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TITLE: Identifying Novel Therapeutic Targets and Combination Strategies for Patients with BPDCN

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Project 1: We measured characteristics of leukemia cells before and after treatment tagraxofusp (SL401) and determined that diphthamide pathway genes are silenced after tagraxofusp exposure. We also measured up-front markers of leukemia cell sensitivity to tagraxofusp and found that CD123 level correlates with baseline sensitivity but ADP-ribosylation activity of diphthamide on eEF2 by TAG does not. This suggests that diphthamide activity mediates resistance but is not a biomarker of baseline sensitivity in untreated leukemias. We also developed a BPDCN cell system for CRISPR/deadCas9 interference and performed a genome-wide CRISPRi screen for genes that affect sensitivity and resistance to tagraxofusp or venetoclax plus azacitidine.

In Project 2, we have demonstrated synergistic activity combining anti-CD123 targeting agents (IMGN632 or SL401) with venetoclax and with VENetoclax and AZAcitidine, in BPDCN and AML cell lines. Mechanistic studies focusing on IMGN632/venetoclax combination have shown upregulation of apoptotic markers, profound cell cycle arrest and induction of DNA damage, which were reduced upon p53 silencing. IMGN632/venetoclax combination eradicated BPDCN in a PDX BPDCN model.

We have determined the recommended phase 2 dose (RP2D) for the TAG/AZA/VEN triplet in AML/MDS trial and have recently received IRB and HRPO approval for amendment to add BPDCN cohort. We plan to initiate BPDCN enrollment in year 2 of funding period.

Project 3: We determined that ZRSR2 mutations in BPDCN cells promote evasion of apoptosis by missplicing of genes in the toll-like receptor pathway, particularly the interferon response gene IRF7. This renders BPDCN cells hypoactive compared to normal pDCs in the setting of inflammation and protects them from activation-induced cell death. We have also made progress in identifying the impact of ZRSR2 mutant induced mis-splicing on immune response and generation of potentially immunogenic proteins that could serve as exciting potential immunotherapy targets.

15. SUBJECT TERMS					
Blastic Plasmacytoid Dendritic Cell Neoplasm (PBDCN), Acute Myeloid Leukemia (AML), CD123, tagraxofusp, IMGN632, venetoclax, azacytidine, combination therapy,					
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1. INTRODUCTION:

BPDCN is a highly aggressive hematologic malignancy characterized by poor clinical outcomes and no approved or standard therapies. It can evolve out of an underlying myelodysplastic syndrome or present de novo. The median age is ~65 years with overall survival of 8-14 months using conventional chemotherapy. Thus, there is an urgent unmet need for rational targeted agents and non-cytotoxic therapies. CD123 (the IL3 receptor) is highly expressed in ~100% of BPDCN cases, generally at higher levels than normal cells or forms of acute myeloid leukemia (AML). This observation led to a multi-center Phase I/II trial of SL-401 (Tagraxofusp) in BPDCN, which our sites led and recent FDA approval of tagraxofusp (Elzonris^{TM}, Stemline Therapeutics, Inc.), a CD123-directed cytotoxin. Among 45 patients, we observed a 72% combined rate of complete response (CR)/clinical complete response in frontline-treated patients and 38% in relapsed patients; 45% of frontline patients were bridged to stem cell transplant.[4] However, many patients experience relapse after initial response to tagraxofusp, and ~30% of patients do not respond to tagraxofusp monotherapy up-front. Preliminary studies indicate that BPDCN is dependent on BCL-2. In BPDCN cell lines, patient samples, patient-derived xenograft (PDX) models, and a two-patient pilot study, we observed a high sensitivity to the BCL-2 inhibitor venetoclax. Since then, there have been subsequent reports of clinical activity against BPDCN with venetoclax as a single agent, or in combination with hypomethylating agents. Although we have observed activity of anti-CD123 and anti-BCL-2 targeted agents as *monotherapies*, relapses still occur in most cases. **This project aims to** investigate mechanisms of response and resistance to the most advanced targeted therapies in BPDCN, assess potential for combination treatment in a clinical trial, and explore novel mechanism-based therapies targeting recurrently mutated genes in BPDCN involved in RNA splicing. **We expect our aims to provide clinical data for the next front-line treatment strategy for BPDCN patients and the preclinical basis to intelligently select the next targeted therapy approach for clinical testing in BPDCN**.

2. KEYWORDS:

Blastic Plasmacytoid Dendritic Cell Neoplasm (PBDCN), Acute Myeloid Leukemia (AML), CD123, tagraxofusp, IMGN632, venetoclax, azacytidine, combination therapy

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Specific Aim 1

Major Task 1: Test the hypothesis that in vivo resistance to tagraxofusp in BPDCN cells is mediated by epigenetic silencing of diphthamide synthesis genes.

Major Task 2: Test the hypothesis that azacitidine cooperates with tagraxofusp in primary BPDCN cells in vivo via its effects on DNA methylation and expression of diphthamide synthesis genes.

