VIP152, a selective CDK9 inhibitor, induces complete regression of high-grade B-cell lymphoma (HGBL) models and depletion of short-lived oncogenic driver transcripts, *MYC* and *MCL1*, with a once weekly schedule

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INTRODUCTION

- High-grade B-cell lymphoma (HGBL), previously known as double-hit lymphoma (DHL), is an aggressive type of B-cell non-Hodgkin lymphoma. HGBL is refractory to standard of care chemotherapy and characterized by rearrangements in *MYC* and *BCL2* or *BCL6*, which drive oncogenic transcription and anti-apoptotic signaling, respectively.
- VIP152 is a well-tolerated and clinically active cyclin-dependent kinase 9 (CDK9) inhibitor that led to complete metabolic remission in 2/7 HGBL patients treated with once weekly (QW) 30 mg intravenous doses.[1,2]
- The carboxy-terminal domain (CTD) serine 2 residue of RNA polymerase II (p-Ser2) is the direct substrate and phosphorylation site of CDK9, allowing transcription elongation and the generation of mRNA (**Fig. 1**).
- *MYC* is consequently dependent on CDK9 phosphorylation of p-Ser2, making it the key biomarker for evaluating the mechanism of action of CDK9 inhibitors.
- Herein, we demonstrate that an *in vitro* dose of VIP152, equivalent to once weekly inhibition of CDK9, induces an "oncogenic shock"-like disruption of *MYC* transcription[3] and other short half-life mRNA gene transcripts.
- Atuveciclib and KB-0742 are both administered orally while VIP152 is intravenous. The pharmacodynamic profiles of routes of administration are compared in this study.
- VIP152 treatment enables durable control of oncogenic protein levels without continuous dosing, resulting in cancer cell apoptosis and antitumor efficacy (**Fig. 1**).

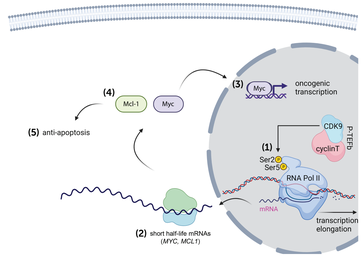

Figure 1. The mechanism of action of the CDK9 inhibitor VIP152. (**1**) Inhibition of CDK9 phosphorylation site Ser2 at RNA polymerase II (RNA Pol II). (**2**) Reduction of short half-life mRNAs *MYC* and *MCL1* regulated by RNA Pol II and (**3**) Myc-dependent genes. (**4**) Control of oncogenic proteins Myc and Mcl-1 delivering "oncogenic shock" without daily dosing. (**5**) Induction of apoptosis markers.

METHODS

- The pharmacodynamic (PD) extent and duration of CDK9 pathway inhibition *in vitro* was evaluated with human diffuse large B-cell lymphoma (DLBCL) cell lines SU-DHL-4 and SU-DHL-10, harboring *MYC* amplification and *MYC* overexpression with P57T mutation, respectively.
  - Cells were treated with three CDK9 inhibitors VIP152, atuveciclib, and KB-0742 using a 4-hour pulse and washout time course (**Fig. 2**).
  - VIP152-induced differential gene expression was analyzed by RNAseq, and short half-life gene transcripts *MYC* and *MCL1* were confirmed by qPCR (Discovery Life Science).
  - PD biomarkers including p-Ser2 RNA polymerase II, Myc, Mcl-1, and cleaved PARP (cPARP) were assessed by Western blot, quantified by near-infrared Western blot detection with background correction of fluorescence intensity using Odyssey Imaging System (LI-COR Biosciences).
- The antitumor efficacy of VIP152 was evaluated in the SU-DHL-10 *in vivo* xenograft mouse model.
  - 10x10^6 SU-DHL-10 cells were inoculated subcutaneously into 6–8-week-old female CB-17 SCID mice.
  - Mice were randomized to treatment groups at a tumor volume of 63–104 mm^3 and treated with vehicle or VIP152 at 10 and 15 mg/kg (i.v., QW).
  - All animal experiments were performed under the national animal welfare laws of Germany and approved by the local authorities.
- Seven HGBL patients treated with VIP152 (30 mg, i.v., QWx3) had blood samples collected to perform the following studies:
  - Longitudinal levels of short half-life transcripts and VIP152 plasma concentrations were measured using qPCR and LC/MS-MS, respectively.
  - The clinical study protocol was approved by the institutional review board of the participating institutions and complied with the Declaration of Helsinki, current Good Clinical Practice guidelines, and local laws and regulations. Written informed consent was provided by all participants prior to the initiation of any study-specific procedure. This study is ongoing and is sponsored by Vincerx Pharma, Inc. (ClinicalTrials.gov Identifier: NCT02635672).

