Selectivity and Safety of VIP943: A Novel CD123 Targeting Antibody-Drug Conjugate (ADC) Using a Proprietary Linker and Payload Class

Poster # 1435

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INTRODUCTION

ADCs as therapeutic class have been highly efficacious but suffer from shortcomings in their safety profile. Most payloads are cytotoxic agents that can inhibit general processes of cellular homeostasis including microtubule polymerization or DNA damage repair. While these are good targets for cytotoxicity, they have the drawback of being non-selective for dividing cancer cells. Here, we report the potent on-target activity and high selectivity of our new CD123-targeting ADC VIP943, which utilizes a kinesin spindle protein inhibitor (VIP716), modified with a CellTrapper® moiety to avoid membrane permeability[1]. The kinesin super family is comprised of motor-proteins involved in various cellular mechanisms. The target of our payload is the kinesin spindle protein (KSP) also known as KIF11 or Eg5. KSP is a motor protein essential for the formation of the bipolar mitotic spindle during cell division in the G2/M phase. Targeting this motor protein enables us to specifically inhibit dividing cells, while at the same time avoiding off-target toxicities in non-dividing differentiated cells.

METHODS

Eg5 on-target and KIF family selectivity biochemical assay: The motor domain of the human Eg5 (KSP, 10 nM) and of other KIF family members were evaluated in a biochemical assay using stabilized microtubules (50 µg/mL). The freshly prepared mixture was incubated with VIP716 (payload of VIP943) starting at 10µM (1:10 dilution); S-Trityl-L-Cysteine (STLC), a reversible first generation KSPi; and ispinesib, a clinical stage KSPi (starting concentration 10 µM) in the presence of 500 µM ATP. ATPase activity was directly analyzed by measuring formed inorganic phosphate by determination of complex formation with Malachite green after 50 min at l=620 nm.

Cell cycle analysis
The effect of VIP943 on the 3 phases of the cell cycle (G0/G1- S- G2/M) was measured with DNA staining (200µL propidium iodide (Merck/Sigma, #P-4170,100 µg/ml in PBS, 4°C) and flow cytometry in MOLM-13 and MV-4-11 cell lines (2x10^5 cells/well). Concentrations of VIP943 of 5 to 500 nM were used for a 48 h treatment of cells. Then DNA staining of the treated and untreated cells was evaluated by flow cytometry (MacsQuant, Millipore, Germany). In addition, cell cycle analysis was measured by flow cytometry after a 4 h incubation with VIP943 (20 nM) in MOLM-13 cells. The corresponding isotype ADC was used as a control. After a final incubation time of 48 h, 72 h and 96 h, the analysis was performed. Flow cytometry was designed to ensure only single cell evaluation.

In vitro cytotoxicity
A cell line panel containing 56 hematologic cell lines was treated in vitro with VIP331 (cell permeable analog of VIP716) starting from a top concentration of 12 µM with 3.16-fold serial dilutions to achieve 9 dose levels where the lowest VIP331 concentration was 120 pM. In this study, the 50% inhibitory concentration (IC_{50}) was determined in cancer cell lines using CellTiter-Glo luminescent cell viability assay. Each cell line was treated with test article VIP331 and culture medium containing 0.25% [v/v] DMSO as vehicle control. Cell viability was determined after a 72-hour incubation. Clinically impactful biomarkers for agents used for the treatment of hematologic malignancies were extracted and mapped from COLE.[2, 3, 4]

Receptor occupancy
MOLM-13 cells or human whole blood were incubated with different concentrations of the cold unlabeled 7G3 antibody in duplicate. To reduce unspecific binding, an Fc block was carried out in advance. After the first staining, PE-Alexa antibody mixture is added to each batch, incubated, washed and measured with FACS Canto II (BD).

Nonhuman primate safety and toxicokinetics:
VIP943 was intravenously (IV) administered to Cynomolgus monkeys at 1, 3, or 9 mg/kg once weekly for 4 weeks in a Good Laboratory Practice (GLP) toxicology study. Plasma samples were collected at predose, 0.5, 1, 2, 4, 8, 24, 72, and 168 h post-dose (n=5/group/sex) on study Day 1 and Day 22. Plasma concentrations of total antibody from VIP943 and VIP716 were determined by LC/MS/MS. Toxicokinetics for individual animals was determined using non-compartmental analysis.

