Addressing Drug Metabolism and Pharmacokinetics (DMPK) Challenges of Small Molecule-Drug Conjugates (SMDCs)

#3197

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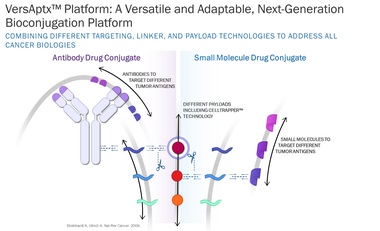
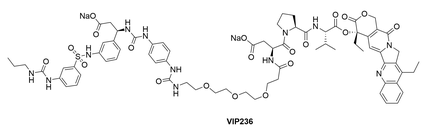
INTRODUCTION

We have developed a novel technology platform VersAptx™ with tailored solutions for ADCs and SMDCs with intracellular and extracellular cleavage options. The specificity of our platform lies on increasing safety by using tumor specific enzymes, ie neutrophil elastase (NE) and legumain, for payload release.

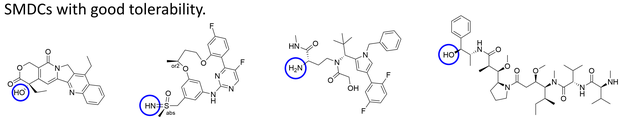


Key features of the SMDC technology comprise of a small molecule ligand such as an αvβ3 integrin binder showing efficient tumor homing and a linker extracellularly cleaved by NE in the tumor microenvironment allowing for seamless release of different payload classes.[1] Our lead SMDC VIP236[2,3] is currently in a Phase 1 clinical trial for the treatment of advanced solid tumors (NCT05712889). Here we provide the study design as well as preliminary safety and efficacy results.



VIP236

In our recent paper[4], we demonstrated the successful extension of our SMDC platform to new payload classes beyond 7-ethylcamptothecin (VIP126). We showed elastase-mediated release of KSPi (kinesin spindle protein inhibitor), PTEFbi (CDK9 inhibitor) and MMAE from SMDCs. Compelling in vivo activity was demonstrated with KSPi and MMAE SMDCs with good tolerability.



Compound 1 VIP126 | **Compound 2 PTEFbi** | **Compound 3 KSPi** | **Compound 4 MMAE**

SMDCs are gaining momentum with potential advantages over antibody-drug conjugates (ADCs), eg, lack of immunogenicity and easier manufacturing. However, SMDCs often have a shorter half-life compared with ADCs, which may result in more frequent dosing schedules.

Here we show significant reduction of clearance and prolongation of half-life of SMDCs achieved by adding a second binding moiety and the impact on in vivo potency.

METHODS

- The in vitro cytotoxicity of the SMDCs in comparison to the respective payloads was tested in a panel of cancer cell lines after a 72h continuous exposure in presence or absence of 20 nM NE using MTT assays (ATCC). IC50 values were determined as the concentration of compound required for 50% inhibition of cell viability.
- Pharmacokinetic properties of SMDCs and their respective payloads were characterized in rats. Briefly, male Wistar rats (n=3) were given a 0.5 mg/kg dose of SMDC formulated in plasma/DMSO (99:1) intravenously (i.v.) via tail vein. Plasma was collected at different time point and stored frozen at < -20ºC until sample analysis. Concentrations of the SMDCs and their respective payloads were measured by LC-MS/MS. Pharmacokinetics of SMDCs and their respective payloads were determined using mean concentration-time profiles. All pharmacokinetic parameters were calculated by non-compartmental methods as previously described[5].
- Pharmacokinetic properties of SMDCs and their respective payloads were characterized in female NMRI nu/nu xenograft mice (Taconic M&B A/S) bearing NCI H69 human small cell lung cancer tumors. The mice were treated i.v. via the tail vein with a single dose. Plasma and tumor samples were collected at different timepoints and stored frozen at < -20ºC until sample analysis. Concentrations of the SMDCs and their respective payloads were measured by LC-MS/MS.
- For in vivo efficacy studies, immunocompromised mice were inoculated subcutaneously with 1.3x10[6] NCI H69 cells in 50% Matrigel/50% media on day 0. Treatment was started at a mean tumor area of 36-37 mm² in the NCI H69 model (n=8/group, compound **10** at 5 mg/kg i.v., QW, and compounds **8** and **9** at 40 mg/kg i.v., QW).