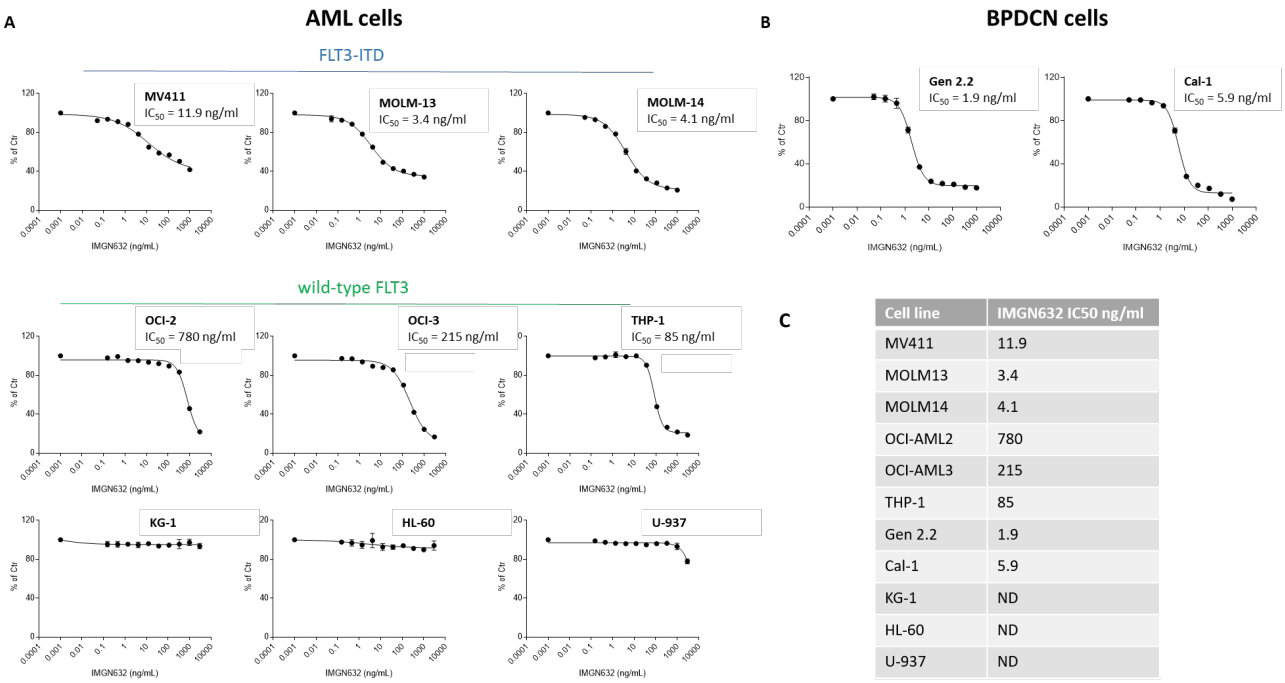
Specific Aim 2
Major Task 1 Test the hypothesis that targeting of CD123 with tagraxofusp or IMGN-623 primes BPDCN cells for apoptosis when combined with BCL2 inhibitor venetoclax and AZA.
Major Task 2 Determine the efficacy of combining anti-CD123 therapy with AZA/venetoclax in vivo
Major Task 3 Conduct a Phase 1b/2 clinical trial combining azacitidine, tagraxofusp, and venetoclax in patients with relapsed/refractory BPDCN and frontline patients unfit for induction chemotherapy

Specific Aim 3
Major Task 1: Determine the effects of perturbing splicing in BPDCN models with or without mutations in *ZRSR2* or *SRSF2*.
Major Task 2: Determine aberrant splicing events generated by mutant *ZRSR2* and *SRSF2* in BPDCN which promote transformation through a positive enrichment CRISPR screen.

What was accomplished under these goals?

MDACC (Konopleva)
Specific Aim 2, Major Task 1: Test the hypothesis that targeting of CD123 with tagraxofusp or IMGN-623 primes BPDCN cells for apoptosis when combined with BCL2 inhibitor venetoclax and AZA.

First, we tested the single agent IMGN632 activity in BPDCN and AML cell lines and correlated cytotoxic effect with the expression of CD123 receptor. Cells were treated with IMGN623 for 48 h and cell viability was analyzed by CTG assay. As shown in **Fig. 1A**, AML cells expressing mutated FLT3-ITD kinase were particularly sensitive to IMGN632 with IC_{50} values placing at low ng/ml range. IMGN632 was highly active also in BPDCN Gen 2.2 and Cal-1 cells (**Fig. 1B**). In contrast, the response of AML cell lines with wild-type FLT3 was significantly lower with IC_{50} values at sub μg/ml level. Three tested AML cell lines, KG-1, HL-60 and U-937 were not responding to IMGN632 treatment.



Cell line	IMGN632 IC50 ng/ml
MV411	11.9
MOLM13	3.4
MOLM14	4.1
OCI-AML2	780
OCI-AML3	215
THP-1	85
Gen 2.2	1.9
Cal-1	5.9
KG-1	ND
HL-60	ND
U-937	ND

Figure 1. The effect of IMGN632 on viability of AML (A) and BPDCN (B) cells. (C) IMGN632 IC_{50} values determined after 48 h exposure.

Next, we examined the expression of CD123 receptor in AML and BPDCN cells. Cells were stained with anti-CD123-PE antibody and the number of CD123 molecules/cell was determined using PE Quantibrite Beads. As shown in **Fig. 2**, the expression of CD123 in tested cell lines was diverse. FLT3-ITD AML and BPDCN cells had high level of CD123 receptor. In AML cells with wild-type FLT3, CD123 expression was significantly lower. Importantly, Pearson's linear correlation analysis revealed that the activity of IMGN632 was significantly negatively correlated with the number of CD123 molecules in cell (**Fig. 3**). Lower IC_{50} values of IMGN632 were obtained for cells with higher expression of CD123, confirming IMGN632 on target activity.