RESULTS

- On-target activity of VIP716, the non-permeable metabolite of VIP943, was demonstrated in comparison to the permeable KSP inhibitor VIP331 on recombinant motor protein Eg5. The described modification of VIP716 did not influence the inhibitory potency as compared to parental compound VIP331.

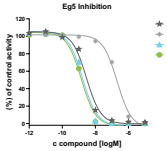


Figure 1. Inhibition of Motor Protein KSP (Eg5):
Dose response curves of payload VIP716 (non-permeable) and VIP331 (permeable) vs ispinesib and STLC.

Table 1. Inhibitory Potency of VIP716 on Eg5

Compound	Inhibition IC_{50} (nM)
Ispinesib	2.0
VIP331	1.7
VIP716	1.4
STLC	206

Selectivity of VIP943 payload on different KIF family members

- The impact of VIP716 (VIP943 payload with CellTrapper) was evaluated on different KIF family members compared to ispinesib and the permeable payload VIP331 at concentrations up to 10 µM. VIP716, VIP331, and ispinesib are highly selective for KSP (Eg5) and showed no activity against other KIF family members (Table 2).

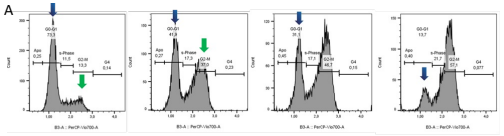
Table 2. Selectivity of VIP716 to 11 KIF family members .

KIF Family Member	Biological Function	VIP716 (IC_{50})	VIP331 (IC_{50})	Ispinesib (IC_{50})
Eg5/KSP	bipolar spindle assembly and alignment of chromosome	1.4	1.7	2.0
KIF 1B	transport of mitochondria and synaptic vesicle towards plus-end	no inhibition (>10 µM)	no inhibition (>10 µM)	no inhibition (>10 µM)
KIF2	transports membranous organelles	no inhibition (>10 µM)	no inhibition (>10 µM)	no inhibition (>10 µM)
KIF3C	axon growth, regeneration by regulating microtubule cytoskeleton in the growth cone	no inhibition (>10 µM)	no inhibition (>10 µM)	low inhibition at 10 µM
KIF 13b	reorganization of the cortical cytoskeleton. regulates axon formation	no inhibition (>10 µM)	no inhibition (>10 µM)	no inhibition (>10 µM)
KIF 26a	repressing cell growth in the enteric nervous system	no inhibition (>10 µM)	no inhibition (>10 µM)	no inhibition (>10 µM)
CENP-E / KIF10	metaphase chromosome alignment	no inhibition (>10 µM)	no inhibition (>10 µM)	no inhibition (>10 µM)
MKLP	spindle pole separation	n.d.	no inhibition (>10 µM)	no inhibition (>10 µM)
KIFC1	cytoskeletal signaling and Golgi-to-ER retrograde transport towards minus-end	no inhibition (>10 µM)	no inhibition (>10 µM)	no inhibition (>10 µM)
KIFC3	translocate cargos along microtubules	no inhibition (>10 µM)	no inhibition (>10 µM)	no inhibition (>10 µM)
MCAK/ KIF2c	destabilize microtubules, catastrophe frequency increase	no inhibition (>10 µM)	no inhibition (>10 µM)	no inhibition (>10 µM)
KHC / KIF5	vesicle transport	no inhibition (>10 µM)	no inhibition (>10 µM)	no inhibition (>10 µM)

n.d.: Not Determined

Cell cycle analysis of AML cell lines MV-4-11 and MOLM-13 after VIP943 treatment shifts cells into the G2/M phase

- The cell cycle consists of 3 phases: the G0/G1 phase, the S phase and the G2/M phase. In healthy cells, the distribution of cells between the respective phases is stable.
- Treatment of MV-4-11 cells with VIP943 causes a significant and dose-dependent increase of cells in the G2/M phase which is consistent with inhibition of KSP by VIP716 the payload of VIP943 (A).
- VIP943 treatment demonstrates a time-dependent shift of MV-4-11 cells from the G0/G1 to the G2/M phase. The number of cells in S-phase remains stable (B).
- In a 4-hour pulse experiment with VIP943, the number of MOLM-13 cells entering apoptosis increased over an incubation period up to 96h. Treatment of VIP943 but not isotype control (VIP156) causes the increase of apoptotic cells (C).



