Comparison of the CXCR5-Antibody Drug Conjugate (ADC; VIP924) to a CD19-ADC and a CD79b-ADC in a Humanized REC-1 Mantle Cell Lymphoma (MCL) Mouse Model

Poster # 2809

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INTRODUCTION

The chemokine receptor CXCR5 is highly expressed in mantle cell lymphoma (MCL), which is a rare subtype of non-Hodgkin lymphoma. VIP924 is a first-in-class CXCR5-targeting ADC composed of a legumain-cleavable linker and a kinesin spindle protein inhibitor (KSPi) payload. We compared VIP924 with CD19- and CD79b-targeting ADCs in a humanized mouse model of MCL. This is to our knowledge the first report of a lymphoma targeting ADC compound tested in a humanized mouse model .

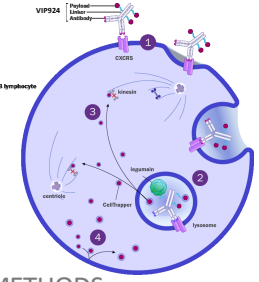


Figure 1. Schematic of VIP924 function. The antibody-drug conjugate VIP924, consisting of a CXCR5-binding monoclonal antibody coupled via a cleavable linker to a kinesin spindle protein inhibitor (KSPi), binds to its target epitope on the chemokine receptor CXCR5 (1) and subsequently is internalized. Inside the cell, the ADC VIP924 is cleaved within the lysosome by legumain (2) and the cytotoxic payload is released into the cell cytoplasm (3). When the cell is dividing, KSPi binds to its target protein and inhibits effective spindle formation. The CellTrapper® modification on the KSPi prohibits the diffusion of the KSPi out of the cell (4).

METHODS

Expression analysis on human patient samples by immunohistochemistry
Expression of CXCR5 and the B-cell targets CD19 and CD79b were analyzed on tumor samples from patients with MCL using immunohistochemistry. Slides were examined by a pathologist and scored for expression.

In vivo REC-1 xenotransplantation model in humanized NSG-SGM3 mice
NSG-SGM3 mice were transplanted with human hematopoietic stem cells from 4 different donors and after engraftment transplanted subcutaneously with REC-1 MCL cells into their flanks. When subcutaneous tumors reached a size of 100 mm³, mice were randomized into 5 groups and treated with either 10 mg/kg of an isotype control ADC, 3 mg/kg polatuzumab vedotin, 0.66 mg/kg loncastuximab tesirine, 3 mg/kg VIP924 or 10 mg/kg VIP924 every 5 days for 4 doses.

Immunophenotyping of peripheral blood, spleen, bone marrow and tumors
Immunophenotyping of peripheral blood was performed by flow cytometry before starting treatment (Day 0) and on Day 5 and Day 18 during the treatment. At the end of the experiment, immunophenotyping was also done on tumors, spleen and bone marrow.

Complete blood counts
Complete blood count was analyzed based on single cell evaluation (Drew Scientific HemaVet 950FS Auto Blood Analyzer).

RESULTS

Ex vivo analysis of patient-derived tumor samples

CXCR5, CD19 and CD79b expression was analysed by immunohistochemistry on 20 MCL tumors. Fig. 2 shows expression of CXCR5 and CD19 was medium to high in all analysed samples, while CD79b showed a slightly lower expression.

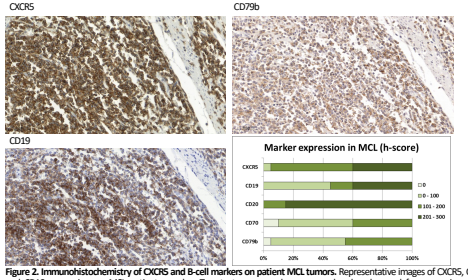


Figure 2. Immunohistochemistry of CXCR5 and B-cell markers on patient MCL tumors. Representative images of CXCR5, CD79b and CD19 expression on MCL patient samples. Twenty tumor samples were analyzed and scored for expression of the respective markers. The respective h-scores are summarized in the graph.

In vivo humanized MCL model

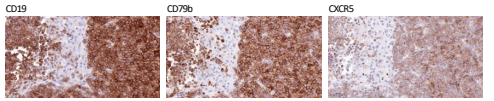


Figure 3. Immunohistochemistry on REC-1 tumors. REC-1 tumors were analysed for CD19, CD79b and CXCR5 expression by immunohistochemistry.

To evaluate the efficacy of VIP924 *in vivo*, we tested a humanized xenotransplantation model with the human REC-1 MCL tumor cell line engrafted into hematopoietic stem cell (HSC) transplanted NSG-SGM3 mice. Using this model with a humanized hematopoiesis, we aimed to also detect potential effects of the different treatments on human hematopoietic cells. After NSG-SGM3 mice were successfully transplanted with HSCs, REC-1 tumor cells were inoculated into the flanks of the mice. The animals were treated when tumors reached a size of 100mm³ with either VIP924, polatuzumab vedotin (Polivy®) or loncastuximab tesirine (Zynlonta®) (Table 1).

