Activity of VIP943 on AML Patient-Derived Leukemic Blasts and Healthy Donor-Derived Bone Marrow Hematopoietic Stem Cells

#629

Beatrix Stelte-Ludwig[1], Tibor Schomber[1], Stephen Strickland[2], Emily Curran[3], Naval Daver[4], Omer Jamy[5], Sebastian Ludwig[1], Melanie M. Frigault[6], Joseph Birkett[1], Amy J. Johnson[6], Anne-Sophie Rebstock[1], Raquel Izumi[6], Ahmed Hamdy[6]

[1]Vincerx Pharma GmbH, Monheim, Germany; [2]SCRI at TriStar Centennial; [3]University of Cincinnati; [4]MD Anderson; [5]University of Alabama at Birmingham; [6]Vincerx Pharma Inc., Palo Alto, CA, USA

INTRODUCTION

CD123 has been shown to have higher expression on blast cells and leukemic stem cells (LSC) in patients (pts) with acute myeloid leukemia (AML) compared with normal hematopoietic stem cells (HSC) and other more mature CD34$^+$ subsets[1]. LSCs are resistant to standard of care chemotherapeutics, and their persistence after chemotherapy is associated with disease relapse. VIP943, the frontrunner ADC from the VersAptx™ platform, consists of an anti-CD123 antibody, a unique linker cleaved intracellularly by legumain, and releases a novel kinesin spindle protein inhibitor (KSPi) payload that accumulates inside the cell due to the novel CellTrapper™ technology. Herein, we evaluated the potential of VIP943 to target LSC and progenitor cells and compare it with cytarabine and gemtuzumab ozogamicin (Gem-Oz). We also present preliminary clinical results from the first-in-human study of VIP943 (NCT06034275).

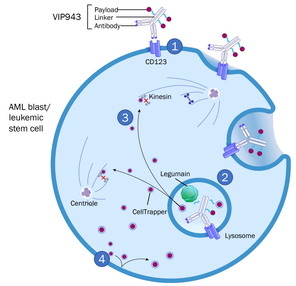

Figure 1: VIP943 mode of action.
(1) The anti-CD123 mAb of the VIP943 ADC binds to the CD123 surface protein, a validated target in myeloid malignancies and a potential LSC target. (2) VIP943 is internalized upon binding to CD123 and delivers the effector chemistry to the lysosomes of the cancer cell. Here, legumain cleaves the linker and releases the KSPi payload. (3) KSPi inhibits the kinesin spindle protein and prevents the formation of the mitotic spindle, leading to mitotic catastrophe and cell death. (4) The CellTrapper® modification of the KSPi payload prevents diffusion of the KSPi, allowing for intracellular accumulation and preventing off-target activity of the payload after cell death[2].

METHODS

Characterization of progenitor and blast cell population: Peripheral blood mononuclear cells (PBMC) and bone marrow (BM) samples from AML pts and from healthy volunteer (HVBM) were stained with a specific monoclonal antibody cocktail to identify progenitor cell populations. A fraction of the sample was stained with specific monoclonal antibodies to identify CD34$^+$CD38$^+$ hematopoietic stem and progenitor cells (CD34-FITC, CD38-PE/Dazzle594), CD123$^+$CD34$^+$CD38$^+$ LSC and blast populations (CD33-APC, CD123-PECy7: all from Biolegend)

Cytotoxicity assay: PBMC from previously untreated pts with primary or transformed myelodysplastic syndrome AML were treated with VIP943 (66 nM = 10 µg/mL) or cytarabine (5 µM) and compared with untreated control. Bone marrow samples derived from untreated AML patients were incubated in proliferation-stimulation medium (Stemline® II Hematopoietic Stem Cell Expansion Medium) and treated with VIP943 and cytarabine. After the end of an 18, 48 or 96 h incubation, CD123$^+$ blasts and the CD34$^+$CD38$^-$ HSPC were detected by flow cytometry.