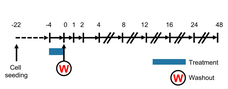

Figure 2. Timeline of the *in vitro* washout experiment. SU-DHL-4 and SU-DHL-10 DLBCL cells were seeded 18 hours prior to treatment with one of three CDK9 inhibitors: VIP152 (0.25 or 1 μM), atuveciclib (1 μM), or KB-0742 (1 μM). Following the 4-hour treatment, the cells were washed and the incubation continued for 48 hours. The samples were collected 4 hours before treatment (-4), immediately after treatment (0), and at 1, 2, 4, 8, 12, 16, 24 and 48 hours after washout for RNAseq, qPCR, and Western blotting analyses.

RESULTS

VIP152 delivers the most robust inhibition of RNA polymerase II Ser2 phosphorylation for up to 48 hours

- RNAseq analysis demonstrated that the two DLBCL cell lines used in this study, SU-DHL-4 and SU-DHL-10, have comparable *MYC* transcript levels, despite their genetic differences (*MYC* gene amplification vs. *MYC* mutation, respectively). The levels of *MCL1* mRNA in SU-DHL-10 cells were almost twice as high compared with the SU-DHL-4 cells (**Fig. 3A**).
- A 4-hour treatment with 0.25 μM VIP152 *in vitro* decreased the levels of p-Ser2 by 50% for 48 hours in the *MYC*-amplified SU-DHL-4 cell line. The extent of p-Ser2 inhibition was greater with the higher 1 μM VIP152 dose and the inhibition was also sustained for 48 post hours (**Fig. 3B-C**).
  - Treatment with KB-0742 elicited a 50% decrease of p-Ser2 for 12 hours, which is in alignment with a previous report using a *MYC*-driven AML xenograft mouse model.[4]
  - Treatment with atuveciclib resulted in a 50% inhibition of p-Ser2 for 16 hours.
- In the SU-DHL-10 cell line, 1 μM VIP152 was the only treatment able to achieve a 50% reduction of p-Ser2 for 24 hours treatment (**Fig. 3D-E**).
  - Atuveciclib and KB-0742 control p-Ser2 in the presence of the treatment compound but not after washout.

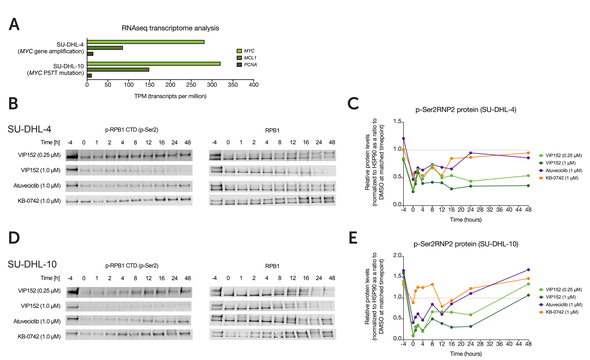

Figure 3. VIP152 delivers the most robust inhibition of RNA polymerase II Ser2 phosphorylation for up to 48 hours. (**A**) The gene expression of *MYC*, *MCL1*, and *PCNA* as determined by RNAseq. Western blots of phosphorylated Ser2 RNA Pol II (p-RPB1 CTD) and quantification of p-Ser2RNP2 (phosphorylated Ser2 RNA Pol II) protein levels in (**B-C**) SU-DHL-4 and (**D-E**) SU-DHL-10 cells. For quantification, the protein levels were normalized to HSP90, expressed as a ratio to DMSO at matched timepoints.

