Anti-Angiogenic Therapy with Bevacizumab Is Effective *In Vivo* in Ibrutinib-Resistant Mantle Cell Lymphoma

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INTRODUCTION

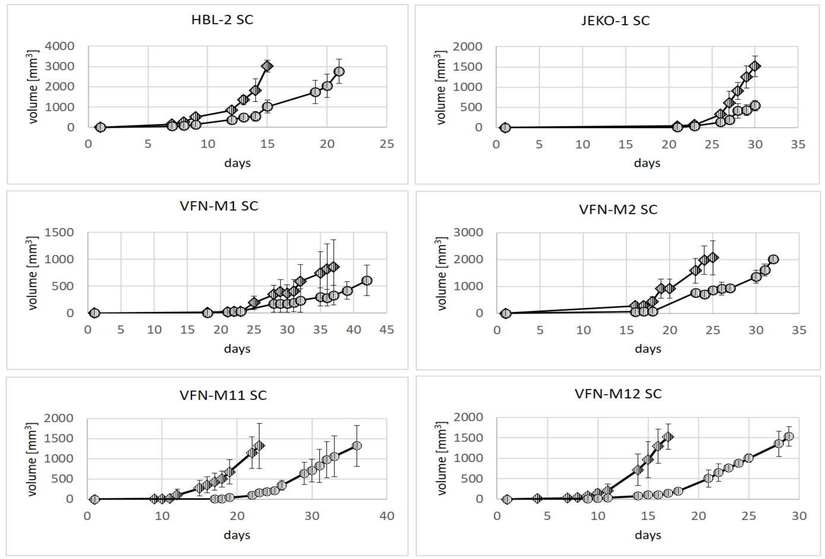
Bevacizumab is a monoclonal antibody against human vascular endothelial growth factor (hVEGF), approved for combinational treatment of selected solid tumors. Mantle cell lymphoma (MCL) is a subtype of B-non-Hodgkin lymphomas characterized by frequent relapses. Despite significant prolongation of survival achieved with ibrutinib, a Bruton tyrosine-kinase inhibitor, majority of patients still relapse often with biologically highly aggressive MCL. Outcome of patients who fail after ibrutinib is dismal. Our aim was to investigate the anti-angiogenic agent bevacizumab in experimental therapy of chemotherapy- and ibrutinib-refractory MCL.

METHODS

Two MCL cell lines (JEKO-1 and HBL-2) and four MCL patient-derived xenografts (PDX) (VFN-M1, VFN-M2, VFN-M11, VFN-M12) were used for *in vitro* and *in vivo* experiments. VFN-M11 and VFN-M12 were derived from patients with ibrutinib-resistant MCL. Cell clones with transgenic hVEGF expression were derived from JEKO-1 and HBL-2 cell lines using pLenti-C-Myc-DDK-P2A-Puro transfection system. Level of human VEGF (hVEGF) was analyzed by ELISA-based method. *In vitro* proliferation was assessed using WST8-based Cell Proliferation Assay. Anti-tumor efficacy of bevacizumab *in vivo* was evaluated by its ability to inhibit growth of subcutaneous (SC) PDX tumors compared to untreated controls.

RESULTS

The most profound inhibition of growth of MCL xenografts with bevacizumab was observed in PDX models derived from patients with ibrutinib-refractory MCL (*bevacizumab-treated – squares; untreated tumors – circles)*

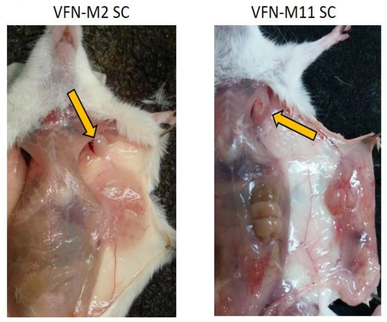


Bevacizumab does not prevent systemic spread of subcutaneous MCL tumours

A

	untreated	bevacizumab
VFN-M2	6 (66,67%)	8 (72%)
VFN-M11	1 (16,67%)	6 (100%)