B

Cell population	MV-4-11 untreated		
(%)	6h	24h	48h
G0-G1	68.1	73.3	73.3
S	11.6	11.6	13.1
G2/M	**18.5**	**13.4**	**11.8**

Cell population	MV-4-11 treated		
(%)	6h	24h	48h
G0-G1	66.2	45.5	13.7
S	**11.9**	**16.9**	**21.7**
G2/M	**20.4**	**35.3**	**57.2**

C

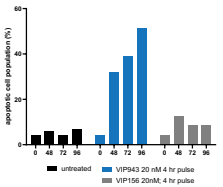


Figure 2. Impact of VIP943 on Cell Cycle: (A) Histograms of MV-4-11 cells treated with VIP943 demonstrating dose-dependent G2/M arrest of cells. Overview of changes in subpopulations after treatment with VIP943 for 48 h. **(B)** Time-dependent distribution of MV-4-11 cells in different cell cycle phases of untreated versus VIP943 (500 nM) treated samples. **(C)** Time-dependent increase of apoptotic MOLM-13 cells after a 4-hr 20 nM VIP943 pulse treatment followed by an incubation up to 96 h in comparison to the corresponding isotype control ADC (VIP156).

Sensitivity of VIP943 cell permeable payload VIP331 is not impacted by genomic alterations in a hematologic cell line panel

- The impact of genomic biomarkers on in vitro sensitivity of VIP331 in a panel of 56 hematologic cell lines was evaluated. Cell lines are ranked from the lowest to highest IC50 with a mean IC50 of 2.495 nM (range 0.51-30 nM) indicating that most hematologic cell lines tested are sensitive to VIP331.
- The presence of DNA alterations: AMP (red), HOMDEL (blue), SNV (white/alteration noted) or RNA expression: HIGH (pink), LOW (light blue) in 16 genes associated with hematologic targets including CD123 (IL3RA) do not impact VIP331 cytotoxicity as shown in the heatmap.

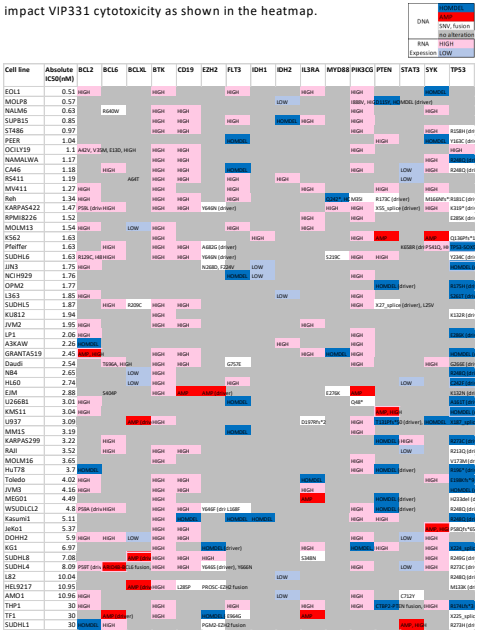


Figure 4. Heat Map of Genomic Biomarkers with In Vitro Cytotoxicity of VIP331. AMP: amplification; HOMDEL: deletion; SNV: single nucleotide variant; HIGH: high mRNA expression; LOW: low mRNA expression

Receptor occupancy competition for binding to CD123 in MOLM-13 AML cell line and in whole blood from healthy human volunteers

- PE-labeled 7G3 Ab clone saturates CD123 in MOLM-13 cells and in whole blood in a competition assay.

