VIP943 Is a Novel CD123 Antibody Drug Conjugate with in Vitro and In Vivo Efficacy in Acute Myeloid Leukemia (AML) Models

Poster # 2650

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INTRODUCTION

VIP943 is a novel αCD123 antibody drug conjugate (ADC), binding to the IL3Rα chain (CD123). VIP943 combines the new payload class of kinesin spindle protein inhibitors (KSPi) with a novel legumain-cleavable linker, which is specifically cleaved in the lysosome. KSP inhibition results in the formation of characteristic monopolar spindles (monoasters) and subsequent mitotic catastrophe. Consequently, KSP selectively acts on cells undergoing cell division. Our KSPi is a mitosis-specific drug optimized for significant retention in the tumor cell resulting in a favorable efficacy and safety profile. Current treatment options (eg, cytarabine with anthracyclines) for patients with acute myeloid leukemia (AML) are associated with severe toxicities, making these treatments unsuitable for elderly and vulnerable patients. For this patient population, the current standard of care (SOC) is a combination of venetoclax and azacitidine, yet novel therapies are still needed.

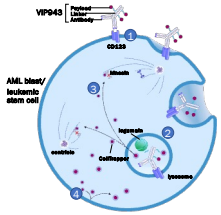


Figure 1. VIP943 mode of action.
(1) The αCD123 mAb of the VIP943 ADC binds to the CD123 surface protein, a validated target in myeloid malignancies and a potential leukemic stem cell target.
(2) VIP943 is internalized upon binding to CD123 and delivered to the lysosomes of the cancer cell. Here, legumain 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.

METHODS

Depletion assay of bone marrow (BM) samples derived from patients with AML
BM samples were treated with VIP943 monotherapy at different concentrations (0.1pM to 1μM). One concentration (16.5nM) of venetoclax was tested as single agent. Combination treatment was performed as a dose-response curve using 8 different concentrations of VIP943 combined with one fixed dose of venetoclax; cell counts were read by microscopy.

Proliferation assay of BM samples derived from patients with AML
Patient-derived BM samples were stained before incubation with a cytokine cocktail (SCF, IL3, IL6, FLT3L) for 72 h.

Flow cytometry analysis
CD123 expression on tumor cells was analyzed by flow cytometry using the anti-CD123 antibody clone, 6H6.

In vitro cytokine release assay
Peripheral blood mononuclear cells (PBMC) were freshly prepared from whole blood of 10 healthy human donors and incubated with Antibodies/ADC coated on a microtiter plate. Cytokine release was measured after 24h.

Immunophenotyping assay in non-human primates
Non-human primates were dosed with 10mg/kg or 20mg/kg VIP943 as a single dose. Peripheral blood populations were analyzed at the indicated time points: B cells (CD20+/CD3-), T cells (CD3/CD20-), Th-cells (CD3+/CD20-/CD8-), Tc-cells (CD3+/CD20-/CD8+), NK-cells (CD3-/CD16+), Basophils (FcεRIa+/CD303-/Lineage-) and pDC (303+/ FcεRIa-/Lineage-).

In vivo study of VIP943 in mono- and combination therapy in patient-derived AML mouse model
VIP943, azacitidine and venetoclax were evaluated in an AML patient-derived mouse model. Tumor was transplanted in NOG mice. VIP943 and isotype-ADC were dosed at 5mg/kg q7D, azacitidine was dosed at 2.5mg/kg qDx5, and venetoclax was dosed at 50mg/kg, qDx5 over 3 weeks. Same dosing was used in the combination arms.

Safety evaluation of VIP943 in comparison with gemtuzumab ozogamicin (Gem-Oz) and gemtuzumab-KSPi ADC
Non-human primates (NHP; 1M/1F) were treated with a non-toxic single dose of Gem-Oz (3mg/kg), gemtuzumab-KSPi-ADC (3mg/kg) or VIP943 (20mg/kg). Collection of whole blood samples was performed on day 1, day 3, day 7 and day 14. Hematology and serum chemistry evaluation were performed. were

RESULTS

Ex vivo Analysis of Patient-Derived Bone Marrow Samples

We used bone marrow (BM) samples from AML patients ex vivo to test our compound VIP943 in a depletion (without cytokine addition) and proliferation assay. Patient characteristics are summarized in Table 1. All analyzed patient samples were positive for CD123 expression (Fig. 2A). In the depletion assay, only the samples which showed spontaneous proliferation were sensitive to VIP943 treatment in accordance with the mode of action of the KSPi payload (Fig 2B). This spontaneous proliferation is generally associated with poor prognosis. Combination with venetoclax did not improve the depletion efficacy of the patient-derived cells.

Table 1. Summary of AML patient BM characteristics and ex vivo efficacy.