B

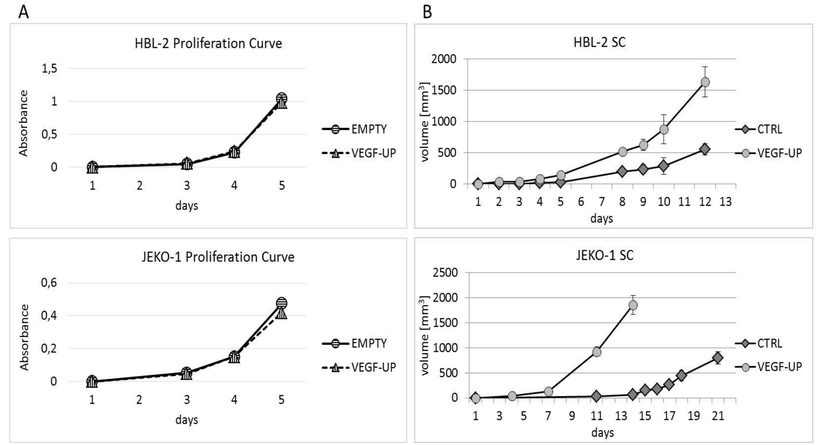


It is generally acknowledged that angiogenesis promotes tumor spread and metastasizing. Despite the observed inhibition of VEGF-dependent local growth of SC MCL tumors, lymphoma spread defined as formation of distant lymph node-like tumors or macroscopically involved murine organs (predominantly the liver and spleen) was observed in both, untreated and bevacizumab-treated.

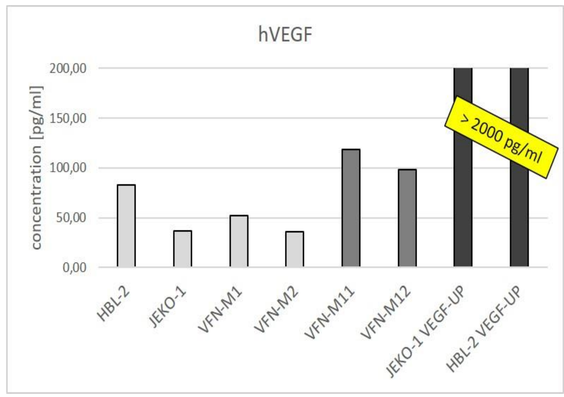
***A** – comparison of number of secondary tumours formation after SC xenotransplant of VFN-M2 and VFN-M11;*
***B** - axillar lymph node-like lymphoma masses in bevacizumab-treated VFN-M2 and VFN-M11*

VEGF-upregulation is associated with biological aggressiveness of the engrafted tumours in vivo

To further investigate the role of VEGF in the engraftment, growth and spread of MCL cells in vivo, JEKO-1 and HBL-2 subclones with stable VEGF upregulation were derived. While no proliferation advantage was observed *in vitro* (**A**), both subclones displayed markedly more aggressive behaviour *in vivo* (**B**). Subcutaneous xenotransplantation of the subclones was associated with prompt engraftment and an invasive pattern of growth compared to the original cell lines.



Levels of hVEGF detected in MCL tumor lysates positively correlate with anti-lymphoma efficacy of bevacizumab



The highest levels of hVEGF were detected in VFN-M11 and VFN-M12, the two PDX models derived from ibrutinib-refractory patients (108.23 +/- 14.35 pg/ml), while hVEGF levels in the ibrutinib-naïve tumours were significantly lower (52.01 +/- 21.99 pg/ml; p = 0.03).

CONCLUSIONS

Our data demonstrated a positive correlation between angiogenesis and aggressive phenotype of MCL. In translation, the data suggest that ibrutinib-refractory patients might profit from implementation of anti-angiogenic agents into post-ibrutinib salvage therapy.

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