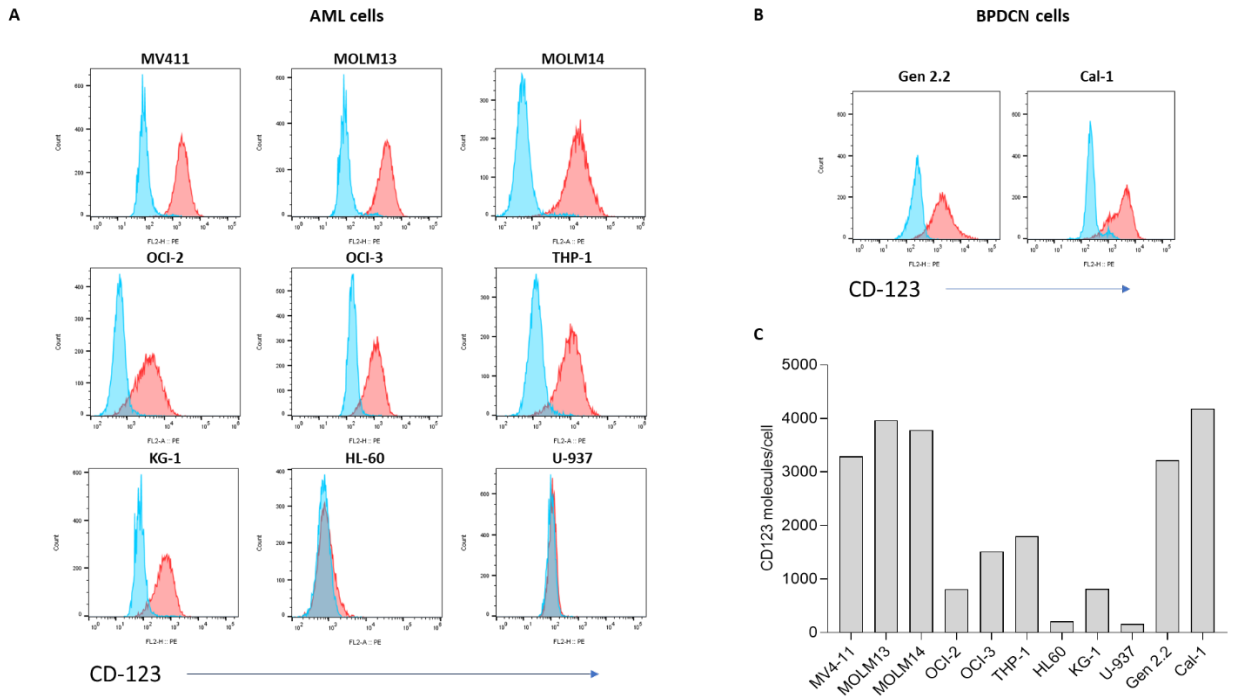


Figure 2. Expression of CD123 receptor in (A) AML and (B) BPDCN cell lines. Cells were stained with IgG-PE or CD123-PE antibodies and analyzed using flow cytometry. The number of CD123 molecules/ cell (C) was calculated based on CD123-PE MFI values in relation to MFI of PE Quantibrite Beads.

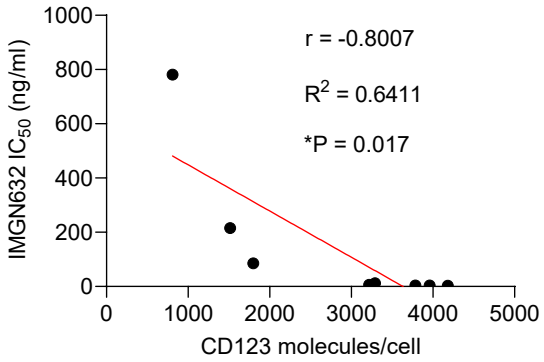


Figure 3. Correlation between expression of CD123 and IC_{50} values of IMGN623. Pearson's correlation coefficient (r) and p-value (p) were determined for AML and BPDCN cell lines. Each point represents one cell line.

Next, we tested the efficacy of IMGN632 or SL401 in combination with VENetoclax and AZAcitidine. The BPDCN cell line CAL-1 was first pre-treated with IMGN623 or SL-401 for 24 h, followed by additional 24 h exposure to VEN/AZA. Pharmacological interactions were determined by the combination index (CI) where values <1.0, =1 and >1 indicate synergism, additive, and antagonistic effects, respectively. After 24 h of triple-drug exposure, cell viability was analyzed by CTG assay. The results indicate synergistic reduction in viability of CAL-1 cells upon sequential exposure, with CI of 0.29 and 0.75 (**Fig. 4A, B**). Data were confirmed by Annexin V/PI/beads flow cytometry (**Fig. 4C**).

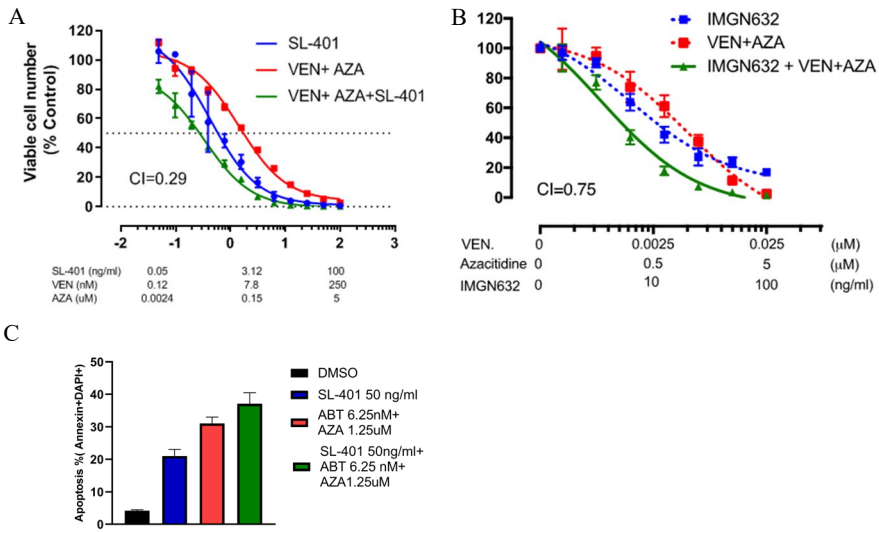


Figure 4. Combined efficacy of SL-401 and IMGN632 with AZA/Ven in CAL-1 cells. CAL-1 cells were pre-incubated with SL-401 (left) or IMGN632 (right) for 24 h followed by 24 h exposure to VEN/AZA. **A-B.** After 24 h of triple-drug exposure, cell viability was analyzed by CTG assay. CI, Combination Index (CI) indicative of synergy (<1). Data are representative of 3 experiments. **C.** Cells were stained with Annexin V-FITC and DAPI to determine the degree of apoptosis by flow cytometry.

We have further tested triple combination also in AML cell lines and confirmed the synergy (**Fig. 5**).