RESULTS

VIP236 first-in-human clinical study design and preliminary results

Study VNC-236-101 is an open-label, multicenter, Phase 1, dose-escalation study to characterize the safety, tolerability, preliminary efficacy, and pharmacokinetics of VIP236 monotherapy in patients (pts) with relapsed/refractory metastatic cancer.

Dose escalation started with a 2 days on/5 days off (2 on/5 off) schedule with a 21-day dose-limiting toxicity (DLT) observation period. Five pts were treated on the 2 on/5 off schedule (0.1 [Cohort 1; n=1], 0.2 [Cohort 2a; n=2], 0.4 [Cohort 3a; n=2] mg/kg); four were men. The median (range) age was 74 (57-80) years, and 80% of these pts had ≥3 lines of prior therapy. Two pts experienced a DLT in Cohort 3a. The DLTs were a delay in dosing >14 days due to Grade (G) 3 white blood count decrease (n=1) and G4 lymphocyte count decrease (n=1). The schedule was changed to once every 3 weeks (Q3W) dosing.

Fifteen pts were treated on the Q3W schedule (0.2 [Cohort 2b; n=2), 0.4 [Cohort 3b; n=5], 0.6 [Cohort 4b; n=5], 0.8 [Cohort 5b; n=3] mg/kg); eight were men. The median age (range) was 58 (35-80) years, and two-thirds of these pts had ≥3 lines of prior therapy.

Table 1 shows a reduction in the overall frequency of G3-G4 treatment-related adverse events (AEs) with the Q3W schedule. No DLTs have occurred on the Q3W schedule. No severe or life-threatening diarrhea has occurred in any pts treated with VIP236. One pt on the 2 on/ 5 off and no pts on the Q3W schedule have discontinued VIP236 due to an AE. Two pts had dose reductions due to neutropenia (1 in Cohort 3a; 1 in Cohort 3b). Enrollment for Cohort 6b (1.0 mg/kg) is open.

Table 1. Treatment-related adverse events occurring in ≥ 15% of pts

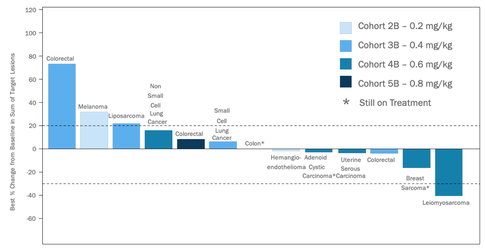
	2 days on/5 days off (N=5)				Q3W (N=15)			
Preferred Term	G1	G2	G3	G4	G1	G2	G3	G4
Alopecia	0	1 (20%)	0	0	5 (33.3%)	2 (13.3%)	0	0
White blood cell count decrease	0	0	3 (60%)	0	0	1 (6.7%)	2 (13.3%)	1 (6.7%)
Fatigue	1 (20%)	0	1 (20%)	0	3 (20%)	1 (6.7%)	0	0
Nausea	1 (20%)	0	0	0	5 (33.3%)	0	0	0
Diarrhea	1 (20%)	0	0	0	3 (20%)	1 (6.7%)	0	0
Neutropenia	0	1 (20%)	1 (20%)	0	0	0	1 (6.7%)	2 (13.3%)
Vomiting	1 (20%)	0	0	0	1 (6.7%)	3 (20%)	0	0
Anemia	0	0	2 (40%)	0	0	1 (6.7%)	1 (6.7%)	0
Thrombocytopenia	1 (20%)	1 (20%)	0	0	0	1 (6.7%)	1 (6.7%)	0
Lymphocyte count decrease	0	0	1 (20%)	1 (20%)	0	1 (6.7%)	0	0

On the Q3W schedule, 7 pts have achieved objective stable disease (hemangioendothelioma/2b, SCLC/3b, breast sarcoma/4b, NSCLC/4b, adenoid cystic carcinoma/4b, colorectal/5b, and a second colorectal/5b). Both pts with colorectal cancer were heavily pretreated and had been previously treated with irinotecan.

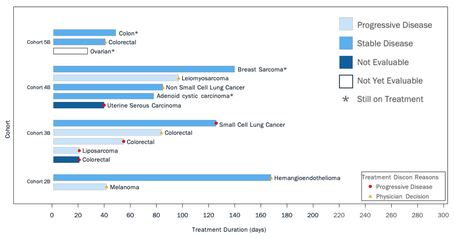
Pharmacokinetic properties were comparable between both schedules and show high variability with a trend toward increasing exposure of parent and payload with increasing dose.

