Synthesis and characterization of novel small molecule drug conjugates with different payloads designed to be released in tumor microenvironment by neutrophil elastase

#2722

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INTRODUCTION

- αvß3 integrins show an abundant expression in the tumor microenvironment of aggressive cancers which is correlated with metastatic disease and poor prognosis[1,2]. Anti-angiogenic therapies targeting αvβ3 showed a good safety profile with optimal homing to the tumor and metastases, but with limited efficacy[3].
- For efficient treatment of aggressive cancers and metastasis, we designed small molecule drug conjugates (SMDCs) consisting of an αvß3 integrin binder linked to an optimized camptothecin payload. Our frontrunner compound VIP236 has just entered a Phase I clinical study (NCT05712889)[4].
- Due to insufficient internalization of αvß3 binders, we developed new mechanisms for activation and payload release using enzymes present in the tumor microenvironment (TME). Neutrophil elastase (NE) belongs to a family of serine proteases that degrades extracellular matrix proteins and contributes to tumor evasion and metastasis. NE expression and neutrophil tumor infiltration have been correlated with metastatic potential and poor prognosis[5].
- Previous studies have shown that NE efficiently cleaves and activates camptothecin conjugates by cleaving an ester bond. In the current study we investigated linker variations and the extension of elastase-mediated payload release to advance further payload classes with different mode of actions.

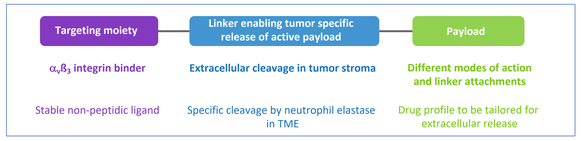

Figure 1. Design of αvß3-targeted Small Molecule Drug Conjugates (SMDCs) for activation and payload release in the tumor microenvironment

METHODS

- Tumor accumulation of the IRDye® conjugates in mice was determined 8, 24, and 48 h after administration using the LI-COR Pearl® imaging system as previously described[4]. Tumor specificity of elastase cleavage was shown with a quenched fluorogenic substrate LYS(QSY21)ANPV-Cys(AlexaFluor647) in a Lewis lung cancer model.
- The in vitro cytotoxicity was evaluated in a panel of cancer cell lines after a 72-h continuous exposure in presence or absence of 10 nM neutrophil elastase using MTT assays (ATCC). IC_{50} values were determined as the concentration of compound required for 50% inhibition of cell viability.
- For the rat plasma stability assay, the test compound was dissolved in ACN/DMSO (1:1, 0.5 ml). The HPLC vial was shaken and sonicated. While vortexing, 20 µl of this solution was added to 1 ml 37°C rat plasma. After 0.17, 0.5, 1, 1.5, 2 and 4h the enzymatic reaction was stopped by adding ACN/buffer pH3 (80:20). After centrifugation, the supernatant was analyzed by HPLC and peak areas were used for quantification. In addition, the starting material and the 4h sample were analyzed by LC/MS.
- Stability of SMDCs in PBS buffer at pH 7.4 over 24 h was determined in a similar way as described above for rat plasma.
- For the elastase cleavability assay, the test compound was added to buffer (150mM NaCl, 10 mM $CaCl_2$, 0.05 % BSA) to a final concentration of 5 µM (0.5 % DMSO). The reaction was started by adding different concentrations of h-Elastase (0, 20, 40, 60 nM) to the reaction vials. After incubation for 1 h at 37° C, the enzymatic reaction was stopped by precipitation in 50 % ACN. Subsequently, the samples were subjected to HPLC-MS analysis to determine the concentration of test compound and of its metabolite.
- For pharmacokinetic studies, male Wistar rats (n=3) were given a 0.5 mg/kg dose of SMDC (2.0 mg/kg for VIP280) formulated in plasma/DMSO (99:1) intravenously (IV) via tail vein. Blood was collected at predose, 2, 5, 15, 30, 45, 60 min, 2, 4, 7, 24 hr post-dose. Plasma was collected and frozen at < -20°C until sample analysis. Plasma samples were analyzed for concentrations of SMDCs and their respective warheads 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[6].
- For in vivo efficacy studies, immunocompromised mice were inoculated subcutaneously with $1x10^6$ MX-1 TNBC cells in 50% Matrigel/50% Media on day 0. Treatment was started at mean tumor volume of 75 mm^3 in the MX-1 model (n=12/group). Tumor and body weight were measured at least twice weekly.

