VIP152, a selective CDK9 inhibitor, demonstrates sensitivity in gynecologic cell lines that are cisplatin sensitive or resistant and delivers *in vivo* antitumor efficacy

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INTRODUCTION

- Ovarian and endometrial cancers are the most lethal and common gynecologic malignancies, respectively (1).
- Current adjuvant chemotherapy for ovarian and endometrial cancer consists of a platinum-based drug (i.e. cisplatin/carboplatin) and a taxane (i.e., paclitaxel).
- However, despite initial high response rates to cisplatin, over 70% of patients with ovarian cancer eventually develop platinum-resistant disease (2), and novel secondary therapies such as PARP inhibitors are not curative.
- Various mechanisms of platinum resistance have been proposed, including the altered expression of the *MYC* oncogene. Mechanisms of *MYC* activation include gene amplification or mutation. They are prevalent in gynecologic malignancies, such as ovarian serous (31.5%) (3) and endometrial (9.7%) (4) cancers.
- CDK9 phosphorylation of RNA polymerase II is required for transcription of *MYC* mRNA regulating Myc driven oncogenic signaling.
- VIP152 is a well-tolerated and clinically active, highly selective CDK9 inhibitor (5). We present the antitumor responses of VIP152 in preclinical models of gynecologic malignancies and preliminary clinical data of VIP152-treated patients with gynecologic cancers.

METHODS

- The cytotoxicity of VIP152 was examined in a panel of 42 gynecologic cancer cell lines treated with 9 dose levels of VIP152 using DMSO and cisplatin as controls (OmniScreen, Crown Bioscience). Cell viability and IC_{50} values were determined after 72 h (CellTiter-Glo®, Promega).
- The association between response to VIP152 and status of baseline mutations, gene expression, and copy number variation was determined by RNA-Seq analysis (DESeq2 (6)) in 33 cell lines for which genomic data was available in DepMap (21Q3) (7). To select variants that are more likely to be functionally important, the data were filtered to include only variants with a "damaging" or "other non-conserving" annotation (Ensembl Variant Effect Predictor (VEP) (8)).
- The cytotoxicity of VIP152 was further evaluated *ex vivo* in cells obtained from tissues of patients with ovarian and endometrial cancer. The cells were treated for 72 h with VIP152 and cytotoxicity was measured by cell count and cell viability. In parallel, cells from patient samples were treated for 0, 2, 4, and 16 h with 500 nM VIP152 and the Myc protein level was determined by immunofluorescence using a Alexa Fluor 594 -conjugated Myc antibody (ab32072, Abcam). The intensity data were normalized using the mean intensity values from the control wells.
- The *in vivo* antitumor efficacy of VIP152 monotherapy was evaluated in a cisplatin-sensitive A2780 human ovarian cancer xenograft model in mice. Female NMRI nude (nu/nu) mice (Taconic) were inoculated subcutaneously with $2x10^6$ A2780 cells and treated (n=10–12/group) with a single intravenous (i.v.) dose of vehicle (30% PEG400, 10% ethanol, 60% water) or VIP152 (17.5 mg/kg) at average tumor volume of 68 mm^3. All animal experiments were performed under the national animal welfare laws of Germany and approved by the local authorities.
- The Phase 1 clinical trial of VIP152 is open and enrolling (NCT02635672) and patient consent was obtained. Patients with gynecologic cancer have received doses of 5, 22.5, or 30 mg of VIP152 as a 30-min, i.v. infusion once weekly. Response assessments, tumor measurements, and reason for end of treatment (EoT) were captured in the clinical trial electronic data capture (EDC) database according to RECIST 1.1. Plasma samples for pharmacokinetic (PK) assessments were collected pre-dosing and at 0.25, 0.5, 0.67, 1, 2, 4, 6, 8, 24, 48, 72, and 168 h after the start of infusion on cycle 1, day 1 (C1D1). Plasma VIP152 was quantified using a validated liquid chromatography coupled to mass spectrometry assay. PK parameters were assessed using non-compartmental analysis. A pharmacodynamic (PD) analysis of target gene mRNAs was performed in total intracellular RNA extracted from whole blood (PAXgene Blood RNA tubes, BD Biosciences) collected from patients on C1D1 (pre-dosing and 0.5, 1, 2, 4, 6, 8, 24, and 48 h after start of infusion) and on C1D15 (pre-dosing and 0.5, 1, 2, 4, 6, and 8 h after start of infusion). Absolute neutrophil counts over time were plotted against each patient's baseline counts.