Flow cytometry of healthy BM: Fresh BM samples were collected at the respective hospital centers following clinical practice. Fresh BM samples from healthy volunteers were stained with specific monoclonal antibodies to identify CD34$^+$ progenitor subpopulations (CD117-APC, CD19-APC-Cy7, CD123-PE and CD38-PE-Cy7).

Depletion assay of BM samples from healthy volunteers: The effect of VIP943 on HVBM was measured as a dose response curve (DRC) starting at 1 µM and the effect of Gem-oz as a DRC starting at 0.2 µM. At the end of the 72 h incubation, red blood cells were lysed, the remaining cells were stained with a cocktail of antibodies to discriminate between progenitor cells and then analyzed by flow cytometry.

Table 1: Overview of AML Patient Characteristics obtained from BioBank (BAYER AG, Berlin)

Sample ID	Specimen	Cell count	Sex	Age	Ethnic background	Clinical diagnosis	Disease status
15-03605	PBMC	$1.2x10^7$	F	72	White	AML transformed from MDS	untreated
15-03618	PBMC	-	-	-	White	Primary AML	untreated
15-03642	PBMC	-	-	-	White	Primary AML	untreated
P160853	BM	5.0×10^6	F	67	White	Primary AML	untreated
P160702	BM	5.0×10^6	M	63	White	Primary AML	untreated
P160774	BM	5.0×10^6	F	73	White	Primary AML	untreated
P160705	BM	1.0×10^7	M	61	White	Primary AML	untreated
P160699	BM	6.7×10^6	M	77	White	Primary AML	untreated
P160698	BM	1.2×10^7	F	67	White	Primary AML	untreated

Table 2: Overview of AML patient material and material from healthy volunteer obtained from HemaCare

Sample ID	Specimen	Sex	Age	Ethnic background	Clinical diagnosis	Disease status
D36377	PBMC	M	24	Asian	Healthy volunteer	
E261	PBMC	F	81	White	Primary AML	untreated

RESULTS

Characterization of progenitor cell populations in peripheral blood of a healthy vs AML pt; VIP943 cytotoxic to AML cells and spares healthy cells

Direct comparison of peripheral blood from a healthy volunteer and an AML patient was performed by multicolor flow cytometry to characterize the CD123$^+$, CD33$^+$, CD34$^+$CD38$^-$ and CD123$^+$CD34$^+$CD38$^-$ AML relevant progenitor cell populations. The AML patient-derived sample showed a CD34$^+$CD38$^-$ population of 25% compared to the 0.4% of CD34$^+$CD38$^-$ cells in the healthy volunteer sample. The CD123$^+$CD34$^+$CD38$^-$ LSC population is more pronounced in the AML pt compared with healthy volunteer with 11.3% to 0.04% respectively (Fig. 2A and B).

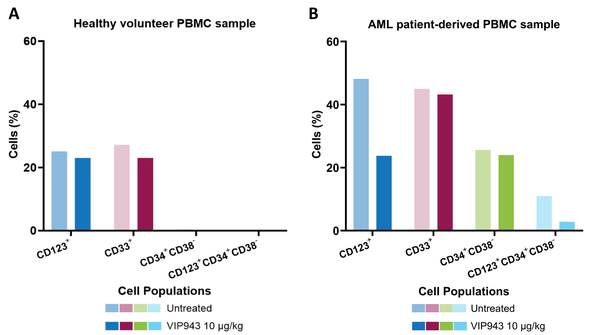

Figure 2: Quantification of VIP943 treated healthy and AML patient-derived material after 18 h. Impact of VIP943 treatment on CD123$^+$, CD33$^+$populations, CD34$^+$CD38$^-$and CD123$^+$CD34$^+$CD38$^-$cells in PBMC derived from a healthy volunteer **(A)** and an AML-patient **(B).**

VIP943 (10 µg/mL) treatment had a cytotoxic effect on the AML patient-derived sample leading to a potent reduction of CD123$^+$ blasts and the CD123$^+$CD34$^+$CD38$^-$ LSC population, while exerting only a minor effect on the CD33$^+$ cell population in both samples. This demonstrates the specificity of CD123$^+$ directed therapy for AML PBMCs.