Group	CD34 donor	N	Treatment	mg/kg	dose volume (ml/kg)	Frequency
1	4 x 3 donor	12	Isotype control ADC	10mg/kg	5	Every 5 days/ x4
2	4 x 3 donor	12	Polatuzumab vedotin	3mg/kg	5	Every 5 days/ x4
3	4 x 3 donor	12	Loncastuximab tesirine	0.66mg/kg	5	Every 5 days/ x4
4	4 x 3 donor	12	VIP924 low dose	4mg/kg	5	Every 5 days/ x4
5	4 x 3 donor	12	VIP924 high dose	10mg/kg	5	Every 5 days/ x4

Table 1. Experimental set-up and dosing schedule. NSG-SGM3 mice were transplanted with HSCs from 3 different donors. After bone marrow engraftment, mice were injected with REC-1 MCL cells into their flanks and randomized into 5 groups with an average tumor size of 100mm³ at treatment start.

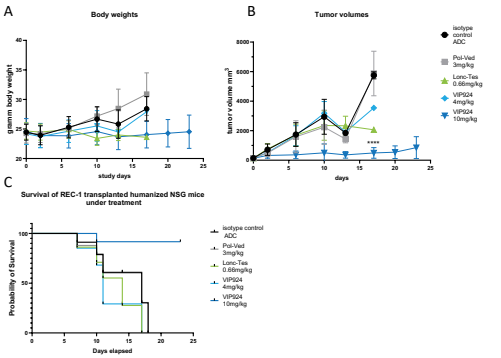


Figure 4. Body weight, tumor growth inhibition and survival curves of VIP924, polatuzumab vedotin (Pol-Ved) and loncastuximab tesirine (Lonc-Tes). (A) Body weights of individual animals was measured twice weekly. No significant body weight reductions were observed throughout the experiment. **(B)** Tumor volumes of individual mice was measured by caliper twice weekly and individual volumes were calculated. The drop in the isotype control ADC, polatuzumab vedotin and loncastuximab tesirine groups is due to removal of mice with large tumors. On day 17, tumor volumes of VIP924 treated animals were significantly lower (p<0.00002, multiple unpaired t-test) compared with control. **(C)** Survival curves for the different treatment groups. Only one mouse in the 10mg/kg VIP924 group was removed due to high tumor burden on Day 10.

Treatment with VIP924 and the two other ADCs had no major effect on body weights in the different cohorts (Fig. 3A). Significant tumor growth inhibition was only observed in the 10mg/kg VIP924-treated animals compared with isotype control ADC treated animals on Day 2 and Day 17 of treatment (p = 0.0087 on Day 2; p< 0.00002 on Day 17). Polatuzumab vedotin and loncastuximab tesirine treatments showed only a non-significant tumor growth inhibition on Day 10 of 25% compared to isotype control ADC. On study Day 10 one mouse from the VIP924 10mg/kg treatment cohort reached tumor volume endpoint (>2000mm³) and was removed. The other 11 mice from this cohort survived until the experiment was terminated at Day 23.

Effects of the treatments on peripheral blood cells

Retro-orbital blood draws were performed to evaluate the potential effects of the different treatments on peripheral blood cells (PB). Analysis of different lineages, were performed before the start of the treatment (d0), at Day 5 and Day 18 of the treatment. At Day 18 of the treatment the peripheral human CD45+ cells were significantly reduced in polatuzumab vedotin-treated animals. Animals treated with loncastuximab tesirine also showed a clear reduction of hCD45+ cells on Day 18, which did not reach significance. The hCD45+ cells in both VIP924-treated cohorts showed no difference to the isotype ADC control animals.

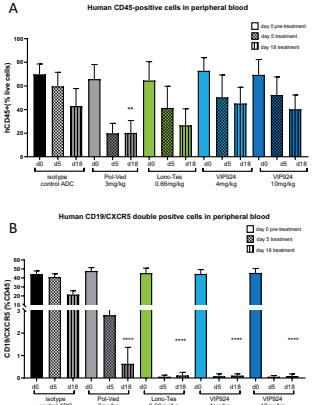
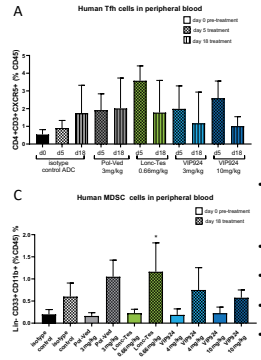


Figure 5. Peripheral blood analysis by flow cytometry. (A) Retro-orbital blood was drawn at the indicated time points of the experiment. Peripheral blood was analyzed for human CD45+ cell content in the different groups. At Day 18 of the therapy, human CD45+ cells were significantly reduced (p= 0.0053) in the Pol-Ved-treated animals. (B) Retro-orbital blood was drawn at the indicated time points of the experiment. Peripheral blood was analyzed for human CD19/CXCR5 double-positive mature B-cells. At Day 18 of the treatment all groups showed a significant reduction in mature human B-cells in peripheral blood compared with the isotype control ADC group. Statistical tests were performed with one-way ANOVA with Dunnett´s multiple comparisons test. Polatuzumab vedotin: Pol-Ved in the figure; loncastuximab tesirine: Lonc-Tes in the figure.