To date, 4 pts remain on study (4b [n=2]; 5b [n=2]) with the longest treated pt on study for 168 days (hemangioendothelioma/2b).

Best response (RECIST 1.1) according to baseline: Waterfall plot



Treatment duration by Q3W dose-level cohort: Swimmer plot



Next-generation bivalent SMDCs with different payload classes demonstrate high neutrophil elastase (NE) dependent potency

- During the exploration of avß3 targeted SMDCs, we investigated payload and linker variation as well as impact of having a second binding moiety.

Figure 1. Monovalent and bivalent conjugates with different payloads.

- Various payloads, PTEFbi **2**, KSPi **3** and MMAE **4** were converted into avß3 targeted monovalent (**5**, **6**, **7**) and bivalent SMDCs **8**, **9** and **10** respectively, via a branched linker to two avß3 integrin binding moieties.

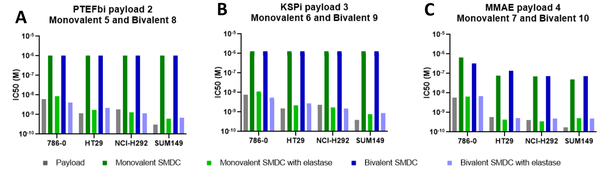


Figure 2. Cytotoxicity in vitro in the presence and absence of elastase: IC_{50} [M] of monovalent and bivalent conjugates with and without elastase compared to payloads **(A)** PTEFbi (**2**), monovalent (**5**) and bivalent (**8**) PTEFbi SMDCs **(B)** KSPi (**3**), monovalent (**6**) and bivalent (**9**) KSPi SMDCs **(C)** MMAE, monovalent (**7**) and bivalent (**10**) MMAE SMDCs

- Monovalent and bivalent conjugates showed similar weak cytotoxicity on the tested cell lines. In the presence of NE, monovalent and bivalent conjugates were highly potent across several cancer cell lines, reaching similar potency to the respective payload. These data demonstrate the ability of NE to release payload from all SMDCs in a selective and efficient manner.

Pharmacokinetics of bivalent small molecule drug conjugates **8**, **9** and **10** in comparison with respective monovalent analogues **5**, **6** and **7**

- Monovalent and bivalent SMDCs were investigated in pharmacokinetic studies in rat measuring the conjugate and the released payload.

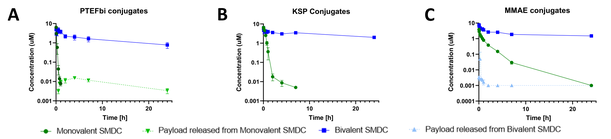


Figure 3. Plasma concentration-time profiles of monovalent (green, solid) and bivalent (blue, solid) SMDCs and payload released (light green, dashed from monovalent and light blue, dashed from bivalent conjugates **(A)** monovalent (**5**) and bivalent (**8**) PTEFbi SMDCs **(B)** monovalent (**6**) and bivalent (**9**) KSPi SMDCs **(C)** Monovalent (**7**) and bivalent (**10**) MMAE SMDCs; the concentration of payload (**3**) as well as (**2**) from (**8**) and (**4**) from (**7**) were below LLOQ and could therefore not be shown.

- No or trace levels of free payload (<0.1% as compared to the respective conjugates) was measured for all bivalent conjugates indicating high stability.

Table 2. Pharmacokinetics of 8, 9 and 10 in rats in comparison to and their monovalent analogues (**5**, **6** and **7** respectively)

	PK Parameter				
	CL_p (mL/min/kg)	V_{ss} (L/kg)	$t_{1/2}$ (h)	$AUC_{0\text{-}inf}$ (µmol*hr/L)	payload/parent AUC ratio
Bivalent SMDC **8**	0.0901 ± 0.0295	0.108 ± 0.023	15.3 ± 1.32	53.6 ± 17.8	NC
Monovalent SMDC **5**	6.60 ± 0.95	0.0366 ± 0.0053	0.240 ± 0.050	0.813 ± 0.108	0.309 ± 0.025
Bivalent SMDC **9**	0.0210 ± 0.0023	0.0433 ± 0.0058	25.5 ± 1.8	144 ± 16	NC
Monovalent SMDC **6**	1.69 ± 0.15	0.0377 ± 0.0050	0.442 ± 0.023	2.97 ± 0.25	NC
Bivalent SMDC **10**	0.0417 ± 0.0090	0.0987 ± 0.0023	28.3 ± 5.0	67.9 ± 12.6	0.0004 ± 0.00004
Monovalent SMDC **7**	1.47 ± 0.06	0.157 ± 0.015	2.30 ± 0.95	3.03 ± 0.11	NC

