře/farmaceuta.

Závěr:
Fytoterapie a koření mohou být skrytým rizikem selhání nebo toxicity onkologické léčby. Role sestry je klíčová v prevenci – dotazováním, edukací a včasným rozpoznáním rizikových kombinací.

S02
Uzdrav mysl - Mindfullnes

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Prezentace se zaobírá problematikou syndromu vyhoření, včetně metody mindfulness. Obsahem je zevrubná historie výskytu syndromu vyhoření, příčiny a projevy. Zakomponovány jsou techniky zvládání stresu, prevence samotného syndromu a technika mindfulness, jenž má mnoho benefitů. Pozitivně ovlivňuje psychické rozpoložení, soustředěnost a zvyšuje radost ze života. Zároveň může být praktikována v různých formách.

S03
Jídlo jako lék

Lucie Růžičková
VFN Praha

Strava nás ovlivňuje od narození až po smrt. Proto jezme dobře, a tělo i duše se nám odmění. Malé hříchy v jídle si ponechme jako odměnu za své laskavé zacházení sami se sebou.

Workshop pro zaměstnance *Jídlo jako lék* je součástí psychologické podpory pro zdravotníky. Jeho cílem je nabídnout praktické nástroje pro zlepšení stravovacích návyků v náročném pracovním prostředí. Účastníci obdrží brožurku s přehlednými informacemi, které jim pomohou snadno se orientovat v potravinách a osvojit si přípravu jednoduchých a zdravých jídel.

Obsah workshopu vychází z myšlenky Hippokrata: „Nechť je tvé jídlo tvým lékem..." a staví na principech středomořské, severské a atlantické stravy. Tyto stravovací vzory podporují zdraví střevní mikrobioty a celkovou vitalitu díky důrazu na čerstvé rostlinné potraviny, celozrnné obiloviny, luštěniny, fermentované produkty a omezenou konzumaci masa. Doporučení zahrnují správné rozdělení talíře, preferované způsoby přípravy jídel, pravidelný pitný režim a omezení cukrů, tuků a soli.

Zvláštní pozornost je věnována výživovým doporučením pro směnný provoz, kde hrozí vyšší riziko únavy, přibývání na váze a poklesu výkonu. Klíčová je pravidelnost stravování, omezení příjmu mezi půlnocí a šestou hodinou ranní, preference lehkých a výživných jídel, příprava vlastních pokrmů a důraz na chutnost i jednoduchost. Doporučuje se snídaně před denním spánkem, rozdělení jídel do 3–5 denních chodů a vyhýbání se potravinám s vysokým obsahem jednoduchých cukrů.

Součástí workshopu jsou konkrétní recepty, tipy na úpravu oblíbených jídel a inspirace pro zdravý jídelníček, který je udržitelný i v náročném pracovním režimu.

S04
Aromaterapie – v práci i doma

Monika Vondrová
VFN Praha

Ráda bych přiblížila aromaterapii zdravotníkům a pomohla bych jim tak k lepšímu pracovnímu prostředí a také ke své vlastní spokojenosti. Aromaterapií

lze zmírnit stres, únavu a přetížení, a to je myslím hlavní téma každého zdravotníka.

V přednášce se budu věnovat teorii aromaterapie a praktickým radám jak jí bezpečně a účelně použít v běžném životě.

S05
Jak dopadl projekt „Prevence syndromu vyhoření“ na Klinice dětské hematologie a onkologie?

Jitka Wintnerová
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Na začátku přednášky připomenu výsledky anonymního dotazníku, se kterými jsem podrobně seznámila účastníky PHD 2025. V rámci prevence syndromu vyhoření a s tím spojeným rizikem různých závislostí většina dotázaných uváděla, že by uvítala víkendový relaxační pobyt se svými spolupracovníky. Respondenti také projevili velký zájem o různé relaxační techniky, základy jógy, dále by se rádi naučili pracovat se stresem a zacházet s emocemi. Vedení Kliniky dětské hematologie a onkologie se rozhodlo zrealizovat pilotní projekt „Prevence syndromu vyhoření“. Projekt byl hrazen z konta darů kliniky určeného na vzdělání. V roce 2025 proběhlo pět víkendových wellness pobytů s odborným programem. Celkem se zúčastnilo víkendového pobytu 70 % sester z kliniky. Tento projekt byl hodnocen zaměstnanci velmi kladně, a proto je snaha pokračovat v projektu i v tomto roce. Do budoucna by bylo dobré, aby víkendový pobyt s wellness a odborným programem byl zařazen do benefitů pro zaměstnance především na exponovaných pracovištích v rámci dlouhodobé péče o zaměstnance.

