CXCR5 is a very promising drug target for the development of antibody-drug conjugates to treat patients with lymphoma

6294

Tibor Schomber[2], Beatrix Stelte-Ludwig[2], Amy J Johnson[1], Oliver v Ahsen[3], Christoph Schatz[4], Raquel Izumi[1], Hans-Georg Lerchen[2], Ahmed Hamdy[1]

[1]Vincerx Pharma Inc., Palo Alto, CA, USA; [2]Vincerx Pharma GmbH, Monheim, Germany, [3]Nuvisan ICB GmbH, Berlin, Germany; [4]Bayer AG, Berlin, Germany

INTRODUCTION

- The chemokine receptor CXCR5 is highly expressed in tumor cells from different lymphoma types, like diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma (MCL), follicular lymphoma (FL) and chronic lymphocytic leukemia (CLL).
- In healthy tissues, CXCR5 is mainly expressed on the B-cell lineage with low to no expression in other tissues.
- Together with its restricted expression in healthy tissues and the broad expression in different lymphoma indications, CXCR5 represents a viable target for the development of antibody-drug conjugates to treat lymphoma patients with unmet medical need.
- Despite fundamental progress in the treatment of B-cell malignancies, there is still a high medical unmet need, especially for patients with refractory or relapsed disease.

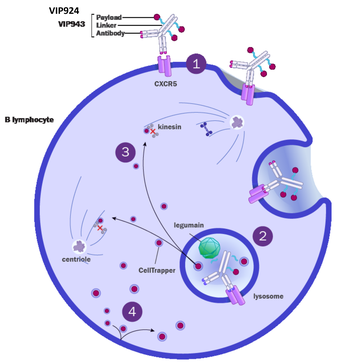

Figure 1. Schematic of VIP924 function. (A) The antibody-drug conjugate VIP924, consisting of a CXCR5-binding monoclonal antibody coupled via a cleavable linker to a kinesin spindle protein inhibitor (KSPi), binds to its target epitope on the chemokine receptor CXCR5 (1) and subsequently gets internalized.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

METHODS

- Immunohistochemistry of human tissues from different lymphoma types were stained using antibodies against human CXCR5, CD19, CD20, CD70 and CD79b. Slides were examined by a pathologist and scored for the respective protein expression.
- On tumor cell lines, CXCR5 expression was detected by flow cytometry. Cell surface receptor density was analyzed by Quantibrite™ PE-beads.
- Evaluation of antibody internalization was performed using the Operetta High Content Imaging System.
- The in vitro cytotoxicity of ADC was evaluated in a panel of cancer cell lines after a 72-h continuous exposure using MTT assay (ATCC).
- For *in vivo* testing of VIP924, female NOD/SCID mice were transplanted subcutaneously with either human HBL-1 lymphoma cells or human lymphoma patient-derived (PDX) tumor material and treated for the indicated time points with either 4mg/kg or 10mg/kg VIP924 CXCR5-ADC or the respective isotype control ADC. PDX models LY2264 and LY2214 were performed at CrownBio. Randomization in the PDX models was done when tumors reached a size of 137mm³. For the large tumor HBL-1 experiment, mice were randomized when tumors reached a size of 350mm³.
- Monoaster detection was performed by IHC and subsequent multiplex analysis.

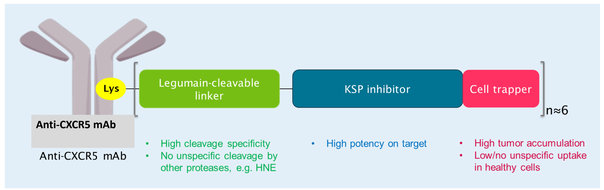

Figure 2. Structure of VIP924. The monoclonal CXCR5 antibody was conjugated to a legumain-cleavable linker, which facilitates intracellular cleavage within the lysosome and not cleaved by other proteases such as for example Human Neutrophil Elastase (HNE). The linker is bound to a highly potent kinesin spindle protein inhibitor (KSPi) which is kept inside the cell by the cell trapper.