VIP943 treatment results in a reduction of CD123+ blasts in AML patient-derived PBMC and BM samples

We compared VIP943 cytotoxicity with cytarabine on a panel of PBMC and BM samples from AML pts (Fig. 3A and 3B). Incubation of the samples for 48 h or 96 h led to a reduction of CD123$^+$ blasts with increasing effect over time. After 48 h, 2 of 3 PBMC and 3 of 3 BM samples demonstrated higher cytotoxicity with VIP943 compared with cytarabine.

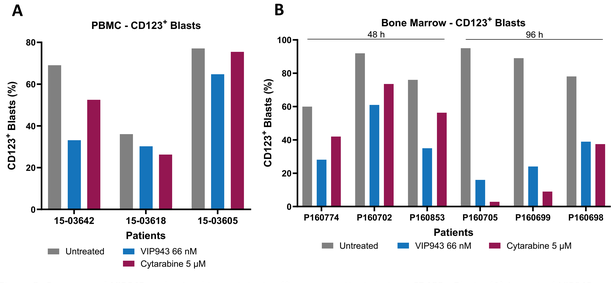

Figure 3: Summary of VIP943 and cytarabine cytotoxic efficacy on patient derived CD123+ Blasts. (A) Impact of VIP943 and cytarabine on CD123$^+$ blasts in AML PBMC after a 48h treatment **(B)** Cytotoxic activity of VIP943 and cytarabine on BM samples from AML pts was evaluated after 48 h or 96 h incubation period.

VIP943 treatment of AML patient-derived BM samples achieved a reduction of the CD34$^+$CD38$^-$ LSC population as seen in Figure 4.

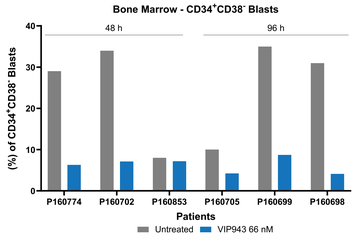

Figure 4: Summary of VIP943 cytotoxic efficacy on patient derived bone marrow CD34$^+$ Blasts. Impact of VIP943 treatment on CD34$^+$ CD38$^-$ blasts in AML patient-derived PBMC after a 48 h and 96 h treatment period

The above depicted in vitro findings (Fig. 3 and 4) demonstrate the favorable toxicity profile of VIP943, showing good efficacy on CD123$^+$ blasts and CD123$^+$CD34$^+$CD38$^-$ LSC.

VIP943 treatment, unlike Gem-Oz, spares healthy BM cells

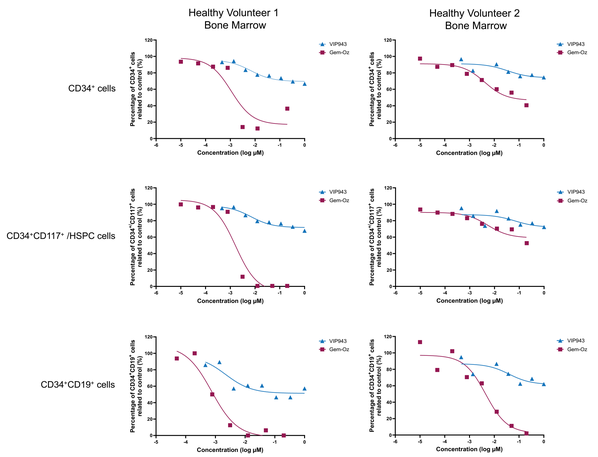

Figure 5: Representative dose-response curves showing changes in the number of CD34$^+$ cells and CD34$^+$ subpopulations in the presence of VIP943 or Gem-Oz on BM cells from Healthy Volunteer 1 and 2.