Strategie dlouhodobé péče o zaměstnance je klíčovým faktorem pro úspěch každé firmy. Mezi dlouhodobé strategie patří vzdělávací programy, školení, kurzy, které podporují neustálý osobní a profesní růst. Nabízení nadstandardních zdravotních benefitů může výrazně přispět k fyzickému i psychickému zdraví pracovníků. Wellness programy, které zahrnují aktivity zaměřené na snižování stresu a podporu zdravého životního stylu jsou velmi efektivní. Také teambuildingové aktivity podporují týmovou spolupráci, posilují mezilidské vztahy a zlepšují komunikaci v týmu, což přispívá k celkovému zlepšení pracovní atmosféry. Podpora duševního zdraví na pracovišti může zahrnovat zavedení programů zaměřených na snížení stresu, jako jsou např. jógové lekce.

Důležitá je také možnost konzultací s psychologem buď přímo na pracovišti, nebo prostřednictvím externích poskytovatelů zdravotní péče. Tyto aktivity pomáhají zlepšit duševní pohodu zaměstnanců, snižují úroveň pracovního stresu a předcházejí výskytu psychických problémů.

Spokojení zaměstnanci jsou základem nízké fluktuace a pozitivního pracovního klimatu.

S06
„Od stigmat k standardům - konopí v moderní ošetřovatelské péči“

Marcel Koňařík
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Konopí pro léčebné použití patří mezi relativně nový léčebný prostředek k zmírnění neztišitelné bolesti u nádorové i nenádorové bolesti. Jeho využití je však mnohem širší – je účinné při spastických stavech, mimovolných kinézách a dalších chronických stavech. Příspěvek vymezuje jeho definici, využití a základní principy preskripce v České republice a dále popisuje historii a vývoj jeho implementace do moderního ošetřovatelství.

SBORNÍK ABSTRAKTŮ

S07

Bertíkovo uzdravení jako nástroj pro komunikaci závažné diagnózy u dětí nízkého věku s onkologickým onemocněním

Petra Keslová
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Efektivní komunikace mezi zdravotníky a dětskými onkologickými pacienty je klíčovým prvkem kvalitní péče, která ovlivňuje nejen průběh léčby, ale i psychickou pohodu dítěte a jeho rodiny. Každá věková skupina vyžaduje odlišný přístup k podávání informací o léčebném procesu i zapojení dítěte samotného.

Na Klinice dětské hematologie a onkologie ve FN v Motole se ročně léčí kolem 250 dětských pacientů s nově diagnostikovaným onkologickým onemocněním. Nejméně třetina dětských pacientů je ve věkové kategorii do 6 let. Předškolní dítě chápe nemoc a léčbu především skrze konkrétní obrazy, příběhy a emoce, nemá abstraktní myšlení. Obrázková knížka může fungovat jako citlivý komunikační nástroj, který napomáhá dětem i jejich rodičům porozumět situaci, vyjadřovat pocity a zmírňovat úzkost spojenou s léčbou.

Ve spolupráci s nadační podporou spolku HAIMA z.s. byla pro KDHO koncem roku 2025 vydána knížka Bertíkovo uzdravení, jejíž autorkou je česká spisovatelka, maminka vyléčeného onkologického pacienta. Kniha se samolepkami pomáhá dítěti i jeho rodičům pomocí obrázků a jednoduchého textu porozumět nové složité situaci i léčebným úkonům. Herní prvky pak umožňují malému dítěti se situaci lépe přizpůsobit.

