A novel small molecule drug conjugate - $\alpha_v\beta_3$ integrin antagonist linked to a cytotoxic camptothecin derivative - for the treatment of multiple cancer types

Hans-Georg Lerchen[1,2], Beatrix Stelte-Ludwig[1], Charlotte Kopitz[3], Melanie Heroult[4], Dmitry Zubov[1], Jörg Willuda[5], Thomas Schlange[1], Antje Kahnert[1], Raquel Izumi[6], Ahmed Hamdy[6]

[1]Bayer AG, Pharmaceuticals R&D, Wuppertal, Germany; [2]Vincerx Pharma GmbH, Germany; [3]NUVISAN ICB GmbH, Berlin, Germany;
[4]Bayer AG, Crop Science Division, Frankfurt am Main, Germany; [5]Bayer AG, Pharmaceuticals R&D, Berlin, Germany; [6]Vincerx Pharma Inc, Palo Alto, CA, USA

INTRODUCTION

- In the tumor microenvironment, $\alpha_v\beta_3$ integrins play critical roles in tumor progression, resistance to cytotoxic therapy, metastasis, and the recruitment of immune and inflammatory cells.
- Neutrophil elastase belongs to a family of proteases, which contribute to cancer progression by enhancing tumor evasion and metastasis in tumor stroma.
- The expression levels of $\alpha_v\beta_3$ integrins and neutrophil elastase are associated with aggressive disease in various cancers.[1,2]
- To improve the tumor selectivity of cytotoxic agents, we designed small molecule drug conjugates (SMDCs) that independently address two mechanisms of targeted delivery: a) binding to $\alpha_v\beta_3$ integrins and b) drug release mediated by the cleavage enzyme neutrophil elastase.
- VIP236 is an SMDC consisting of an $\alpha_v\beta_3$ integrin binder and a neutrophil elastase cleavable linker (**Fig. 1**). The payload of VIP236 is a modified camptothecin (CPT) derivative of SN38, a well-known moderately cytotoxic drug and the active metabolite of irinotecan. The CPT payload was further tailored to permeate cells in an optimal manner (i.e., high permeability and low efflux) after extracellular release.
- Here, we characterize the efficacy of VIP236 in preclinical cancer models *in vitro* and *in vivo*.

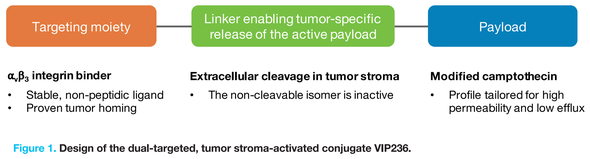

Figure 1. Design of the dual-targeted, tumor stroma-activated conjugate VIP236.

METHODS

- BALB/c nude mice were s.c. inoculated with 786-O renal cell adenocarcinoma (RCC) cells. At an average tumor size of 0.7 cm in diameter, the mice were injected with 20 nmol of BAY810, an $\alpha_v\beta_3$ binder coupled to IRDye® 800CW (LI-COR Bioscience), according to manufacturer's instructions or BAY813, a non-binding control conjugate of IRDye® 800CW. Tumor accumulation of the IRDye® conjugates in mice was determined 8, 24, and 48 h post-administration using the LI-COR Pearl® imaging system.
- The efflux properties of the modified CPT payload were compared with SN38, a known P-glycoprotein (P-gp) substrate, using a flux assay and LLC-PK1 cells transfected to express P-gp.[3] The cell permeability was investigated by an *in vitro* flux assay using Caco-2 human colorectal adenocarcinoma cells.[4]
- The cytotoxicity of VIP236 and the payload was measured in a panel of cancer cell lines 72 h after treatment using the MTT Cell Proliferation Assay (ATTC).
- For the determination of drug metabolism and pharmacokinetics (DMPK) properties, VIP236 (4 mg/kg) and modified CPT (1 mg/kg) were administered intravenously to 786-O tumor-bearing mice. Plasma and tumor samples were collected at specific time points and the concentration of modified CPT was measured by liquid chromatography with tandem mass spectrometry (LC-MS/MS).
- To study the antitumor efficacy of VIP236 *in vivo*, immunocompromised female NMRI nu/nu mice were subcutaneously inoculated with human MX-1 triple-negative breast cancer (TNBC), NCI-H69 small cell lung cancer (SCLC) or SW480 colorectal cancer (CRC) cells. Tumors were allowed to establish before the animals were assigned to different groups by stratified randomization. Tumor area (length x width) and body weight were assessed at least twice weekly. Statistical analyses were performed using one-way ANOVA followed by Dunnett's test.