RESULTS

CXCR5 expression and *in vitro* characterization of VIP924

- Based on the high expression of CXCR5 in different lymphomas and the nearly absent expression in normal tissue (Fig. 3), we generated the new antibody-drug conjugate VIP924. The CXCR5 antibody in VIP924 shows excellent cell internalization capacity as compared to other B-cell binding antibodies (data not shown).

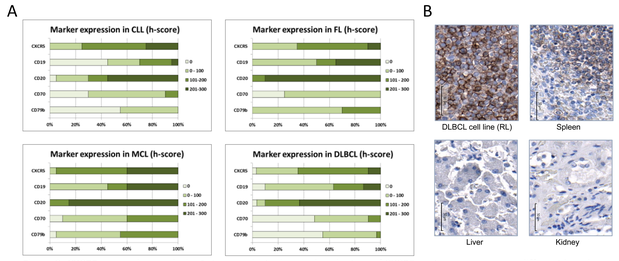

Figure 3. CXCR5 expression analysis. A. Immunohistochemical evaluation of CXCR5 protein expression in different lymphoma patient samples from CLL, FL, MCL and DLBCL patients. **B**. Protein expression of CXCR5 in healthy tissue (kidney, spleen and liver) and in a DLBCL cell line.

- VIP924 was tested on different lymphoma cell lines for its cytotoxic capacity and showed efficacies in the picomolar range.

Table 1. VIP924 vs. other B-cell Target-KSPi-ADCs. Different B-cell binding antibodies were conjugated with the VIP924 chemistry and tested in cytotoxicity assays on different lymphoma cell lines.

KSPi ADCs (same effector chemistry)	DAR	REC-1 (MCL)	Pfeiffer (DLBCL)	RI-1 (B-NHL)	BL-70 (Burkl.)	SU-DHL-6 (GC-DLBCL)
CXCR5-KSPi	3.7	2.62E-10	1.50E-11	1.56E-10	5.05E-11	3.30E-10
CD19-KSPi	3.1	5.38E-08	1.79E-09	2.16E-07	2.56E-10	<3.00E-11
CD22-KSPi	3.2	1.06E-07	4.73E-08	1.88E-08	3.05E-09	2.70E-10
CD70-KSPi	3.4	5.69E-09	8.54E-08	1.62E-07	4.60E-09	8.83E-08
CD79b-KSPi	3.4	9.75E-11	3.04E-10	9.24E-11	4.89E-11	<3.00E-11
Isptype contro-KSPi	4.1	2.82E-07	5.90E-08	1.88E-07	5.58E-08	1.39E-07

In vivo evaluation of VIP924 in patient-derived xenograft (PDX) and cell line-derived xenograft tumor models

- Different PDX samples from DLBCL of the ABC type and the GCB type were analyzed by immunohistochemistry and flow cytometry for CXCR5 expression.

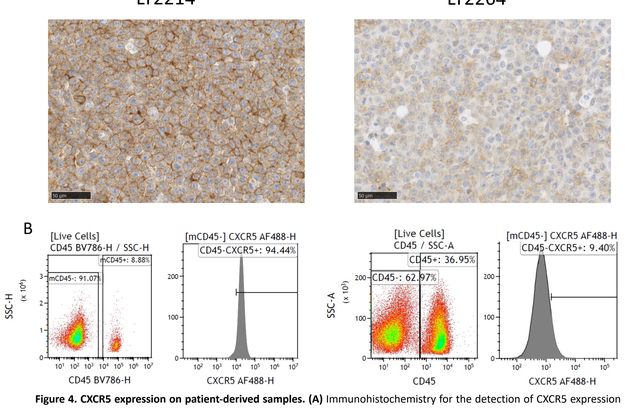

Figure 4. CXCR5 expression on patient-derived samples. (A) Immunohistochemistry for the detection of CXCR5 expression on human patient-derived samples LY2264 and LY2214. **(B)** Flow cytometry analysis on human patient-derived samples LY2214 and LY2264 showing a high number of CXCR5-positive cells in the LY2214 sample and low number in the LY2264 sample.