RESULTS

VIP152 demonstrates sensitivity in cisplatin-sensitive and cisplatin-resistant gynecologic cancer cell lines

- A 3-log range of sensitivity to VIP152 was observed in gynecologic cancer cell lines, with IC_{50} values ranging from 38–593 nM (**Fig. 1**). The median IC_{50} values for VIP152 and cisplatin were 0.11 µM and 5.22 µM in gynecologic cancer cell lines (n=42) and 0.11 µM and 4.95 µM in ovarian cancer cell lines (n=20), respectively.
- VIP152 showed low IC_{50} values (<100 nM) in both cisplatin-sensitive (<5 µM) and cisplatin-resistant (>5 µM, dashed line) cell lines (**Fig. 1**).
- VIP152 sensitivity was observed in cell lines with *MYC* and/or *MYCN* genetic alterations (gene amplification, mutation) (**Fig. 1**).

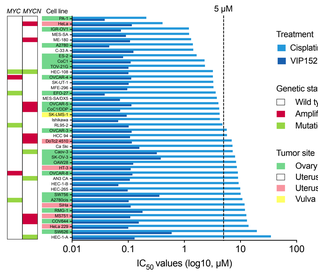

Figure 1. Response to VIP152 and cisplatin treatments in ovarian (n=20), uterus (n=15), uterus/cervix (n=6) and vulva (n=1) cancer cell lines, presented as IC_{50} values. Cell lines were assigned as sensitive and resistant to VIP152 or cisplatin based on the obtained IC_{50} values. The *MYC* and *MYCN* genetic statuses of cell lines were obtained from DepMap (7) (white, wild type; red, gene amplification; green, mutation). Dashed line at 5 µM represents the cut-off value for cisplatin resistance.

VIP152 sensitivity is associated with high number of gene mutations identifying a gene signature that could predict response in gynecologic malignancies

- The genomic features potentially predicting sensitivity to VIP152 were identified by plotting the top 100 most differentially abundant gene mutations as heatmaps.
- The most VIP152-sensitive gynecologic cancer cell lines (n=33) were associated with multiple gene mutations (bottom quartile in **Fig. 2A**), whereas the least VIP152-sensitive cell lines were associated with only a few mutations (top quartile in **Fig. 2A**). Similar mutation patterns were observed in the subset of 15 ovarian cancer cell lines, where the IC_{50} range was 38–79 nM for the bottom quartile and 131–379 nM for the top quartile (**Fig. 2B**).
- Cell lines with the highest tumor mutational burden (TMB) were associated with VIP152 sensitivity. Cell lines with high TMB and intermediate VIP152 sensitivity but narrow IC_{50} ranges could also be considered as sensitive to VIP152.