RESULTS

The $\alpha_v\beta_3$ integrin-targeting moiety mediates tumor homing

- The $\alpha_v\beta_3$ integrin-targeting moiety coupled to a fluorescent IR800 dye showed strong tumor homing in mice bearing 786-O RCC tumors, as detected by near-infrared imaging (**Fig. 2**).
- No tumor-specific accumulation was detected with the non-binding control conjugate.

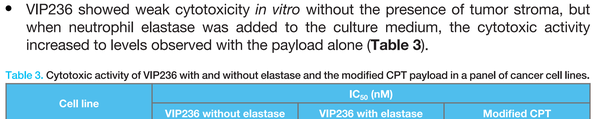

Figure 2. (**A**) The $\alpha_v\beta_3$ integrin-targeting moiety (in blue) coupled to a fluorescent IR800 dye, (**B**) Tumor homing of the IR800-coupled $\alpha_v\beta_3$ binder and the non-binding control conjugate in mice as detected by near-infrared imaging.

The modified CPT payload of VP236 shows an improved *in vitro* profile compared with SN38

- To overcome the potential transporter-mediated resistance observed in the clinic with SN38, the CPT derivative was specifically optimized to be highly permeable with low efflux potential; two features considered beneficial for extracellular release in the tumor microenvironment.
- The modified CPT payload of VP236 showed high permeability and low cellular efflux ratio when compared to the control SN38, the active metabolite of irinotecan. This indicated that the modified CPT payload is not a transporter substrate (**Table 1**).
- The modified CPT demonstrated comparable cytotoxicity in the parental NCI-H1975 cells and the P-gp or BCRP transporter-expressing mutants (**Table 2**). In contrast, SN38 showed lower cytotoxicity in the transporter-expressing mutants.

Table 1. Permeability and efflux ratio measured in flux assays with P-gp-expressing LLC-PK1 cells and Caco-2 cells.

Cell line	Payload	Permeability (nm/s)	Efflux ratio
LLC-PK1	Modified CPT	196	0.6
	SN38	10	16
Caco-2	Modified CPT	171	1
	SN38	8	36

Table 2. Cytotoxic activity of modified CPT and SN38 in NCI-H1975 parental and P-gp or BCRP transporter-expressing mutant cells.

Compound	IC_{50} (nM)		
	NCI-H1975	NCI-H1975 – P-gp	NCI-H1975 – BCRP
SN38	45	141	512
Modified CPT	19	34	27

In vitro cytotoxic activity of VIP236 against tumor cell lines is elastase-dependent

- VIP236 is a conjugate consisting of the $\alpha_v\beta_3$ binder, the modified CPT payload, and a neutrophil elastase linker (**Fig. 1**).
- VIP236 showed weak cytotoxicity *in vitro* without the presence of tumor stroma, but when neutrophil elastase was added to the culture medium, the cytotoxic activity increased to levels observed with the payload alone (**Table 3**).

Table 3. Cytotoxic activity of VIP236 with and without elastase and the modified CPT payload in a panel of cancer cell lines.

Cell line	IC_{50} (nM)		
	VIP236 without elastase	VIP236 with elastase	Modified CPT
786-O	188	1.1	1.2
HT29	245	8.7	6.8
LoVo	91	2.9	1.8
SW480	41	1.2	1.8
NCI-H292	209	1.8	1.5
NCI-H69	486	3.0	2.9

Administration of VIP236 results in higher tumor-to-plasma ratio of the CPT payload

- Tumor and plasma exposures of the modified CPT were determined after i.v. administration of the VIP236 conjugate or the payload alone.
- Administration of VIP236 (**Fig. 3**, blue lines) resulted in a tumor-to-plasma ratio of 6.5 of the modified CPT, whereas a ratio of 0.6 was observed with an equimolar dose of the payload alone (**Fig. 3**, green lines). This indicates a 10.8-fold increase in the ratio when using the conjugate.

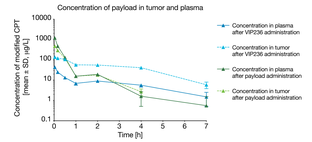

Figure 3. Concentration of the modified CPT in tumor and plasma following the application of VIP236 or the payload alone.

VIP236 shows high antitumor efficacy and good tolerability *in vivo*

- VIP236 showed comparable or superior efficacy compared with reference chemotherapy drugs in the MX-1 TNBC, NCI-H69 SCLC, and SW480 CRC models (**Fig. 4A-C**).
  - Complete tumor responses were observed in 100% of mice in the MX-1 TNBC model at all VIP236 doses (26–40 mg/kg; **Table 4**).
  - Additionally, partial responses were observed in 8/8 mice both in the NCI-H69 SCLC and SW480 CRC models at a VIP236 dose of 40 mg/kg.
- VIP263 showed less than 5% mean body weight loss and demonstrated superior tolerability as compared to the CPT derivatives and reference chemotherapy drugs tested (**Fig. 4D-F**).