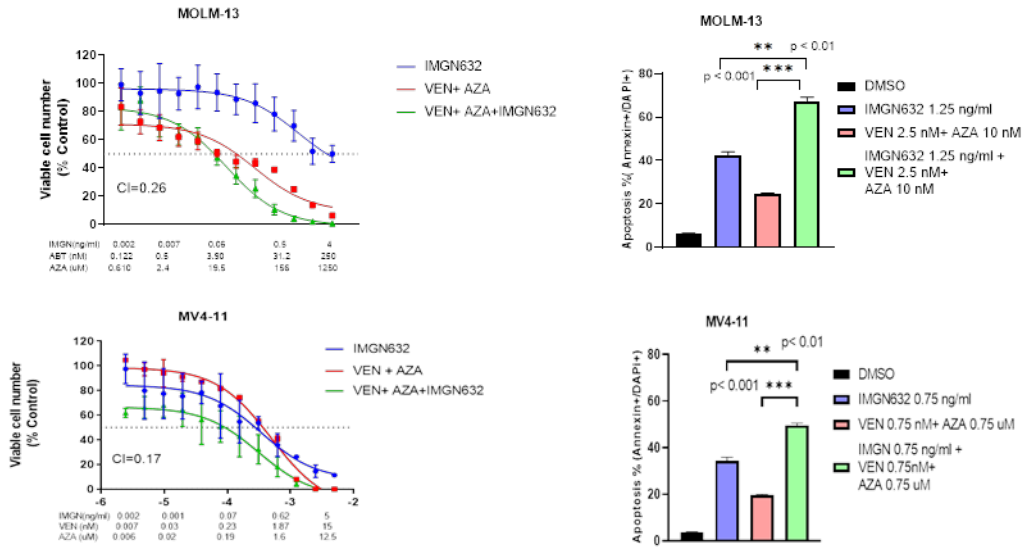


Figure 5. Combined efficacy of IMGN632 with AZA/Ven in AML cells.
We tested the cytotoxic efficacy of IMGN632 alone, VEN+AZA and the triple combination of IMGN632+VEN+AZA in AML cells lines. Cells were treated with IMGN632 for 48 h followed by VEN+AZA for 24h. Treatment with the triple combination induced greater apoptosis and reduced viable cell numbers when compared to IMGN632 alone or VEN+AZA, with the triple combination demonstrating synergism with CI value of 0.26 in MOLM13 cells and 0.17 in MV4-11 cells.

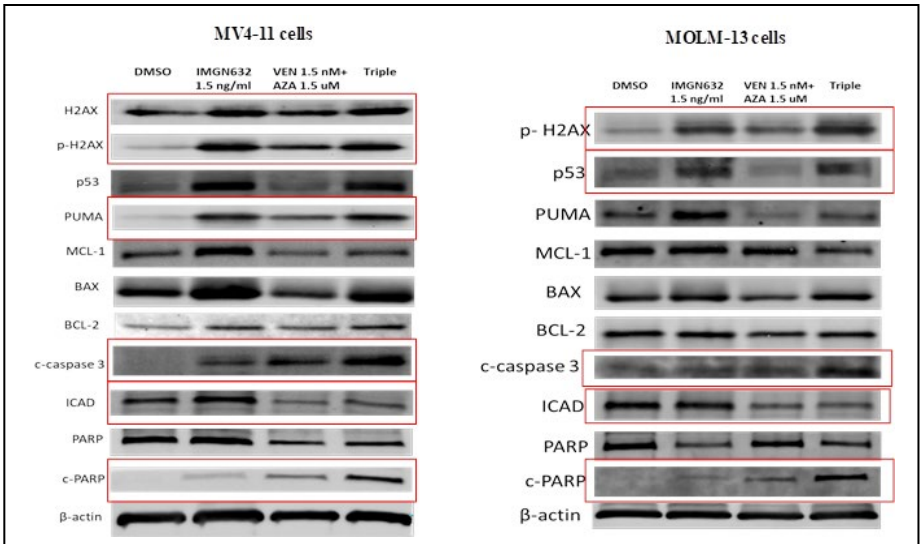


Figure 6. MV4-11 and MOLM13 cells were treated with IMGN632 for 48h followed by VEN+AZA for 24h. Whole cell lysates were collected and analyzed by western blotting to measure the expression of pro-apoptotic proteins and DNA damage markers.

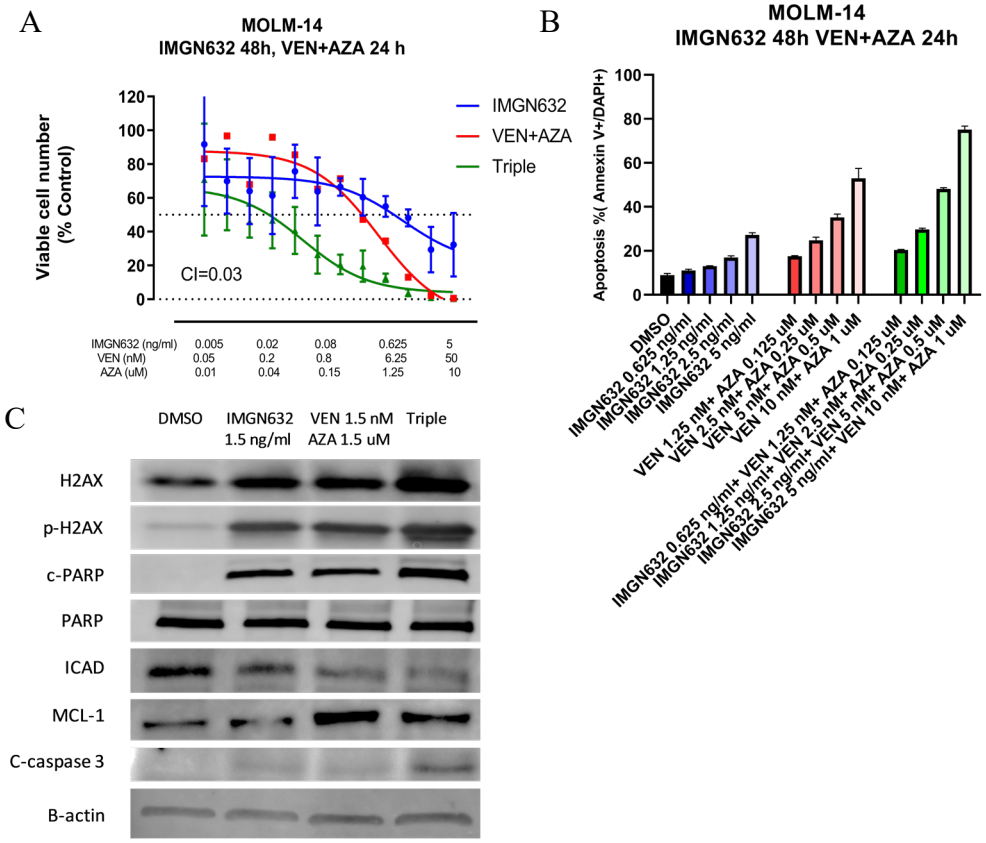


Figure 7. Combined efficacy of IMGN632 with AZA/Ven in AML cells. MOLM-14 cells were pre-incubated with IMGN632 for 24 h followed by co-incubation with VEN+AZA for additional 24 h. **A.** Cell viability was analyzed by CTG assay. CI, Combination Index (CI) indicative of synergy (<1). Data are representative of 3 experiments. **B.** Cells were stained with Annexin V-FITC and DAPI to determine the degree of apoptosis by flow cytometry. **C.** Western blot analysis of apoptotic and DNA damage markers.