Table 3: Overview of the calculated mean IC_{50} values for respective subpopulations

Compound	IC_{50} values (µM)			
	CD34$^+$	CD34$^+$CD38$^+$	CD34$^+$CD117$^+$	CD34$^+$CD19$^+$
VIP943	8.83	3.37	8.27	2.42
Gem-Oz	0.16	0.04	0.02	0.03

This in vitro assay further verifies the low impact of VIP943 on normal hematopoietic stem cells and progenitor cells. In this assay, we demonstrated a calculated 55-fold lower impact of VIP943 on the CD34$^+$ population compared with Gem-Oz. For the CD34$^+$CD38$^+$ common myeloid progenitor subpopulation we detected a 112-fold lower IC_{50} value of Gem-Oz in comparison to VIP943. Additionally, a 413-fold lower effect of VIP943 compared to Gem-Oz on CD34$^+$CD117$^+$ HSPC subpopulation and an 80-fold lower effect on CD34$^+$CD19$^+$ lymphoid progenitor cells was observed.

VIP943 first-in-human clinical study design and preliminary results

- Study VNC-943-101 is an open-label, multicenter, Phase 1, dose-escalation study to characterize the safety, tolerability, preliminary efficacy, and pharmacokinetics of VIP943 monotherapy in pts with CD123$^+$ hematologic malignancies.
- Three pts (1 de novo AML, 1 secondary AML, and 1 B-ALL) were dosed on Cohort 1 (0.2 mg/kg) and 4 pts (1 de novo AML, 2 secondary AML and 1 MDS) were dosed in Cohort 2 (0.4 mg/kg).
- All pts (n=7) completed the 28-day dose-limiting toxicity (DLT) evaluation period. Five out of 7 received a cycle 2 dose and 2 of these started cycle 3. One pt with MDS is still on study on cycle 3.
- No DLTs occurred in Cohort 1 and 2. Two pts had drug-related adverse event (AEs). One pt had Grade 2 dry eye. One pt had Grade 1 hot flush, Grade 1 confusion, and Grade 3 diarrhea. The Grade 3 diarrhea was a serious AE, and no other drug-related serious AEs were reported. No pts discontinued VIP943 due to an AE. No pts had a reduction in VIP943 dose.
- Four pts have been enrolled in Cohort 3 (0.7 mg/kg) and are undergoing DLT assessment.

Pharmacokinetic analysis shows low levels of free payload in circulation

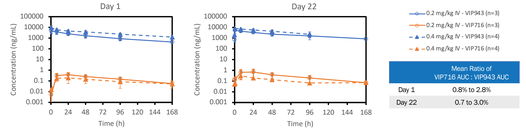

Figure 6: Pharmacokinetic data for Cohorts 1 and 2. Subjects were given VIP943 as a 0.5 h infusion QW for 4 weeks. Timepoints for detailed PK collection on days 1 and 22: pre-dose (0), 0.5, 8, 24, 48, 96, and 168 h postdose.

- Pharmacokinetic analysis shows 0.7% - 3.0% free payload in circulation after 4 weekly doses, which is indicative of our stable and selective legumain cleavable linker.
- Low free payload after multiple doses is consistent with the favorable clinical safety profile observed to date and consistent with our preclinical studies.

CONCLUSIONS

- Preclinical in vitro analysis of primary samples from AML pts demonstrates a significant cytotoxic effect of VIP943 on patient-derived CD123$^+$ blasts as well as chemoresistant LSCs.
- At anticipated pharmacologically active levels, VIP943 shows no adverse effects on HSPCs in vitro, unlike Gem-Oz, suggesting an improved safety window.
- VIP943 is currently being investigated in a FIH clinical trial in CD123$^+$ AML, higher-risk MDS, and B-ALL (NCT06034275).
- Dose-escalation Cohorts 1 and 2 have shown that VIP943 is well tolerated and no DLTs have been observed. Remarkably, in the context of this difficult to treat pt population in a Phase 1 study, all 7 pts who were dosed completed the 28-day DLT review period. Also 71% continued into Cycle 2.
- VIP943 preclinical results and preliminary clinical data support the differentiated profile of VersAptx platform ADCs. This data supports continued clinical development of this next-generation bioconjugation technology.

REFERENCES

1. Zhou J, World J Stem Cells 2014
2. Kirchhoff D. et al. Cancer 2020

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Corresponding author: Beatrix Stelte-Ludwig (beatrix.stelte-ludwig@vincerx.com)

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