Sample ID	Age	Sex (M/F)	Previously Treated (Y/N)	Gene Mutations	EC$_{50}$ Proliferation Assay (μM)
13040	67	F	N	TET2	NA
13044	61	F	Y	IDH2, NPM1, DNMT3A	NA
16341	70	F	N	unknown	0.49
15988	77	F	N	JAK2	0.102
15358	55	M	N	TP53, DNMT3A	0.004
16363	56	M	N	ASXL1, SRSF2	0.005
15946	65	M	Y	TET2	0.01
16161	55	M	N	RUNX1/RUNX1T1	NA
16420	38	F	N	unknown	0.01
16421	57	F	N	no mutations	0.0006
16452	32	M	N	no mutations	0.01
16453	48	F	N	CEBPA	0.01
16454	74	M	N	FLT3, NRAS, WT1	0.01
16463	80	M	N	FLT3, DNMT3, TET2, NRAS, NPM1	0.01
16464	52	M	N	no mutations	0.06
16470	68	M	N	TET2, ASXL1	0.05

All samples were sensitive to VIP943 treatment, but only sample 15358 showed sensitivity to venetoclax. No improved efficacy was observed by co-treatment of VIP943 with venetoclax.

Efficacious EC$_{50}$ values in low as well as high CD123 expressing patient samples. This confirms the benefit of our KSPi payload designed to allow accumulation in the tumor cell resulting in significant cell death also at low target expression (Fig. 2A, B). NA: Not analyzed.

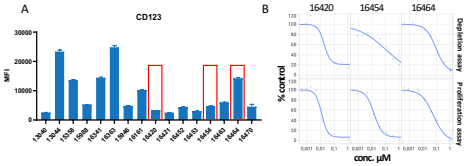
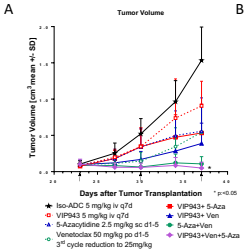


Figure 2. Ex vivo evaluation of AML patient BM (A) Cell surface expression of CD123 was analyzed by flow cytometry. All patient samples in the ex vivo assay express CD123. **(B)** Three examples of EC$_{50}$ curves in the depletion and proliferation assay. Sample 16453 showed low, sample 16454 medium, and sample 16464 high CD123 expression on the cell surface.

Efficacy of VIP943 in a Patient-Derived AML Mouse Model delivers Complete Response in Combination

In this low-moderate CD123 expressing PDX mouse model, all monotherapies demonstrated inferior efficacy compared with combination (Fig. 3A). While combination of VIP943 and venetoclax (Ven) improved efficacy and survival, the combination of VIP943 with azacitidine (Aza) only improved survival. Ven+Aza combination revealed significant tumor regression resulting in two complete responses (CR, 22%) at the end of treatment. The triple combination of Ven+Aza+VIP943 enhanced the impact on tumor regression achieving 5 CRs (56%) and significantly prolonged survival time (Fig. 3A and B) without increased toxicity.



B

	Treatment Response (CR)	Survival (days)
Iso-ADC	-	37
VIP943+Ven	-	57
VIP943+Aza	-	76
Ven+Aza	2 (22%)	83
VIP943+Ven+Aza	5 (56%)	>107

Figure 3. PDX AML model 6252. (A) Tumor growth inhibition of monotherapies and combinations on tumor growth after 3 doses **(B)** Depth of treatment response and survival analysis of combination therapies

Cytokine Release and Immunophenotyping Profile of VIP943

In a human PBMC cytokine release assay, VIP943 did not induce the release of cytokines, compared to positive controls (Fig. 3A). In an immunophenotyping study in non-human primates, one dose of VIP943 led to a reversible reduction in CD123$^+$ basophils (Fig. 3B). Other blood cell populations showed no change.

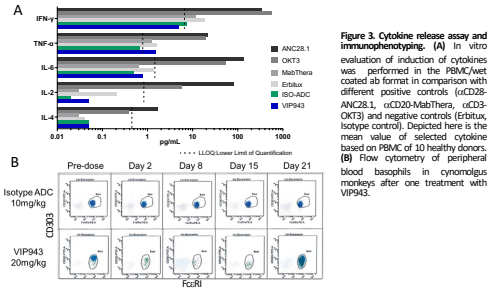


Figure 3. Cytokine release assay and immunophenotyping. (A) In vitro evaluation of induction of cytokines was performed in the PBMC/wet coated ab format in comparison with different positive controls (αCD28-ANC28.1, αCD20-MabThera, αCD3-OKT3) and negative controls (Erbitux, Isotype control). Depicted here is the mean value of selected cytokine based on PBMC of 10 healthy donors. **(B)** Flow cytometry of peripheral blood basophils in cynomolgus monkeys after one treatment with VIP943.