- Phenotypes of DLBCL patient samples LY2214 (GCB, CXCR5 high, CD20+, CD79a+, CD10-, Bcl6-, MUM1-) and LY2264 (ABC, MYD88 L265P, CXCR5 low, CK-, Vim+/-, CD79a +/-, HMB45-, Mart-1-, Pax-5-, Bcl6+, EMA-, CK7-). Tumor samples were transplanted subcutaneously into female NOD/SCID mice and treated with either 4mg/kg or 10mg/kg VIP924.
- In the CXCR5-low PDX model LY2264, mice were dosed at days 1, 11, 18 and 21 after randomization by intravenous injection of VIP924. Tumor growth inhibition was at 68% as compared to the isotype control ($p<0.0001$). Survival rate was at 100% in the VIP924 10mg/kg group at the time point when all controls had to be terminated due to tumor size at day 29 after randomization.
- No body weight loss was observed in the 10mg/kg VIP924 treated animals throughout the experiment indicating high tolerability of VIP924.

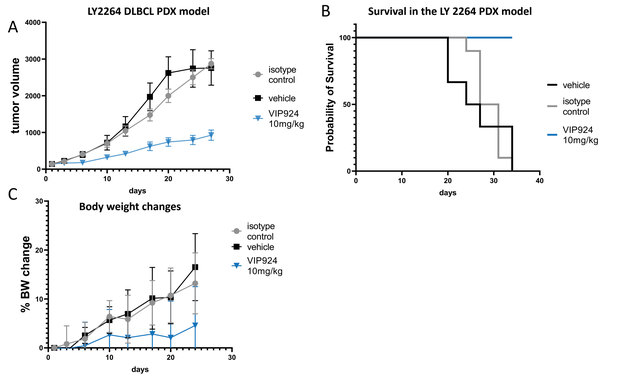

Figure 5. Tumor growth in the LY2264 PDX model. (A) Tumor growth of the transplanted LY2264 PDX specimen was analyzed by caliper twice weekly. Treatment with VIP924 and isotype control ADC started at day 1 after randomization. **(B)** Survival of the tumor-bearing mice was monitored until all mice in the control groups were terminated due to high tumor burden. All VIP924 treated mice were alive at this time point. **(C)** Body weights were analyzed twice weekly for all groups. No adverse effects of VIP924 and isotype control ADC was observed throughout the in-life phase of the experiment.

- The high CXCR5 expressing LY2214 model was treated with either 10mg/kg VIP924 on days 1 and 5 followed by 4mg/kg VIP924 on days 10, 15 and 20 after randomization or with 10mg/kg VIP924 once weekly for 3 weeks.
- Both treatment arms showed major antitumor activities. Significant TGI of 87% in the 10mg/kg VIP924 treated group and 91% in the 4mg/kg treated group, respectively was observed ($p< 0.0001$).
- VIP924 treatments were well tolerated with no body weight loss observed in both treatment groups.

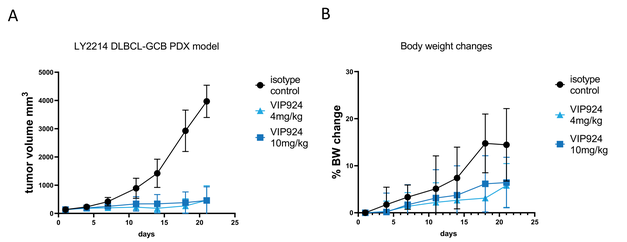

Figure 6. Tumor growth in the LY2214 PDX model. (A) Tumor growth of the transplanted LY2214 PDX specimen was analyzed by caliper twice weekly. Patient-derived DLBCL lymphoma samples were transplanted into flanks of female NOD/SCID mice. Randomization was performed when tumor size reached 137mm3. Treatment with VIP924 and isotype control ADC started at day 1 after randomization. **(B)** Body weights were analyzed twice weekly for all groups. No adverse effects of VIP924 and isotype control ADC was observed throughout the in-life phase of the experiment.