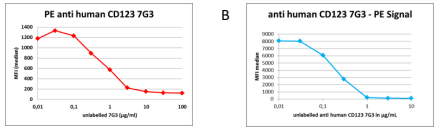


Figure 5. CD123 Receptor Occupancy Assay. (A) MOLM-13 cells **(B)** whole blood, gated on CD123 positive cells

Nonhuman primate safety and toxicokinetics profile of VIP943 and payload

- VIP943 up to 9 mg/kg repeated doses (5 males/5 females per group; QWx4) administered IV were well-tolerated (no mortalities) without adverse events typically observed with ADCs containing other payload classes such as thrombocytopenia, neutropenia, or anemia.
- Mild signs of fully reversible liver enzyme increases were observed (no morphologic changes).
- At the highest dose level, ocular findings (corneal pigmentation, single cell necrosis and hypertrophy of the basal cell layer epithelium) were observed at the terminal and recovery ophthalmologic examinations and microscopically at the terminal and recovery necropsy intervals. No cardiovascular effects were observed with VIP943 treatment.
- VIP943 and VIP716 exposures increased with increasing dose in a dose-proportional or greater than dose-proportional manner. No consistent differences in toxicokinetics were observed with sex (combined male and female data is presented). Based on area under the concentration curve (AUC), no accumulation of VIP943 was observed. As well, little to no accumulation was observed for VIP716. Payload to parent ratios (≤3%) were consistent with a low level of non-specific release of VIP716 from VIP943.

Table 3. Plasma toxicokinetic parameters (mean ± SD) of total antibody from VIP943* on Days 1 and 22 after weekly IV bolus administration of 1, 3, and 9 mg/kg VIP943 to Cynomolgus monkeys (n=5/sex/dose group)

VIP943 Dose (mg/kg)	Day	T_{max}** (hr)	C_0 (µg/mL)	$AUC_{0\text{-}168}$ (µg·hr/mL)
1	1	0.500(0.500 - 0.500)	28.9 ±3.29	1270 ±230
	22	0.500(0.500 - 0.500)	17.6 ±10.5	213 ±442
3	1	1.00(0.500 - 4.00)	110 ±37.3	5040 ±1200
	22	0.500(0.500 - 2.00)	73.5 ±26.7	2280 ±2470
9	1	0.500(0.500 - 2.00)	235 ±46.7	12800 ±1290
	22	0.500(0.500 - 2.00)	223 ±99.9	12300 ±11700

Table 4. Plasma toxicokinetic parameters (mean ± SD) of VIP716 on Days 1 and 22 after weekly IV bolus administration of 1, 3, and 9 mg/kg VIP943 to Cynomolgus monkeys (n=5/sex/dose group)

VIP943 Dose (mg/kg)	Day	VIP716 T_{max}** (hr)	VIP716 C_{max} (ng/mL)	VIP716 $AUC_{0\text{-}168}$ (ng·hr/mL)
1	1	2.00(2.00 - 2.00)	183 ±45.9	7570 ±2710
	22	2.00(1.27 - 4.00)	649 ±344	7120 ±3500
3	1	2.00(2.00 - 4.00)	420 ±133	19000 ±3710
	22	4.00(2.00 - 8.00)	943 ±712	23900 ±11200
9	1	8.00(2.00 - 8.00)	926 ±266	53900 ±8990
	22	4.00(1.61 - 8.00)	2670 ±3560	88600 ±70700

$AUC_{0\text{-}168}$ = Area under the concentration-time profile from time 0 to 168 hours post-dose; C_0 = Concentration at time 0; C_{max} = Maximum observed plasma concentration; T_{max} = Time of maximum plasma concentration.
*Plasma concentrations of total antibody from VIP943 serve as surrogate for VIP943 concentrations as VIP943 appears to be very stable. ** Median (minimum-maximum)

CONCLUSIONS

- On-target selectivity of VIP943 for KSP (Eg5) versus several other KIF family members was confirmed.
- The on-target activity translates to G2/M arrest in a dose- and time-dependent manner, resulting in apoptosis in AML cell lines.
- VIP943 permeable payload VIP331 sensitivity in a 56 hematologic cell line panel is independent of common AML genomic alterations such as TP53.
- Receptor occupancy can be measured using 7G3 Ab clone against CD123 in cell lines and in human whole blood.
- Based on high selectivity of the targeting Ab and low payload (VIP716) release in the plasma, repeat dosing of VIP943 was well tolerated in Cynomolgus monkeys.
- VIP943 is being evaluated in a Phase 1 dose-escalation study in patients with CD123+ hematologic malignancies (NCT06034275). VIP943 completed the first dose cohort (n=3) and is now enrolling in the second dose cohort; consistent with preclinical findings, no safety signals have been observed.

REFERENCES

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