Effects on T-follicular helper cells, T-regulatory cells and MDSCs

Peripheral blood was also analyzed for CD4+CD3+FoxP3+ T-regulatory cells (Tregs), CD4+CD3+CXCR5+ T-follicular helper cells (Tfh) and Lin-CD33+CD11b+ myeloid-derived suppressor cells (MDSCs) (Fig. 5A-C).



- The number of peripheral CD4/CD3/CXCR5-positive Tfh cells increased over time in the isotype control ADC group, but was reduced by Lonc-Tes and VIP924 treatment (Fig. 5A).
- A reduction of Tfh was also detected in spleen and tumor of VIP924-treated animals (Fig. 6).
- VIP924 treatment significantly increased the numbers of Tregs in peripheral blood (Fig. 5B).
- Lonc-Tes treatment led to a significant increase of MDSCs compared with control (Fig. 5C).
- The increase of Tregs in VIP924-treated animals might mediate tumor growth inhibition.

Figure 6. Peripheral blood analysis by flow cytometry. (A) Peripheral blood analysis for CD4/CD3/CXCR5-positive T-follicular helper cells. After an initial increase, Lonc-Tes and VIP924 treatment decreased this cell population **(B)** Detection of T-regulatory cells in peripheral blood. VIP924 treatment significantly (p <0.0001) increased this population on Day 18. **(C)** Analysis of Lin-CD33+CD11b+ myeloid-derived suppressor cells. On Day 18, this population was significantly (p=0.0467) increased in the Lonc-Tes-treated group. Statistical tests were performed with one-way ANOVA with Dunnett´s multiple comparisons test.

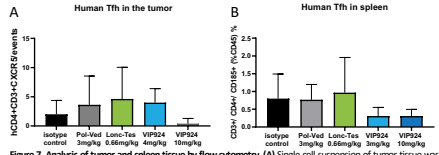


Figure 7. Analysis of tumor and spleen tissue by flow cytometry. (A) Single cell suspension of tumor tissue was analyzed for CD4/CD3/CXCR5-positive T-follicular helper cells. Compared to control, VIP924-treated tumors had almost no detectable events for Tfhs. **(B)** The same detection was performed on spleen tissue, where a reduction of Tfh cells was also evident. Polatuzumab vedotin: Pol-Ved; loncastuximab tesirine: Lonc-Tes.

Complete blood counts

Terminal blood draws were used for complete blood counts. In loncastuximab tesirine-treated animals, white blood counts, monocytes, lymphocytes, hemoglobin and red blood cell counts were reduced. VIP924 treatment showed only minor to no effects on these cell populations (Fig. 8 A-F).

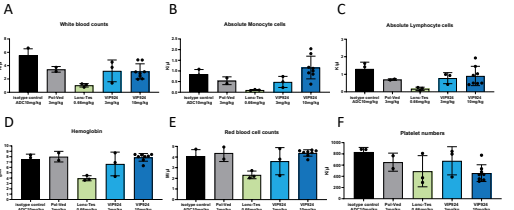


Figure 8. Complete blood counts from treated animals. (A-F) Terminal blood was analyzed on a HemaVet 950FS Auto Blood Analyzer for total white blood counts (A), absolute monocyte counts (B), absolute lymphocyte counts (C), hemoglobin values (D), red blood cell counts (E) and total platelet counts (F). Polatuzumab vedotin: Pol-Ved; loncastuximab tesirine: Lonc-Tes

CONCLUSIONS

- The novel anti-CXCR5 ADC VIP924 demonstrates significant efficacy (ie, tumor growth inhibition and increased survival) in an *in vivo* MCL xenotransplantation model in the background of a human hematopoietic system.
- The commercially available ADCs, loncastuximab tesirine and polatuzumab vedotin, used for treating B-cell malignancies showed no improvement tumor growth inhibition or survival in this setting.
- The observed reduction of T-follicular helper cells in the VIP924-treated animals might be therapeutically beneficial as these results suggest that homing of malignant B lymphocytes to lymphoid structures is reduced.
- The increase of peripheral Tregs might also help to inhibit tumor cell proliferation as Tregs recognize malignant B cells and reduce their proliferation.
- The presented data warrants further evaluation of VIP924 in clinical trials.

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