VIP152 treatment confers a robust shift in transcriptional activity in *MYC+* lymphoma cell lines

- According to principal component analysis (PCA), the transcriptional profiles of VIP152 at both doses resembled atuveciclib treatment, whereas the transcriptional profile of KB-0742 was closer in variability to the DMSO treatment in SU-DHL-4 (**Fig. 4A**) and SU-DHL-10 cell lines (**Fig. 4B**).
- Since CDK9 regulates RNA polymerase II transcription elongation, most differentially expressed genes with any CDK9 inhibitor were decreased as seen in volcano plots for SU-DHL-4 (**Fig. 4C**) and SU-DHL-10 (**Fig. 4D**) cell lines.

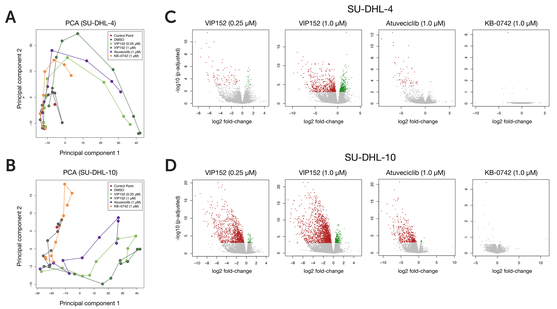

Figure 4. VIP152 treatment confers a robust shift in transcriptional activity in *MYC+* lymphoma cell lines. (**A-B**) PCA of SU-DHL-4 and SU-DHL-10 cell lines. Each timepoint is plotted and lines are drawn to connect treatment conditions to compare the variability by treatment. (**C-D**) Volcano plots plotting the $\log_2$ fold-change and the negative of the $\log_{10}$ of the adjusted p-value. Both $\log_2$ fold-change and adjusted p-value are reported by DESeq2.[5] Red or green points corresponding to genes identified as significantly differentially expressed (p-adjusted<0.001) indicate decreased or increased expression, respectively.

VIP152 depletes oncogenic short half-life mRNA transcripts, *MYC* and *MCL1*, downregulates Myc and Mcl-1 protein levels and activates cell death

- Quantitative PCR studies confirmed that *MYC* and *MCL1* mRNA levels were decreased with all three CDK9 inhibitors initially, however, only atuveciclib and VIP152 were able to drive the depletion of *MYC* and *MCL1* mRNA transcript levels after treatment washout in the SU-DHL-10 (**Fig. 5A**) and SU-DHL-4 cell lines (**Fig. 5B**).
  - In *MYC* overexpressing SU-DHL-10 cells, atuveciclib and KB-0742 were able to maintain ~50% depletion of the *MYC* and *MCL1* mRNA transcripts for 16 hours post treatment, whereas VIP152 inhibited *MYC* and *MCL1* mRNA levels ~75% for 16–48 hours post treatment (**Fig. 5A**).
  - In *MYC*-amplified SU-DHL-4 cells, only 1 μM VIP152 was able to drive durable depletion of *MYC* and *MCL1* mRNA levels over the 48-hour time course (**Fig. 5B**).
- Unlike the other treatments, 1 μM VIP152 delivers sustained Myc and Mcl-1 protein depletion for 48 hours in the SU-DHL-10 (**Fig. 5C**) and SU-DHL-4 cell lines (**Fig. 5D**).
- Maximal cPARP induction in SU-DHL-10 cells was observed 24 hours post treatment washout. In VIP152 and atuveciclib-treated cells, PARP cleavage was 3–5-fold higher at peak in comparison to KB-0742 (**Fig. 5E**).