Western blot analysis of the MV4-11 cells and MOLM13 cells showed that the triple combination upregulated pro-apoptotic proteins such PUMA and BAX and reduced anti-apoptotic MCL-1 expression. The triple combination also induced DNA damage as evidenced by increase in phosphorylated form of gamma-h2AX and decreased expression of ICAD. These effects were accompanied by activation of caspase-3 and cleavage of PARP, both indicative of apoptosis **(Fig. 6).** A strong synergistic activity was also observed for additional AML cell line, MOLM14. Reduction in viability of MOLM14 cells, with CI of 0.03 was accompanied by apoptosis (**Fig. 7 A, B**). Like in MV-411 and MOLM13 cells, triple combination synergized to activate caspase-3, leading to profound PARP cleavage. Importantly, this effect was observed despite elevated levels of ant-apoptotic MCL-1 protein (**Fig. 7 C**).

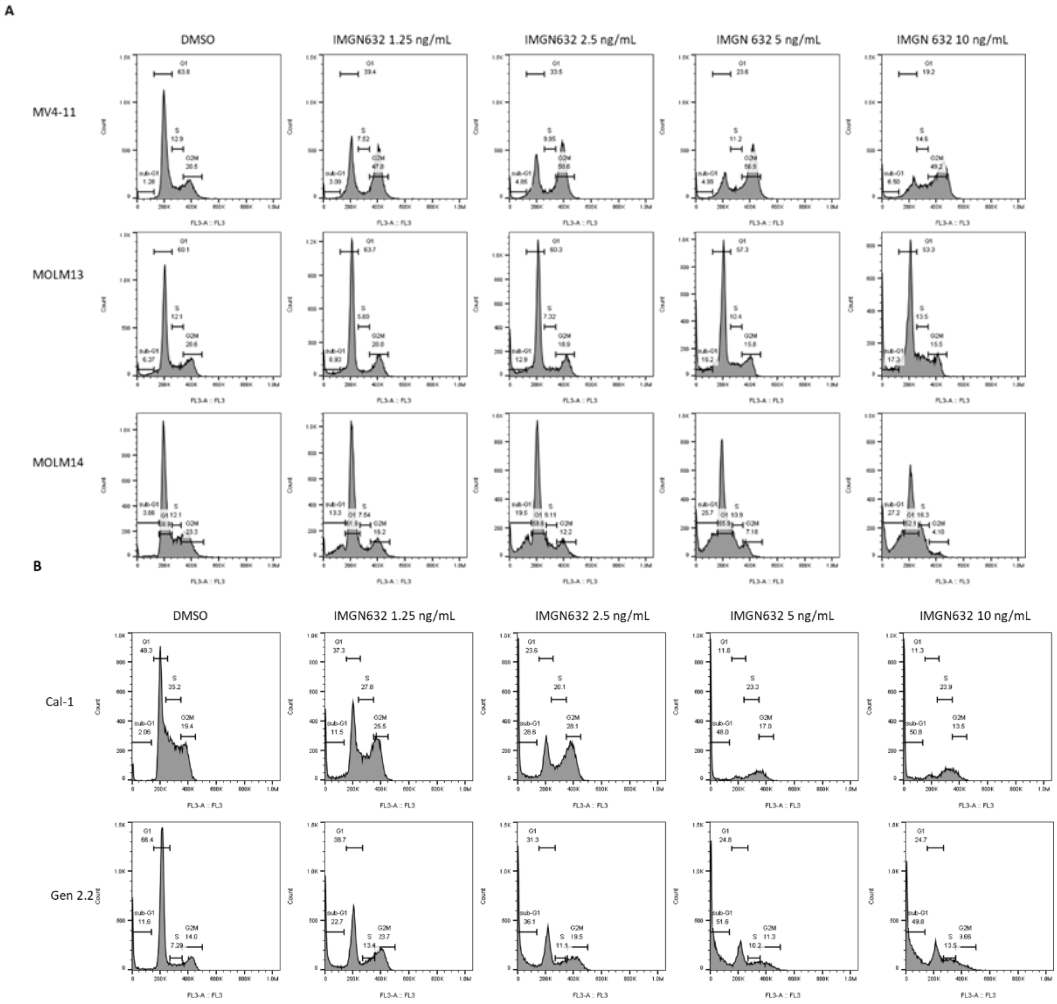


Figure 8. The effect of IMGN632 on the cell cycle of (A) AML and (B) BPDCN cells after 48 h exposure.

Western blot analysis revealed that IMGN632 alone, as well as in combination with VEN/AZA, induced profound DNA damage (evidenced by increase in phosphorylated gamma-H2AX). Thus next, we examined how IMGN632-induced DNA damage affected cell cycle of selected AML and BPDCN cells. Cells were treated with different doses of IMGN632 for 48 h,

stained with propidium iodide and analyzed by flow cytometry. As shown in **Fig. 8**, low doses of IMGN632 induced G2/M arrest in MV411 AML cells as well as in Cal-1 and Gen 2.2 BPDCN cells. MOLM13 and MOLM14 AML cells accumulated in the G1 phase. Higher doses of the drug led to apoptosis. Next, we analyzed the effect of IMGN632/VEN/AZA combination on the cell cycle of MV411 AML cells. Cells were pre-treated with IMGN632 for 48h and VEN/AZA was added at the last 24 h. As shown in **Fig. 9**, IMGN632-induced G2/M arrest was sustained in the presence of VEN/AZA combination. VEN and AZA alone did not affect cell cycle progression. Together, these results suggest that although IMGN632/VEN/AZA combination induces profound cell cycle arrest, this effect is driven mainly by IMGN632 activity.