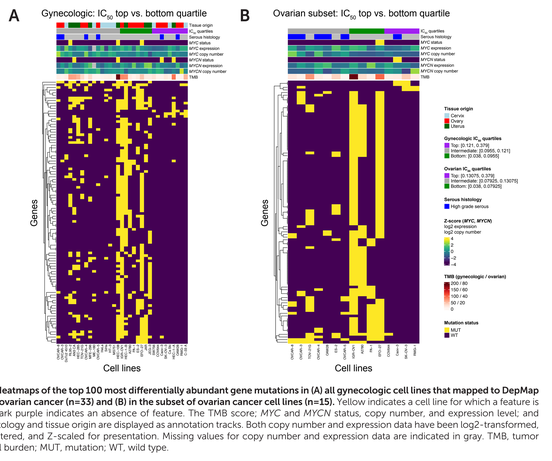

Figure 2. Heatmaps of the top 100 most differentially abundant gene mutations in (A) all gynecologic cell lines that mapped to DepMap including ovarian cancer (n=33) and (B) in the subset of ovarian cancer cell lines (n=15). Yellow indicates a cell line for which a feature is present; dark purple indicates an absence of feature. The TMB score; *MYC* and *MYCN* status, copy number, and expression level; and serous histology and tissue origin are displayed as annotation tracks. Both copy number and expression data have been log2-transformed, mean-centered, and Z-scaled for presentation. Missing values for copy number and expression data are indicated in gray. TMB, tumor mutational burden; MUT, mutation; WT, wild type.

VIP152 shows efficacy and Myc protein regulation in patient-derived gynecologic cancer tissue samples *ex vivo*

- Sensitivity of nine patient-derived platinum-resistant or treatment-naïve samples to VIP152 treatment was determined *ex vivo*.
- VIP152 IC_{50} values ranged from 6 to 12 nM in cancerous tissues from patients with platinum-resistant ovarian cancer. Higher (40–45 nM) IC_{50} values were observed for VIP152 in endometrial cancer tissues. At this level, the tissues were nonetheless considered as sensitive to VIP152.
- VIP152 demonstrated efficacy in both treatment-naïve and platinum-resistant cancerous tissues from patients with ovarian or endometrial cancer (**Fig. 3A–B**).
- A 50–65% reduction in Myc protein expression was observed upon *ex vivo* VIP152 treatment in cancerous tissues when compared with pre-treatment levels (**Fig. 3C**).

A

Patient ID	Cancer type	Treatment history							Other treatments
		CB	PTX	BVZ	DXR	LET	TAM	TRA	
IP 200190	Endometrial	x	x				x	x	Radiotherapy
IPSA 19032	Endometrial								
IB 200150-T3	Ovarian	x	x	x	x				
IB 200165	Ovarian	x	x	x		x			
IB 200156	Ovarian	x	x				x	x	EPR, CPD, DTX
IB 200162-T3	Ovarian	x	x	x	x				GEM, CIS, ETP
IB 200226	Ovarian	x	x			x			
IB 210006	Ovarian	x	x						
IB 210007	Ovarian								

BVZ, bevacizumab; CB, carboplatin; CIS, cisplatin, CPD, cyclophosphamide; DTX, docetaxel; DXR, doxorubicin; EPR, epirubicin, ETP, etoposide; GEM, gemcitabine; LET, letrozole; PTX, paclitaxel; TAM, tamoxifen; TRA, trastuzumab.

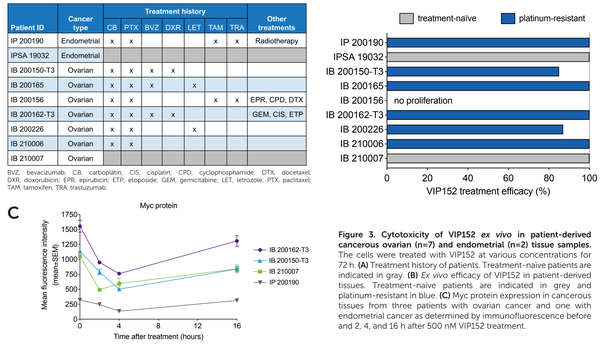

Figure 3. Cytotoxicity of VIP152 *ex vivo* in patient-derived cancerous ovarian (n=7) and endometrial (n=2) tissue samples. The cells were treated with VIP152 at various concentrations for 72 h. **(A)** Treatment history of patients. Treatment-naïve patients are indicated in gray. **(B)** *Ex vivo* efficacy of VIP152 in patient-derived tissues. Treatment-naïve patients are indicated in grey and platinum-resistant in blue. **(C)** Myc protein expression in cancerous tissues from three patients with ovarian cancer and one with endometrial cancer as determined by immunofluorescence before and 2, 4, and 16 h after 500 nM VIP152 treatment.