Díky nadačním projektům mohou zdravotníci efektivně využívat komunikační nástroje. Sdílené čtení a rozhovor nad obrázkovou knížkou podporují otevřenou komunikaci mezi dítětem, rodiči a zdravotníky, posilují vzájemnou důvěru a napomáhají dítěti nízkého věku zvládat náročné emoční situace.

VŠEOBECNÉ INFORMACE

MÍSTO KONÁNÍ KONFERENCE

26. pražské hematologické dny – HEMATOLOGIE 2026
se uskuteční v Clarion Congress Hotel Prague.

Adresa:
Freyova 33
190 00 Praha 9
www.clarioncongresshotelprague.com/cs

Čtyřhvězdičkový Clarion Congress Hotel Prague se nachází na Praze 9 ve Vysočanech, přímo na trase linky B Vysočanská. Od historického centra Prahy je vzdálen pouhých 15 minut jízdy městskou hromadnou dopravou. Hotel je výborně dostupný jak MHD, tak i automobilem, který je možné zaparkovat na hotelovém parkovišti.

REGISTRACE

Po celou dobu konference je registrace umístěna ve vstupním foyer ve 3 patře hotelu.
Registrační přepážka bude otevřena v následujících časech:

středa 21. 1. 2026	8:00 – 18:00 hodin
čtvrtek 22. 1. 2026	8:00 - 19:00 hodin
pátek 23. 1. 2026	8:00 - 13:30 hodin

Registrační poplatky na místě:

Lékař, VŠ	3.000 Kč
NLZP (všeobecná sestra, laborant)	900 Kč
Student, důchodce	700 Kč
Aktivní účastníci (prezentující autoři)	zdarma
VIP host, výbor, čestný člen ČHS	zdarma
Zástupce nevystavující firmy	15.000 Kč

Veškeré platby na místě bude možné provést pouze v českých korunách.

Registrační poplatek zahrnuje:

* vstup na odborný program během celé konference
* kongresovou tašku s materiály
* volný vstup na výstavu firem
* účast na posterové sekci s občerstvením
* občerstvení během přestávek, obědy, večeře

CERTIFIKÁTY

Akreditovaná akce systému celoživotního vzdělávání je garantována ČLK a hodnocena 18 kreditními body. Vzdělávací akce je pořádána dle Stavovského předpisu ČLK č. 16.
Pro NLZP je akce garantována ČAS.

Potvrzení o účasti, certifikáty ČLK a ČAS budou zaslány elektronicky do 14 dnů po skončení konference.

Konference HEMATOLOGIE 2026, 26. pražské hematologické dny je prověřen organizací EthicalMedTech a akci je přidělen status SCHVÁLENO (EMT-25-06144).

STRAVOVÁNÍ

Kávové přestávky:
foyer
konferenčních sálů,
sál Meridian

Obědy:
hotelová restaurace Veduta (2. patro)
Obědy budou vydávány:
čtvrtek 22. 1. 2026 / pátek 23. 1. 2026 v určených časech

Večeře:
restaurace Veduta
(2.patro),
čtvrtek 22. 1. 2026 od 19:30 hodin

Každý registrovaný účastník obdrží v kongresové tašce 2 obědové lístky.

JMENOVKY

Každý účastník konference včetně vystavovatelů obdrží při registraci jmenovku, která ho opravňuje ke vstupu do daných prostor.

Barvy jmenovek:

Červená	Modrá	Žlutá	Hnědá	Šedá
lékař, VŠ	NLZP (všeobecná sestra, laborant)	VIP, čestní členové, hosté kongresu	vystavovatelé	organizační tým

Bez jmenovky nebude vstup povolen – při ztrátě bude vystavena kopie jmenovky proti úhradě 300 Kč.

DOPRAVA

Clarion Congress Hotel je snadno dostupný linkou B metra (žlutá linka) do **stanice Vysočanská**.
Bližší informace o dopravě v Praze najdete na www.dpp.cz

PARKOVÁNÍ

Parkování není zahrnuto v registračním poplatku a náklady na něj si hradí každý účastník sám. Nákupní galerie Fenix disponuje 600 místy.
Parkování zdarma: 3 hodiny během týdne a 5 hodin o víkendu.
K parkování je možné využít hotelové garáže, PATRO -3 (značeno jako P2). Bližší informace na registrační přepážce.