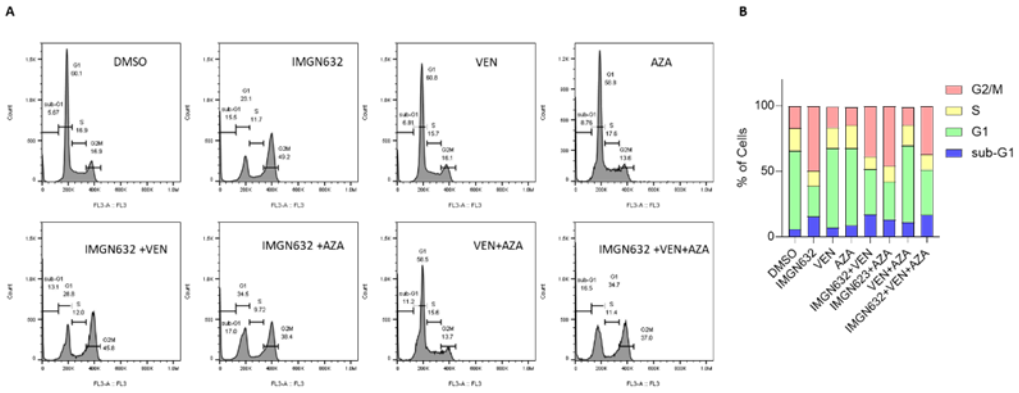


Figure 9. The effect of IMGN632/VEN/AZA combination on the cell cycle of MV411 AML cells.

Western blot analysis showed that IMGN623-induced DNA damage was accompanied by stabilization of p53. Given the involvement of p53 in apoptotic response upon DNA damage, next we examined whether p53 may play functional role in AML response to IMGN632/VEN/AZA combination. MOLM13 cells with wild-type p53 and MOLM13 cells transfected with lentivirus carrying shRNA specific to knockdown p53 were pre-treated with IMGN632 for 24 h followed by co-treatment with VEN/AZA for additional 24 h. CTG assay revealed that the effect of triple combination on cell viability was suppressed in MOLM13 p53 KD cells in comparison to their wild-type p53 counterparts (**Fig. 10 A**). Similarly, loss of p53 profoundly reduced the potential of triple combination to induce apoptosis (**Fig. 10 B**). Together, these results suggest that the synergistic effect of IMGN632/VEN/AZA combination may depend on functional p53.

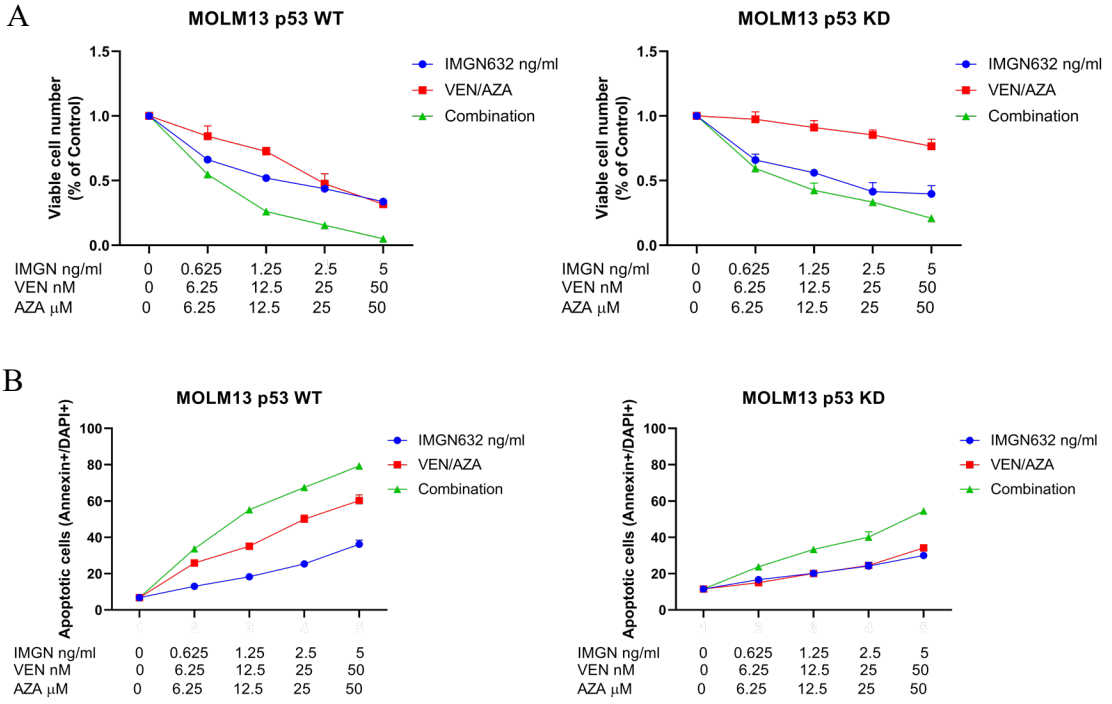


Figure 10. Combined efficacy of IMGN632 with AZA/Ven is suppressed in p53 knock-down AML cells. MOLM13 p53 wild-type and MOLM13 p53 knock-down cells were treated with IMGN632 for 24 h followed by additional co-incubation with VEN+AZA for 24h. **A.** Cell viability was determined by CTG assay. **B.** Level of apoptotic cells was measured by Annexin V-FITC/DAPI staining followed by flow cytometry.

To further understand the role of p53 and the mechanistic pathway of apoptosis in p53 knockdown and wild-type MOLM13 cells, we examined the expression of DNA damage markers such as p-H2AX and ICAD and apoptotic markers, cleaved PARP and cleaved caspase-3. Preliminary western blot analysis revealed increased expression of p-H2AX and downregulation of ICAD in both p53 wild-type and p53 KD MOLM13 cells exposed to triple combination (**Fig. 11**), suggesting that these effects were independent of p53 status. Consistent with flow cytometry analysis of Annexin V-positive apoptotic cells, the induction of apoptosis by IMGN632/VEN/AZA combination was less pronounced in p53 KD cells, as evidenced by decreased ratio of cleaved PARP to the level of full length PARP. Importantly, in comparison to p53 wild-type cells, p53 KD cells expressed significantly higher level of anti-apoptotic MCL-1, which was not affected by either IMGN632 and VEN/AZA alone, or by triple combination. In addition, p53 KD cells had also decreased expression of pro-apoptotic BAX. Together, these results suggest that the decreased efficacy of IMGN632/VEN/AZA combination in cells lacking functional p53 may result from an inability to fully execute apoptosis due to compensatory upregulation of MCL-1 and/or decrease of BAX.