Efficacy of VIP924 in large established tumors

- To evaluate the effect of VIP924 on large tumors, the HBL-1 lymphoma cell line was transplanted into female SCID beige mice.
- Single application of 10mg/kg VIP924 resulted in a complete response in 67% of the treated mice with no measurable tumors on treatment day 26.
- Metabolite exposure was analyzed by LC/MS in satellite animals after 24 and 48 hours showing high enrichment in tumors versus plasma.

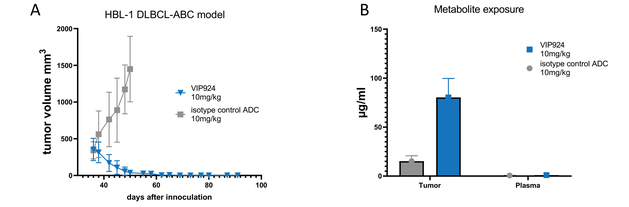

Figure 7. HBL-1 lymphoma CDX model. (A) Tumor cells were transplanted with 5x10⁶ in Matrigel into the flanks of SCID beige mice. Tumors reached sizes of 350mm³ after 26 days and mice were treated with one dose of 10mg/kg VIP924. or isotype control ADC. Tumor sizes were monitored in the VIP924 treated group until day 90 after cell inoculation. **(B)** Enrichment of the VIP924 metabolite in tumor versus plasma.

- To detect target engagement of the KSPi, VIP924 treated tumor sections were stained for monoaster formation. VIP924 showed durable monoaster formation compared to the permeable small molecule KSPi ispenisib.

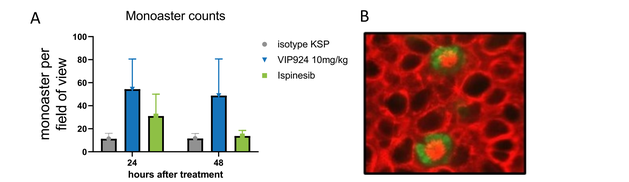

Figure 8. Monoaster detection in tumors. (A) Tumor sections were co-stained for tubulin (red) and phospho-Histone H3 to detect monaster formation. Monaster formation was quantified per field of view. **(B)** Example for a monoaster.

CONCLUSIONS

- The chemokine receptor CXCR5 is an attractive target for an antibody-drug conjugate due to the broad expression in different B-cell malignancies and limited expression in healthy tissues.
- We generated VIP924, a CXCR5 monoclonal antibody conjugated to a legumain-cleavable linker and a cell-trapper modified KSP inhibitor.
- VIP924 showed superiority in *in vitro* cytotoxicity assays on different lymphoma cell lines compared to other B-cell binding antibodies conjugated to the same chemistry.
- VIP924 was tested *in vivo* in two DLBCL patient-derived tumor models, one with low CXCR5 expression (LY2264) and one with high CXCR5 expression (LY2214). In both models significant tumor growth inhibition and prolonged survival of VIP924 treated animals was observed.
- In the HBL-1 xenograft tumor model treatment with one application of 10mg/kg VIP924 led to complete responses in two out of three mice with no measurable tumors left.
- Monoaster formation in REC-1 tumors showed durable responses to targeted KSP inhibition by VIP924 compared to ispinesib.

REFERENCES

1. Johannes S. *et al*. AACR 2019, Poster 4825

ACKNOWLEDGMENTS

We thank CrownBio for *in vivo* support performing some of the mouse tumor models.

Corresponding author: Tibor Schomber (tibor.schomber@vincerx.com)

Presented at the AACR Annual Meeting, April 14-19, 2023, Orlando, Florida