INFORMACE K AKTIVNÍ ÚČASTI

Jednací jazyk

Jednacím jazykem konference bude čeština, slovenština a angličtina.

Informace pro přednášející

Přednášky prezentované přes PC (dataprojektor) je nezbytné předat na USB flash. Přednášky můžete předávat technikům ve Vašem přednáškovém sále **od středy 21. 1. 2026 od 10:00 hodin, nejpozději 60 minut před zahájením programu vašeho přednáškového bloku,** jinak nemůžeme garantovat bezproblémový průběh Vaší prezentace.
Média si, prosíme, vyzvedněte po ukončení Vašeho programového bloku.

POSTERY

Postery budou umístěny na panelech o maximálním rozměru 200 cm (šířka) x 200 cm (výška), v sále Meridian (3. patro).
Posterové tabule budou označeny čísly, které odpovídají posterové části programu (P01 – P84).
Postery budou vystaveny po celou dobu konání konference.
Instalace posterů: středa 21. 1. 2026 od 10:00 hod.
Deinstalace posterů: pátek 23. 1. 2026 po 13:30 hod.
Pomůcky k instalaci budou k dispozici v prostoru posterové sekce.

MODEROVANÁ POSTEROVÁ SEKCE

Moderovaná posterová sekce bude prezentována v deseti blocích formou poster walk.

Jednotlivé bloky:
Akutní lymfoblastová leukémie
Akutní myeloidní leukémie I
Akutní myeloidní leukémie II
Chronická myeloidní leukémie
Lymfomy I
Lymfomy II
CLL / Lymfomy III
Myelomy
Vzácná krevní onemocnění
Experimentální a translační hematologie

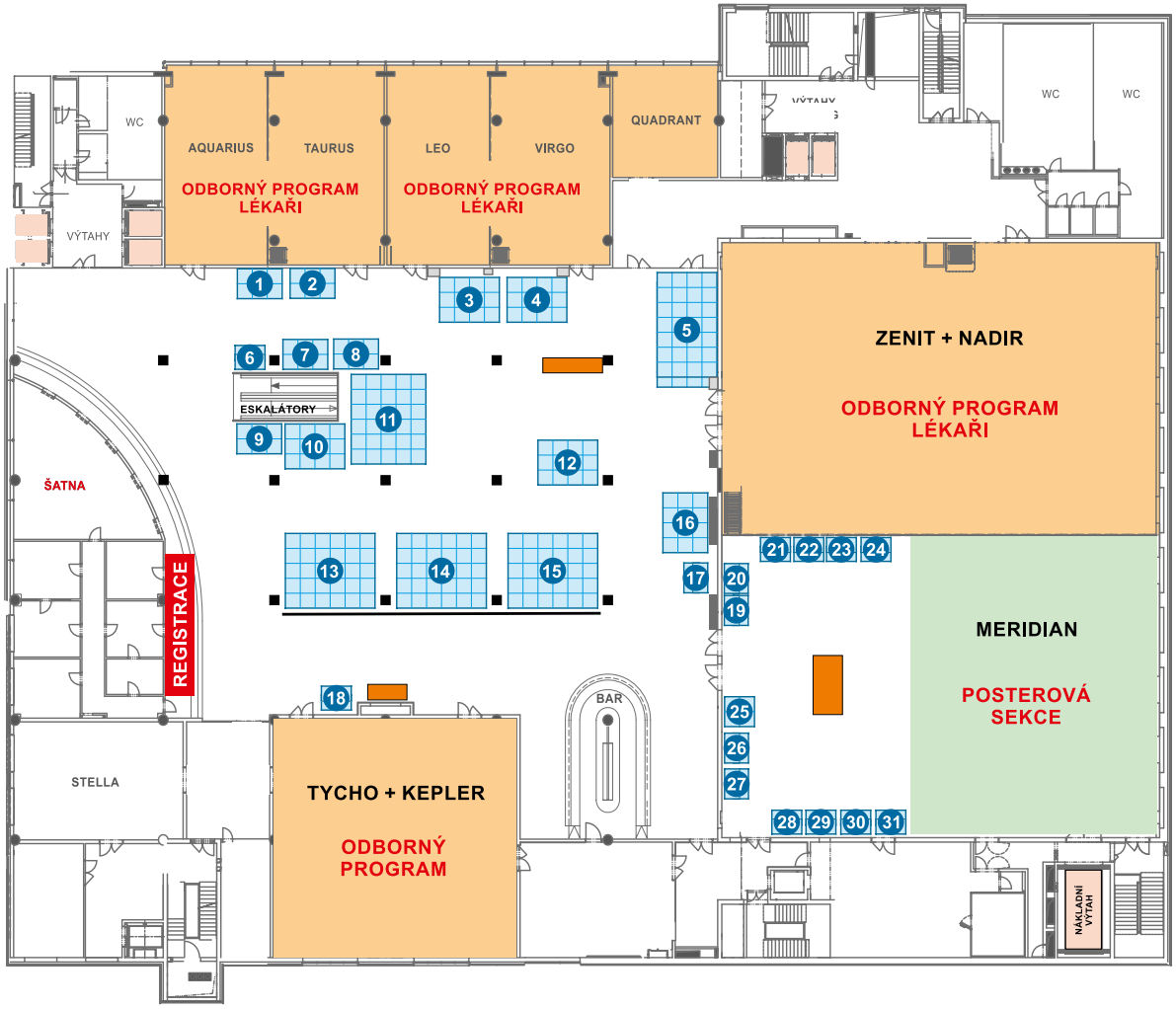
Poster walk každého bloku povede moderátor. Máte možnost se k nim připojit a poslechnout si krátké prezentace pro jednotlivé postery.
V rámci samotné posterové sekce nebo mimo čas k tomu určený budete mít také možnost prohlédnout si postery individuálně.