Safety of VIP943 in Non-Human Primates (NHP) Compared to Gem-Oz

In a NHP toxicology study, VIP943 was compared with Gem-Oz. Additionally, we included a newly generated ADC using a gemtuzumab biosimilar as the targeting antibody conjugated to our linker and payload (Gem-KSPi ADC). This allowed the direct comparison of the effector chemistry of Gem-Oz to our linker and payload since the target (αCD33) was the same. The effect on hematology and serum chemistry after a single application of nontoxic doses was evaluated.

- CD33+/CD123+ basophils showed an expected decrease but treatment with VIP943 and Gem-KSPi-ADC demonstrates a full recovery over the observation period, whereas Gem-Oz showed increasing severity.
- Significant impact of Gem-Oz on platelets, WBC count, reticulocytes and lymphocytes was seen with increasing severity over time. In contrast, the Gem-KSPi-ADC shows no effect on WBC count, lymphocytes or platelet counts.

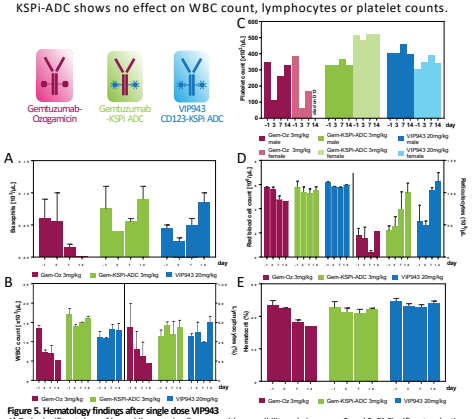


Figure 5. Hematology findings after single dose VIP943
A) Early significant drop of basophil counts in all groups with reversibility only in groups 2 and 3. **B)** Significant reduction in WBC count and lymphocytes with Gem-Oz. **C)** Critical drop of platelet counts in both animals of the Gem-Oz group only. **D)** Reduction of RBC count and of reticulocytes detected only in the Gem-Oz group. **E)** Corresponding decrease in hematocrit in the Gem-Oz group.

Gem-Oz Toxicity Ameliorated by Substituting Proprietary Linker - Payload

VIP943 and Gem-Oz showed mild increase in liver enzymes (ALT and AST), which were normalized with VIP943 to pre-dose levels on day 14, whereas in the Gem-Oz group the elevated levels were persistent. At the end of the observation period total bilirubin levels are doubled in the Gem-Oz with no changes observed in the other treatment groups. A significant increase in urea nitrogen was detected only in the Gem-Oz group. One Gem-Oz-treated animal died on day 13 (female) and the male animal showed signs of liver and kidney toxicity and a critical decrease in RBC count. This animal was euthanized due to morbidity.

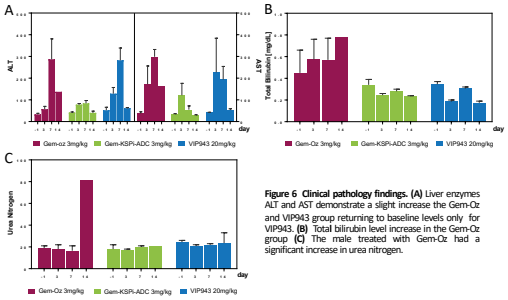


Figure 6 Clinical pathology findings. (A) Liver enzymes ALT and AST demonstrate a slight increase the Gem-Oz and VIP943 group returning to baseline levels only for VIP943. **(B)** Total bilirubin level increase in the Gem-Oz group **(C)** The male treated with Gem-Oz had a significant increase in urea nitrogen.

CONCLUSIONS

- The novel αCD123 ADC VIP943 demonstrates efficacy in an ex vivo AML patient-derived BM proliferation assay with various levels of CD123 cell surface expression.
- VIP943 is not expected to cause cytokine release syndrome based on results from an in vitro cytokine release assay.
- In a patient-derived AML xenograft model, the triple combination of VIP943 with the SOC Aza+Ven increased the number of complete responses and the overall survival.
- In a NHP toxicology study, VIP943 (20mg/kg) showed no signs of toxicity as measured by hematology, serum chemistry and survival. No adverse effects were observed other than a reversible decrease in basophils. In contrast, NHP treated with Gem-Oz had significant toxicity including death and mandatory euthanasia. The toxicity of Gem-Oz was ameliorated by substituting our proprietary linker and payload on an αCD33 antibody as measured by hematology, serum chemistry and survival.
- These results provide compelling evidence that VIP943 represents a substantial advancement in ADC technology and warrants evaluation in clinical trials.

REFERENCES

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