RESULTS

Imaging studies show specific binding to activated αvß3 integrin in tumor/TME and neutrophil elastase cleavage in the TME

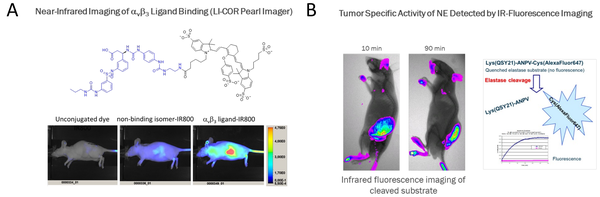

Figure 2. Imaging Studies . (A) 786-O RCC model: Highly specific binding of αvß3-dye-conjugate to activated αvß3 in the tumor in contrast to the isomeric non-binding control. **(B)** Lewis lung cancer model: Tumor specific presence of NE allows for targeted release of the cargo (fluorescent substrate or payload)

Integrin αvß3 targeted SMDCs with different payload classes demonstrate high elastase dependent potency

- Various payload classes, namely an optimized camptothecin (opt. CPT; Topoisomerase I inhibitor), a PTEFbi (CDK9/CycT inhibitor) and a KSPi (Kinesin Spindle Protein inhibitor) were successfully converted into αvß3 targeted SMDCs using different chemical handles (alcohol, sulfoximine and primary amine).

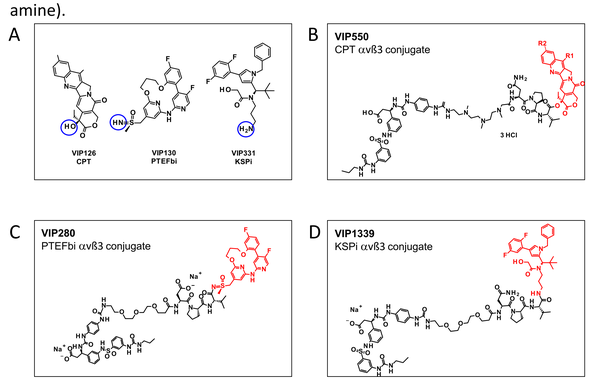

Figure 3. Chemical structures. (A) Payload classes selected: opt. CPT (Topoisomerase inhibitor), PTEFbi (CDK9/cycT inhibitor) and KSPi (Kinesin Spindle Protein inhibitors). **(B)** Structure of VIP550 releasing optimized CPT payload (VIP126). **(C)** Structure of VIP280 releasing PTEFbi payload (VIP130). **(D)** Structure of VIP1339 releasing KSPi payload (VIP331).

- Cytotoxicity in cancer cell lines was clearly dependent on elastase and IC_{50} values were in the nanomolar range across several cancer cell lines reaching similar potency as compared to the respective payloads alone.
- Large scope of potential payloads and tolerated conjugation chemistries demonstrated broad applicability of the Neutrophil Elastase SMDC platform

Table 1. Cytotoxicity in vitro in the presence and absence of elastase. IC_{50} [M] of conjugates VIP550, VIP280 and VIP1339 in comparison to their payloads (VIP126, VIP130 and VIP331 respectively) at 4 cell lines with and without elastase.

Compound	786-0	786-0 + Elastase	HT29	HT29 + Elastase	NCI-H292	NCI-H292 + Elastase	SUM149	SUM149 + Elastase
VIP126	3.7E-9	3.2E-9	2.2E-8	1.9E-8	3.8E-9	4.6E-9	3.8E-9	4.2E-9
VIP550	5.4E-7	3.5E-9	1.0E-6	2.0E-8	1.7E-7	6.0E-9	3.1E-7	4.8E-9
VIP130	2.8E-9	3.3E-9	2.2E-9	2.1E-9	3.9E-9	4.5E-9	2.4E-9	2.4E-9
VIP280	1.0E-6	4.9E-9	1.0E-6	2.5E-9	5.6E-7	2.1E-9	7.8E-7	2.9E-9
VIP331	2.5E-9	2.5E-9	4.1E-10	4.1E-10	4.6E-10	2.7E-10	3.3E-10	1.7E-10
VIP1339	1.0E-6	4.5E-9	1.0E-6	2.4E-9	9.9E-7	1.5E-9	3.3E-7	3.3E-10

Excellent plasma stability and elastase mediated release of opt. CPT, PTEFbi and KSPi payloads from αvß3 conjugates

- αvß3 conjugates VIP280, VIP550 and VIP1339 demonstrated excellent stability in rat plasma as well as in buffer at pH7.4 (data not shown).
- Elastase cleavability assay showed elastase mediated release of VIP130, VIP126 and VIP331 although with slower kinetic for the amide linked KSPi-SMDC.

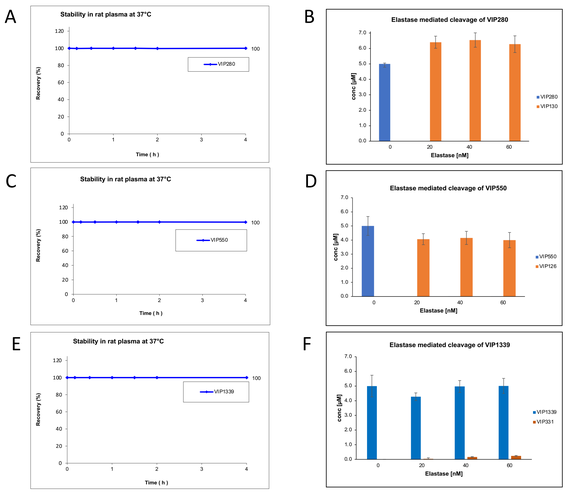

Figure 4. High stability of αvß3 conjugates in rat plasma and elastase mediated release of payload. (A) Stability of VIP280 in rat plasma. **(B)** Elastase mediated cleavage of VIP280 **(C)** Stability of VIP550 in rat plasma. **(D)** Elastase mediated cleavage of VIP550. **(E)** Stability of VIP1339 in rat plasma. **(F)** Elastase mediated cleavage of VIP1339.