Prosíme autory sdělení, aby byli přítomni u svého posteru během celé doby vymezené k diskusi:
ve středu 21. 1. 2026 od 17:45 do 19:00 hodin (sál Meridian).
Každý prezentující autor představí svůj poster souhrnem v délce maximálně 2 minuty.

SBORNÍK ABSTRAKTŮ

Sborník neprošel jazykovou úpravou.
Autoři odpovídají za obsahovou i formální stránku svého příspěvku.

PLÁN PROSTOR A VÝSTAVY



Legenda k plánku výstavní plochy

1. AOP Orphan Pharmaceuticals GmbH
   Member of the AOP Health Group“
2. Sanofi s.r.o.
3. CSL BEHRING s.r.o.
4. GlaxoSmithKline, s.r.o.
5. Gilead Sciences s.r.o.
6. Česká lékařská společnost Jana Evangelisty Purkyně, z.s.
7. AstraZeneca Czech Republic s.r.o.
8. ROCHE s.r.o.
9. Pfizer, spol. s r.o.
10. Amgen s.r.o.
11. Novartis s.r.o.
12. Swixx Biopharma s.r.o.
13. AbbVie s.r.o.
14. Bristol-Myers Squibb spol. s r.o.
15. Johnson&Johnson
16. ELI LILLY ČR, s.r.o.
17. European Hematology Association
18. Lymfom Help
19. Astellas Pharma s.r.o.
20. Sandoz s.r.o.
21. Octapharma CZ s.r.o.
22. Grifols s.r.o.
23. Medac GmbH - organizační složka
24. Pragostem s.r.o.
25. Takeda Pharmaceuticals Czech Republic s.r.o.
26. Zentiva, k.s.
27. Steiner, s.r.o.
28. Swedish Orphan Biovitrum s.r.o.
29. SERVIER s.r.o.
30. Herbacos Recordati s.r.o.
31. HEMATOLOGIE-online.cz
    Konzultační a vzdělávací portál pro lékaře

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astellas

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medac
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For the safe and optimal use of human proteins

Pragostem

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