VIP152 monotherapy inhibits tumor growth in A2780 human ovarian cancer xenograft model in mice

- A single 17.5 mg/kg dose of VIP152 monotherapy resulted in robust tumor growth inhibition (p=0.011) with a treatment/control ratio of 0.21 (day 7) in a cisplatin-sensitive A2780 ovarian cancer xenograft model when compared with vehicle control (**Fig. 4A–B**).
- VIP152 treatment was well-tolerated, as no marked reduction in body weight was observed during the study (**Fig. 4C**).

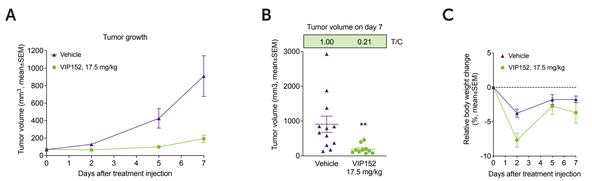

Figure 4. Antitumor efficacy of VIP152 monotherapy in A2780 human ovarian cancer xenograft model in mice. (A) Growth curves of subcutaneous A2780 tumors in mice were treated with a single i.v. injection of vehicle (30% PEG400, 10% ethanol, 60% water) or 17.5 mg/kg VIP152 (n=10–12/group). **(B)** Tumor volumes on day 7 post injection. T/C (treatment/control) ratios were calculated for each group. The statistical analysis was performed using Mann-Whitney test (**, p=0.011). **(C)** Relative body weight change of mice during the treatment period.

VIP152 treatment results in stable disease in four out of seven patients with gynecologic cancers

- Clinical characteristics of seven gynecologic cancer patients participating in the VIP152 Phase 1 trial (NCT02635672) are summarized in **Table 1**.
- Four out of seven patients have achieved stable disease (SD) as their best overall response (BoR) per RECIST 1.1 criteria. Two patients remain on treatment and are pending their second follow up tumor assessment visit.
- Of the ovarian cancer patients, 3 ended treatment due to radiological progression and 1 due to clinical progression without radiological confirmation. The patient with metastatic high grade serous fallopian tube carcinoma ended treatment due to a Grade 1 adverse event of nausea which triggered a voluntary withdrawal from study. Of note, the assessment of SD was performed 22 days after the last VIP152 dose.
- The histology and biomarker profiles were available for 3/4 patients with SD (Foundation One). All three were of serous histology (2 ovarian and 1 fallopian tube), microsatellite stable (MSS), and had *MYC* amplification and TMB scores ranging from 4–9 mutations/Mb.

Table 1. Clinical summary of patients with gynecologic cancers treated with VIP152 to date (DCO March 15 2022).