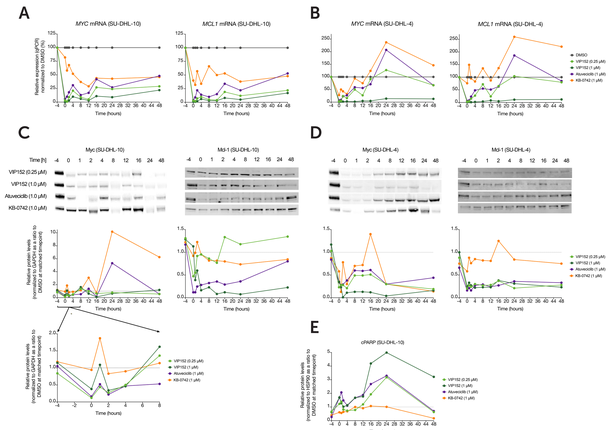

Figure 5. VIP152 depletes oncogenic short half-life mRNA transcripts, *MYC and MCL1*, downregulates Myc and Mcl-1 protein levels and activates cell death. *MYC* and *MCL1* mRNA levels were determined in (**A**) SU-DHL-10 and (**B**) SU-DHL-4 cells using qPCR. The protein levels of Myc and Mcl-1 were quantified in (**C**) SU-DHL-10 and (**D**) SU-DHL-4 cells by near-infrared Western blot detection and background corrected quantification of fluorescence intensity. The inset (lower left corner) shows Myc protein levels in SU-DHL-10 cells until 8 hours post treatment. (**E**) The protein levels of cPARP were quantified in SU-DHL-10 cells. For quantification, the protein levels were normalized to GAPDH or HSP90, expressed as a ratio to DMSO at matched timepoints.

VIP152 treatment results in complete regression of *MYC* overexpressing SU-DHL-10 lymphoma growth *in vivo*

- A dose-dependent antitumor efficacy was observed in the SU-DHL-10 xenograft model, resulting in tumor growth control at 10 mg/kg VIP152 and tumor regressions at 15 mg/kg VIP152 during the 3-week treatment period (**Fig. 6A-B**). All treatments were well tolerated.
- After 3 weeks of treatments, tumors in mice treated with 15 mg/kg VIP152 were allowed to regrow until a new treatment cycle with VIP152 (15 mg/kg, QW) was introduced on study day 29, leading to regression of tumors (**Fig. 6A**).
- The antitumor effect of VIP152 *in vivo* was reflected by the observation that in SU-DHL-10 cells cPARP was induced as soon as 8 hours post treatment *in vitro* (**Fig. 5E**).

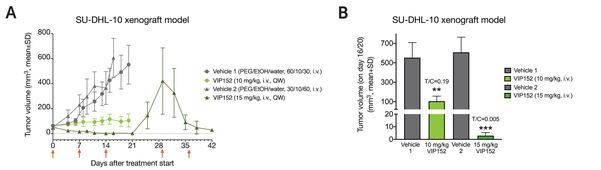

Figure 6. VIP152 treatment results in complete regression of *MYC* overexpressing SU-DHL-10 lymphoma growth *in vivo*. (**A**) Growth curves and (**B**) tumor volumes of SU-DHL-10 tumors treated with either vehicle (30–60% PEG400, 10% ethanol (EtOH) water for infusion) or VIP152 at 10 mg/kg or 15 mg/kg (QW, i.v., n=12/group) for 3 weeks. On days 16 and 20, the T/C (treatment/control) ratios for 10 mg/kg and 15 mg/kg VIP152 were 0.19 and 0.005, respectively. Arrows indicate dosing days. Statistics were performed using unpaired *t*-test in comparison with the corresponding vehicle treatment: **, p=0.011; ***, p<0.001.

The pharmacodynamic effect of VIP152 is detected in the whole blood (tumor surrogate) of VIP152-treated HGBL patients

- In blood of VIP152-treated patients, the maximum extent of inhibition for *MYC*, *MCL1*, and *PCNA* mRNAs was 88–96%, 56–76%, and 78–92%, respectively. The maximal inhibition of all mRNAs occurred within 4 hours of VIP152 treatment (**Fig. 7A**).
- The modeled maximal inhibition of *MYC* and *PCNA* mRNA control by VIP152 was 98% and 88%, respectively, in seven HGBL patients (**Fig. 7B**), whereas for *MCL1*, the modeled maximal inhibition was 72% (**Fig. 7B**).