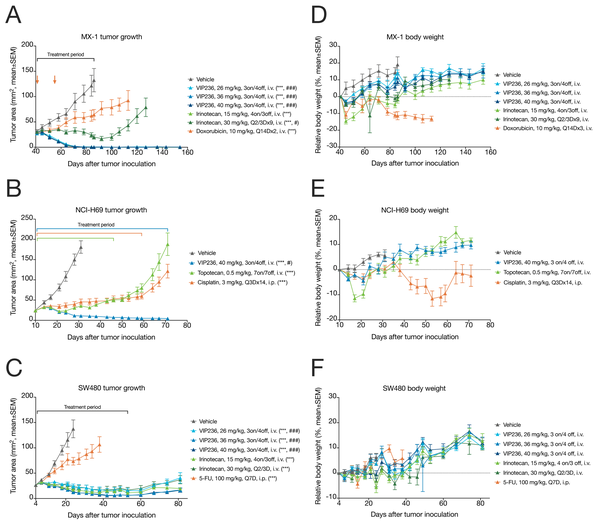

Table 4. Antitumor efficacy of VIP236 and reference chemotherapy drugs in the MX1 breast cancer model.

Treatment	Corresponding payload dose (mg/kg)	Complete responses	Fatal toxicities
VIP236, 26 mg/kg, 3on/4off	6.5	7/8	1/8[a]
VIP236, 36 mg/kg, 3on/4off	9	8/8	0/8
VIP236, 40 mg/kg, 3on/4off	10	8/8	0/8
Irinotecan, 15 mg/kg, 4on/3off	9.5	8/8	0/8
Irinotecan, 30 mg/kg, Q2/3Dx9	19	0/8	1/8[b]
Doxorubicin, 10 mg/kg, Q14Dx2	n/a	0/8	2/8[c]

[a] One animal was found dead on d132, 47 days after the last treatment; no body weight loss observed (not treatment-related)
[b] One animal was found dead on d50; relative body weight loss of >10% observed; treatment holidays needed
[c] Two animals were found dead (d59 and d97); relative body weight loss of >15% observed

CONCLUSIONS

- A novel small molecule drug conjugate VIP236 was developed and optimized to bind to the $\alpha_v\beta_3$ receptor and to release its CPT payload when selectively cleaved by neutrophil elastase in the tumor microenvironment.
- The $\alpha_v\beta_3$ integrin-targeting binder demonstrated efficient tumor homing *in vivo*.
- The CPT payload is a novel, modified SN38 derivative with higher cellular permeability and significantly reduced efflux potential. In contrast to SN38, VIP236 also retained its cytotoxic activity in transporter-expressing mutant cell lines.
- A 10.8-fold higher tumor-to-plasma ratio of the modified CPT payload was shown after conjugate administration compared to i.v. administration of the payload alone.
- Treatment with VIP236 resulted in tumor regressions in all xenograft models tested (TNBC, SCLC, CRC) *in vivo* with good tolerability.

References

1. Hieken TJ, *et al. J Surgical Res*. 1996;63:169-173.
2. Sato T, *et al. Surgical Oncol.* 2006;15:217-222.
3. Schinkel AH, *et al. J Clin Invest.* 1995;96:1698-1705.
4. Troutman MD and Thakker DR. *Pharm Res.* 2003;20:1210-1224.

Acknowledgments

We thank S. Bendix, T. Boldt, A.-M. DiBetta, K. Henschel, K. Jaensch, B. Koenig, B. Oelmez, C. Schade, K.-D. Schroeder, B. Timpner and D. Wolter for excellent technical assistance. Aurexel Life Sciences Ltd. (www.aurexel.com) is thanked for editorial assistance in the preparation of this poster, funded by Bayer AG.

Figure 4. Antitumor efficacy of VIP236 and reference compounds in mouse xenograft models (n=8/group). (A-C) Tumor growth in the (A) MX-1 TNBC, (B) NCI-H69 SCLC, and (C) SW480 CRC models during the course of the study. Statistical analyses were performed using one-way ANOVA followed by Dunnett's test on day 86 in (A), day 31 in (B), or day 24 in (C). ***, p<0.001 compared to vehicle; #, p<0.05 compared to cisplatin; ###, p<0.001 compared to doxorubicin in (A) or 5-FU in (C). **(D-F) Relative body weight change of mice** in the (**D**) MX-1 TNBC, (**E**) NCI-H69 SCLC, and (**F**) SW480 CRC models during the course of the study.

Corresponding author: Hans-Georg Lerchen, hans-georg.lerchen@vincerx.com