Baseline information						Treatment		Tumor measurements (RECIST 1.1, mm)		Clinical outcome	
Patient ID	Enrollment	Age, sex	Diagnosis	*MYC* status	Concurrent biomarkers	Prior therapies	VIP152 dose (mg)	Baseline	Follow-up	BoR to VIP152	Current status	EoT reason
***MYC*-aberration required for enrollment**												
1	Vincerx-enrolled	60, F	Ovarian cancer	*MYC* amp	MSS, *TMB6*, *LOH*, *BRCA1*, *PIK3R1/TR3*	carboplatin, paclitaxel, bevacizumab; doxorubicin; olaparib; gemcitabine; cyclophosphamide, doxorubicin liposome	30	24	28	SD	On treatment	-
2	Vincerx-enrolled	74, F	Ovarian cancer	*MYC* amp	MSS, *TMB9* *CCNE1* amp, *NBN* rearrangement intron 8, *NF1* loss, *TP53* mut	carboplatin, paclitaxel; letrozole; taxol; niraparib/placebo study; anastrazole; aromasin; bevacizumab, gemcitabine; avelumab, doxorubicin; topotecan; cyclophosphamide; XMT 1536; APG115, pembrolizumab; ABBV155; NC318; BMS8502; M1774; NBMBMX; ZM-Q-001; CBX-12	30	49	41	SD	On treatment	-
3	Vincerx-enrolled	69, F	Fallopian tube cancer	*MYC* amp	MSS, *TMB4*, *ARID1A* mut, *CDKT2* mut, *MCL1* amp, *MDM4* amp, *PIK3CB* amp, *PIK3CB* amp, *PRKC1* amp, *TERC* amp, *TP53* mut, *VEGFA* amp	carboplatin, paclitaxel; doxorubicin, bevacizumab; gemcitabine; docetaxel	30	40	45	SD	Off treatment	Adverse event (nausea, grade 1)
No biomarker selection, all-comers												
4	Bayer-enrolled	64, F	Ovarian cancer	n/a	n/a	cisplatin, carboplatin, paclitaxel; doxorubicin; ipilimumab, durvalumab	15*	125	1-124 2-150	PD	Off treatment	Radiologic progression
5	Bayer-enrolled	67, F	Ovarian cancer	n/a	n/a	carboplatin, paclitaxel, bevacizumab; doxorubicin; gemcitabine	22.5	n/a	n/a	PD	Off treatment	Radiologic progression
6	Bayer-enrolled	46, F	Ovarian cancer	n/a	n/a	carboplatin, paclitaxel; doxorubicin; bevacizumab	5	47	1-47 2-47	SD	Off treatment	Clinical progression
7	Bayer-enrolled	52, F	Ovarian cancer	n/a	n/a	carboplatin; paclitaxel; gemcitabine, cisplatin, topotecan, bevacizumab; pemetrexed; oral etoposide; PF-06654178	5	159	178	PD	Off treatment	Radiologic progression

30d, 30 days; BoR, best overall response: EoT, end of treatment; F, female; MSS; microsatellite stability; n/a, not available; PD, progressive disease; PR, partial response; SD, stable disease. *, VIP152 dose was reduced from 30 mg to 15 on the 3rd dose

- Neutropenia is an on-target (CDK9) toxicity and is monitorable and manageable with supportive care. Once weekly dosing of VIP152 allows for recovery of neutrophils before the next dose (**Fig. 5**).

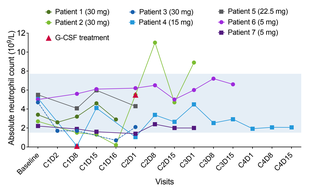

Figure 5. Neutrophil count of patients with gynecologic cancers treated with VIP152. Absolute neutrophil count (ANC, 10^9/L) was recorded for patients at each treatment visit. Light blue area indicates normal range of ANC. Red triangles indicate additional G-CSF treatment given at the specific timepoint.

Downregulation of CDK9-regulated genes *MYC, MCL1,* and *PCNA* is observed in the blood of gynecologic patients treated with VIP152

- VIP152 exhibited linear pharmacokinetics in the five gynecologic cancer patients who had already ended treatment. Clearance and volume of distribution estimates were within the previously reported ranges (9) (**Table 2**).
- The PD effect of VIP152 was determined as changes in RNA expression of three CDK9-regulated target genes (*MYC*, *MCL1* and *PCNA*) in blood after first dose on C1D1.
- In whole blood of five patients, *MYC*, *MCL1* and *PCNA* mRNA levels are modulated with treatment (**Fig. 6**).

Table 2. The PK properties of VIP152 in five patients with gynecologic cancers. The values were determined following VIP152 administration as a 30-min intravenous infusion on C1D1.