NC - not calculated as payload not detected (LLOQ< 0.0005 µM)

With each SMDC, independent of the conjugated payloads, a remarkable impact on exposure was observed when attaching a second binding ligand. The SMDCs with 2 ligands showed a 37-81-fold reduced plasma clearance as compared to the respective monovalent SMDCs, which goes in parallel with a 12-57-fold increase in half-life.

In vivo efficacy of bivalent SMDC **8**, **9** and **10** in NCI H69 mouse model

- Bivalent conjugates **8**, **9** and **10**, releasing respectively PTEFbi **2**, KSPi **3** and MMAE **4** were evaluated in the NCI H69 mouse model.

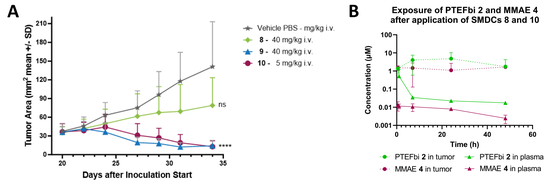


Figure 5. (A) Efficacy in NCI H69 model. Growth curve of NCI H69 tumors treated with 2 applications of **8** (40mg/kg iv, weekly), **9** (40mg/kg iv, weekly) and **10** (5 mg/kg iv, weekly); p<0.0001; ns = not significant. **(B) Tumor vs plasma exposure of payloads 2**, **3** and **4** after application of SMDCs **8** (40mg/kg), **9** (40mg/kg) and **10** (5 mg/kg) respectively.

- Monotherapy treatment in a once weekly schedule with 40 mg/kg iv (**9**) and 5 mg/kg iv (**10**) achieved tumor regression. Once weekly application of 40mg/kg of **7** achieved moderate T/C superior to its monovalent analogue **5**[3].
- No significant impact on mean body weight of mice was observed, indicating good tolerability of SMDCs with PTEFbi, KSPi and MMAE payloads.
- PK study in tumor bearing mice was performed after a single dose of SMDCs **8** (40 mg/kg) and **10** (5 mg/kg). The tumor to plasma ratio of respective payload **2** and **4** was very high (50 to >100 respectively) indicating excellent stability of conjugates in plasma and selective tumor release and accumulation.
- Further in vivo studies are planned to evaluate the minimum effective dose of the next-generation bivalent conjugates **9** and **10**.

CONCLUSIONS

- VIP236—our first-in-class, clinical-stage SMDC—exemplifies the innovation and power of our VersAptx™ platform. Preliminary clinical data for VIP236 shows an improved safety profile versus irinotecan, topotecan and belotecan[6,7,8]. Irinotecan and the other camptothecin derivatives are efficacious anticancer agents used alone and as part of many important polychemotherapy regimens, though are also associated with severe and life-threatening toxicity.
- VIP236 is showing early evidence of monotherapy clinical activity, including tumor reduction and disease control, in a heavily pretreated pt population with a broad range of metastatic solid tumors. VIP236 has the potential to provide clinical activity consistent with camptothecin derivatives with an improved safety profile due to optimized tumor penetration and anticipated tumor accumulation.
- Bivalent SMDCs showed similar potency in vitro in the presence of NE as compared with monovalent analogues, indicating efficient and selective payload release.
- Addition of a second binding moiety, regardless of payload, showed a strong effect in clearance reduction and half-life prolongation of up to 57-fold.
- Tumor regressions in vivo were shown with the bivalent conjugates **9** and **10** with good tolerability.
- Exposure data of **10** showed accumulation of MMAE in the tumor with a factor 100 compared with plasma exposure, indicating excellent stability of conjugates in plasma and selective tumor release and accumulation.
- Our modular VersAptx™ technology platform now comprises a robust solution to increase half-life of conjugates compatible with various payload classes. This offers flexibility to select the best payload for a given tumor indication.
- VNC-236-101 clinical study continues with dose escalation to be followed by expansion cohorts in select populations and combination studies.

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