Pharmacokinetics of small molecule drug conjugates VIP550, VIP280, and VIP331 in male Wistar rats

- Plasma clearance of all 3 small molecule drug conjugates was low with clearance being in the following rank ordering: VIP1339>VIP550>VIP280. Half-life was longer for SMDCs with the lowest clearance estimates.
- Volume of distribution was similar for SMDCs being approximately total body water 0.7 L/kg.
- The ratio between AUC of the payload and parent SMDC was decreasing in the order of VIP280>VIP550>VIP1339. A 15-fold reduction in this ratio between VIP280 and VIP550 suggest a large increase in in vivo stability of the payload linker for VIP550 compared with VIP280.

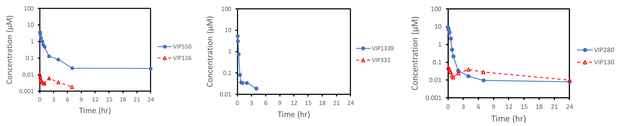

Figure 5. Plasma concentration-time profiles of SMDCs (blue solid line) and their respective payloads (red dashed line) in rats at 0.5 mg/kg IV. (Note that VIP280 was dosed at 2 mg/kg IV and data has been scaled to 0.5 mg/kg IV)

Table 2. Pharmacokinetics of VIP550, VIP280, and VIP331 in rats

PK Parameter	Compound		
	VIP550	VIP1339	VIP280
CL_p (mL/min/kg)	2.33 ± 0.44	5.50 ± 2.35	1.64 ± 0.12
V_{ss} (L/kg)	0.627 ± 0.344	0.600 ± 0.515	0.283 ± 0.155
$t_{1/2}$ (h)	8.31 ± 5.49	4.88 ± 4.37	19.1 ± 5.4
$AUC_{0\text{-}inf}$ (µmol*hr/L)	2.52 ± 0.61	1.09 ± 0.49	3.44 ± 0.13*
payload/parent AUC ratio	0.0105	NC	0.162

NC - not calculated as payload not detected (LLOQ< 0.0005 µM)
* Dosed at 2.0 mg/kg but normalized to 0.5 mg/kg

In vivo efficacy of SMDC VIP550 in the subcutaneous TNBC MX1 CDX mouse model

- VIP550 monotherapy exhibits tumor regression in the 20mg/kg iv 2on5off treatment schedule (PR: 12/12). Once weekly application of VIP550 achieved stable disease (T/C: 0.34) slightly less efficacious compared with VIP236 (T/C: 0.24).
- No significant impact on mean body weight of mice was observed indicating good tolerability of VIP550 and VIP236.
- In vivo studies with SMDCs with PTEFbi and KSPi payloads are ongoing.

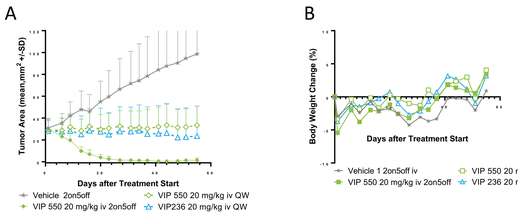

Figure 5. VIP550 efficacy in TNBC MX1 CDX model. (A) Growth curve of MX1 tumors treated with VIP550 in two schedules and VIP236 with once weekly dosing. VIP550 monotherapy demonstrated significant tumor regression in the 20mg/kg 2on5off schedule. **(B)** Body weight changes based on VIP550 monotherapies and VIP236 treatment are minor. All treatment groups show statistical significance ****p<0.0001 vs vehicle (unpaired t-test).

CONCLUSIONS

- Imaging studies with fluorescent conjugates indicate efficient tumor homing of the αvß3 binder and tumor-associated cleavage by NE.
- SMDCs with NE-cleavable linkers show high elastase-dependent potency.
- NE-mediated SMDC activation achieves optimal cytotoxic activity of the respective payloads employed.
- SMDCs are highly stable in rat plasma and in buffer at pH 7.4.
- Activation and traceless release of payloads with different MoA and linkages is shown for SMDCs with opt. CPT, PTEFbi and KSPi payloads, the latter with slower kinetics.
- In DMPK studies all SMDCs show low plasma clearance and a low volume of distribution.
- Strong in vivo efficacy is demonstrated with the SMDC VIP550 with an opt. CPT payload in the MX1 TNBC model.

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