Patient ID	VIP152 dose (mg)	C_{max} (µg/L)	AUC_{0-t} (µg•h/L)	AUC_{0-inf} (µg•h/L)	t_{max} (h)	$t_{1/2}$ (h)	CL (L/h)	V_{ss} (L)
3	30	564	2580	2630	0.25	4.23	11.4	61.1
4	30	803	5900	5910	0.583	5.06	5.08	42
5	22.5	868	3060	3060	0.5	5.18	7.35	47.3
6	5	92.1	216	236	0.27	2.16	21.2	56.9
7	5	96.1	347	350	0.6	3.32	14.3	57.2

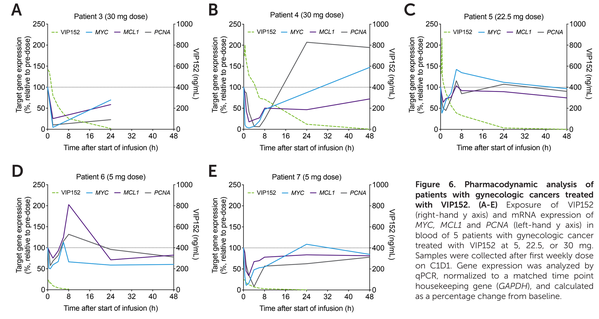

Figure 6. Pharmacodynamic analysis of patients with gynecologic cancers treated with VIP152. (A-E) Exposure of VIP152 (right-hand y axis) and mRNA expression of *MYC*, *MCL1* and *PCNA* (left-hand y axis) in blood of 5 patients with gynecologic cancer treated with VIP152 at 5, 22.5, or 30 mg. Samples were collected after first weekly dose on C1D1. Gene expression was analyzed by qPCR, normalized to a matched time point housekeeping gene (*GAPDH*), and calculated as a percentage change from baseline.

CONCLUSIONS

- VIP152 demonstrates sensitivity in gynecologic cell lines independent of cisplatin sensitivity.
- Tumor mutational burden (TMB) is associated with VIP152 sensitivity in gynecologic cell lines. These findings will be validated in an independent cohort.
- VIP152 induces cellular cytotoxicity and reduces Myc protein expression in patient-derived, platinum-resistant and treatment-naïve ovarian and endometrial cancer tissues *ex vivo*.
- VIP152 shows tumor growth inhibition in the clinically translatable A2780 human ovarian cancer xenograft model *in vivo*.
- In a Phase 1 trial (NCT02635672), four out of seven patients with gynecologic cancers treated with VIP152 have achieved stable disease (SD) as their best overall response. Two of these four patients remain on treatment. Neutropenia is observed and manageable with supportive care and once weekly dosing of VIP152.
- VIP152 has predictable PK properties in gynecologic cancer patients and gene expression modulation is observed (n=5). Intermittent dosing provides modulation of CDK9-regulated oncogenic targets such as *MYC* and *MCL1*.
- These preclinical and preliminary clinical data warrant further evaluation of VIP152 as monotherapy and in combination with other antineoplastic agents in gynecologic cancers.

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REFERENCES

1. Siegel *et al. CA Cancer J Clin*. 2021;71(1):7-33.
2. Bowtell *et al. Nat Rev Cancer*. 2015;15(11):668-79.
3. Cancer Genome Atlas Research Network. *Nature*. 2011;474(7353):609-15.
4. Levine and Cancer Genome Atlas Research Network. *Nature*. 2013;497(7447):67-73.
5. Lücking *et al. J Med Chem*. 2021;64(15):11651-74.
6. Love *et al. Genome Biol*. 2014;15(12):550.
7. Broad. DepMap 21Q3 Public 2021. doi: 10.6084/m9.figshare.15160110.v2.
8. McLaren *et al. Genome Biol*. 2016;17(1):122.
9. Diamond *et al. Clin Cancer Res*. Jan 19 2022;doi: 10.1158/1078-0432.CCR-21-3617.

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