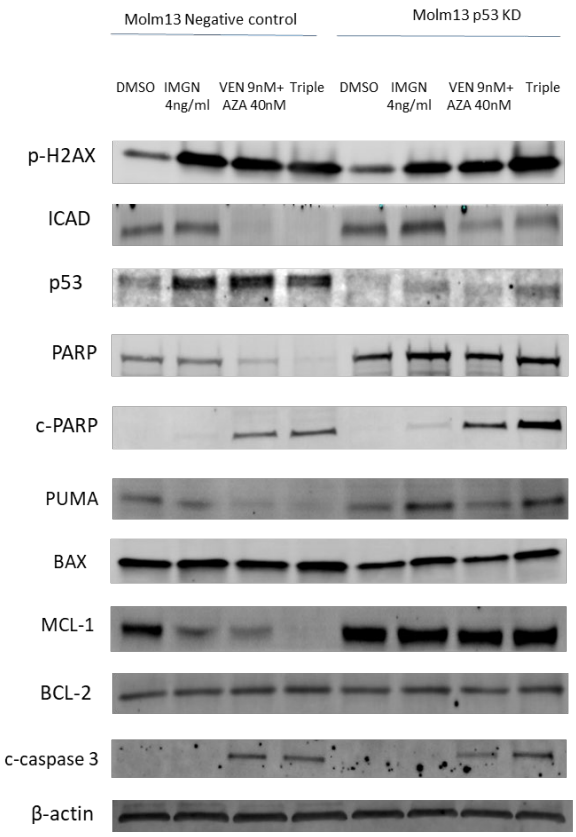


Figure 11. MOLM13 wild-type p53 and MOLM13 p53 KD cells were treated with IMGN632 for 24h followed by additional co-incubation with VEN+AZA for 24h. Whole cell lysates were collected and analyzed by western blotting to measure the expression of pro-apoptotic proteins and DNA damage markers.

(Konopleva)

Specific Aim 2, Major Task 2: Determine the efficacy of combining anti-CD123 therapy with AZA/venetoclax in vivo.

Here, we examined anti-leukemic efficacy of IMGN632, alone or in combination with VEN, *in vivo* in a BPDCN PDX from a relapsed patient. We observed more than doubling of survival in mice co-treated with low dose IMGN632 and VEN (**Fig. 12**; a 10-times higher dose of IMGN632 alone was curative). These findings demonstrate cooperative anti-leukemia efficacy of co-targeting BCL-2 and CD123.

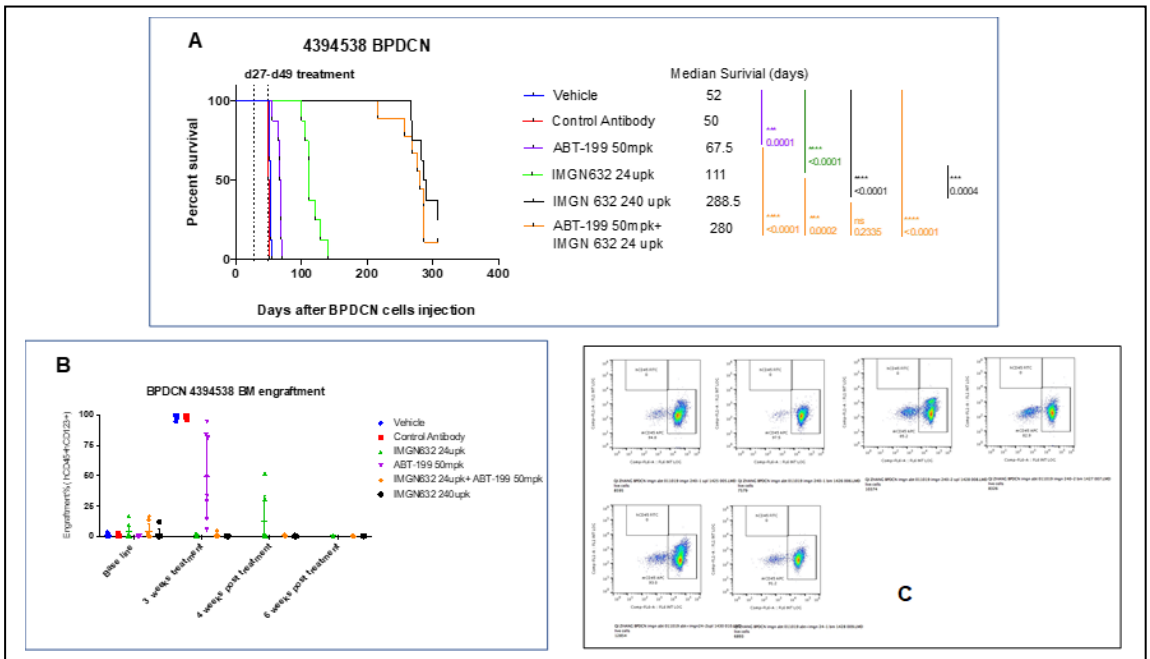


Figure 12. IMGN632 + ABT-199 in BPDCN PDX model. NSG mice injected with BPDCN PDX 24 h post sub-lethal irradiation. After 27 days, bone marrow (BM) engraftment was confirmed by flow cytometry ($hCD45^+/hCD123^+$), and mice randomized to receive Vehicle; VEN (50mg/kg PO QD); control antibody 240µg/kg IV weekly x 3; IMGN632 24µg/kg IV weekly x 3; and combination of VEN with IMGN632. **A,** survival, **B-**BM tumor burden at the end of therapy (3 weeks). **C:** No engraftment in bone marrow and spleen collected from mice received the IMGN632 240ug/kg and the ABT-199+ IMGN632 at 24ug/kg.