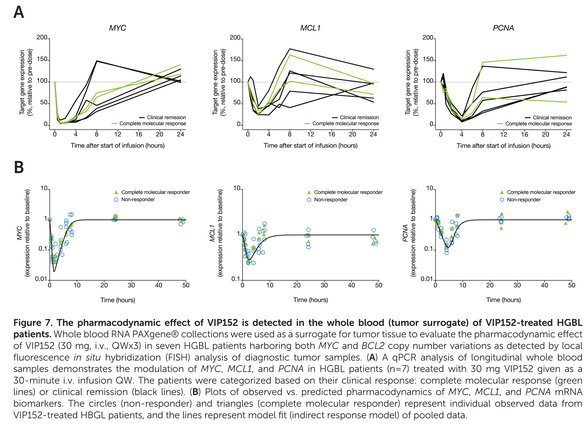

Figure 7. The pharmacodynamic effect of VIP152 is detected in the whole blood (tumor surrogate) of VIP152-treated HGBL patients. Whole blood RNA PAXgene® collections were used as a surrogate for tumor tissue to evaluate the pharmacodynamic effect of VIP152 (30 mg, i.v., QWx3) in seven HGBL patients harboring both *MYC* and *BCL2* copy number variations as detected by local fluorescence *in situ* hybridization (FISH) analysis of diagnostic tumor samples. (**A**) A qPCR analysis of longitudinal whole blood samples demonstrates the modulation of *MYC*, *MCL1*, and *PCNA* in HGBL patients (n=7) treated with 30 mg VIP152 given as a 30-minute i.v. infusion QW. The patients were categorized based on their clinical response: complete molecular response (green lines) or clinical remission (black lines). (**B**) Plots of observed vs. predicted pharmacodynamics of *MYC*, *MCL1*, and *PCNA* mRNA biomarkers. The circles (non-responder) and triangles (complete molecular responder) represent individual observed data from VIP152-treated HBGL patients, and the lines represent model fit (indirect response model) of pooled data.

CONCLUSIONS

- Compared with KB-0742 and atuveciclib at equimolar concentrations, VIP152 demonstrated more potent and durable downregulation of p-Ser2 RNA polymerase II (50% reduction for 24–48 hours) and depletion of short half-life *MYC* and *MCL1* transcript levels up to 48 hours.
- VIP152 treatment conferred a shift in transcriptional program, supporting an oncogenic shock mechanism of action, and sustained robust reduction and near clearance of Myc and Mcl-1 proteins in *MYC* expressing lymphoma cell lines.
- Once weekly VIP152 treatment showed antitumor efficacy as demonstrated by dose-dependent tumor regression and tumor-outgrowth control in the SU-DHL-10 xenograft model.
- The pharmacodynamic effect demonstrated in the blood of HGBL patients treated with VIP152 suggests that the effect may translate to the clinic. Tumor-based pharmacodynamic studies are planned to confirm these findings.
- VIP152 is currently being evaluated in HGBL patients and other MYC expressing indications in the clinic (ClinicalTrials.gov Identifiers: NCT02635672 and NCT04978779).

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ACKNOWLEDGMENTS

Dr. Gerhard Siemeister and the technical team at Nuvisan Innovation Campus Berlin (www.nuvisan.com) are acknowledged for performing the *in vitro* cellular investigations. Michael Sykes and Discovery Life Science team are acknowledged for performing the RNAseq and bioinformatic analyses. Aurexel Life Sciences Ltd. (www.aurexel.com) is thanked for editorial assistance in the preparation of this poster. These activities were funded by Vincerx Pharma.

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Presented at the ASH Meeting 2021, December 11-14, 2021