Next, we have performed *in vivo* "triple" combination studies of IMGN632/VEN/AZA in 2 AML PDX models refractory to VEN+AZA (**Fig. 13 A, B**). In both models, the triple combination exceeded efficacy of IMGN632 monotherapy indicating synergy and potential to overcome VEN+AZA resistance with the IMGN632/VEN/AZA combination. Triple combination was also efficacious against PDX model of AML sensitive to VEN and AZA (**Fig. 13 C**). Consistent with in vitro results, western blot analysis of enriched hCD45+ AML cells from the spleen of mice showed an upregulation of apoptotic proteins, cleaved-PARP and cleaved caspase-3, and upregulation of the DNA damage marker p-H2AX in the triple combination group (**Fig. 13 D**).

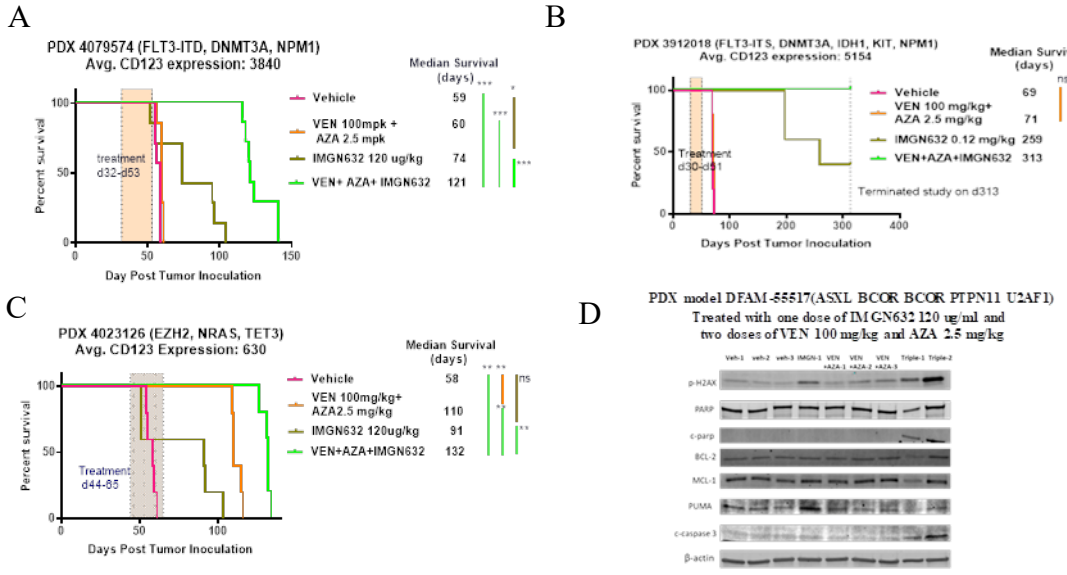


Figure 13. IMGN632+VEN+AZA in AML PDX Models. NSG mice were injected with AML PDX 24 h of sub-lethal irradiation. After confirming engraftment in peripheral blood by flow cytometry, mice were randomized to receive vehicle, VEN 100mg/kg (PO QD x3 weeks) + AZA 2.5 mg/kg (IP x 1 week), IMGN632 120 ug/kg (IV once per week x 3 weeks) and the triple combination. **A.** In AML PDX model 4079574 refractory to VEN+AZA, the combination of IMGN632+ VEN+ AZA significantly prolongs survival when compared to vehicle, VEN+AZA, and IMGN632 (p=0.0004, p=0.0002, and p=0.0001, respectively). **B.** In PDX 3912018 refractory to VEN+AZA, the triple combination enhances survival significantly when compared to IMGN632 alone and VEN+AZA with all the mice surviving in the triple combination (p=0.05 and p=0.0026, respectively). This study was terminated on d313 (37 weeks post last dose) with tumor burden <1% in peripheral blood in the triple combination group **C.** In AML PDX 4023126 model sensitive to VEN and AZA, combination of IMGN632+ VEN+ AZA enhances survival significantly when compared to IMGN632 and VEN+AZA alone (p=0.0017 and p=0.0018, respectively). Survival curves were plotted in Kaplan-Meier survival curves. P values were calculated using log-rank test: *p≤0.05, **p≤0.01, ***p≤0.001 **D.** Western blot analysis of enriched hCD45+ cells from the spleen of mice with PDX DFAM-55517 (treated with one dose of IMGN632 0.120 mg/kg and two doses of VEN 100 mg/kg+ AZA 2.5 mg/kg) shows upregulation of apoptotic proteins.

Specific Aim 2, Major Task 1: (Konopleva)

The goal of this task is to obtain approval, activate, and enroll additional patients with BPDCN on a clinical trial testing the triplet combination of tagraxofusp, azacitidine, and venetoclax. We have made good progress on this task during the funding period. Our SOW goal is to open the trial to enrollment in Year 2, with Year 1 being assigned to regulatory work.

During the founding period, the clinical trial testing TAG/AZA or TAG/AZA/VEN for patients with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS) completed dose escalation and the recommended phase 2 dose (RP2D) was determined for the TAG/AZA/VEN triplet. This allowed us to submit for IRB approval the expansion cohort for patients with BPDCN using the RPD2 of TAG/AZA/VEN, which will be AZAcitidine 75 mg/m2 on days 1-7, VENetoclax 400 mg daily on days 1-21, and TAG 12 ug/kg/day on days 4-6. The protocol amendment to add the BPDCN cohort has been reviewed and approved by the drug supplier and the FDA. The DoD HRPO reviewed the clinical trial protocol and fully approved the DFCI lead site for the study and the MDACC and COH subsites are in review. This task remains on schedule as our original SOW called for the first year to be used for regulatory approvals.

DFCI

Specific Aim 1, Major Tasks 1 and 2 (Lane):

We performed RNA sequencing on BPDCN PDXs treated in vivo with tagraxofusp, azacitidine, the combination of both drugs, or vehicle alone. The objective was to understand the different mechanisms of resistance to each treatment as a way to inform eventual clinical trial combinations in patients. Briefly, recipients were NSG NOD.Cg-Prkdc IL2rg/SzJ mice; Jackson Lab 005557) that were treated with 200 cGy of total body irradiation conditioning. They were then injected with 10^6 BPDCN PDX cells and monitored weekly starting at day 21 for peripheral engraftment (human CD45+CD123+ cells). Once 50% of mice had >0.2% detectable BPDCN, they were randomized to receive tagraxofusp (0.1 mg/kg/d x5 days), azacitidine (2.5 mg/kg/d x7 days) or both or vehicle. When mice succumbed to fatal BPDCN, we collected human CD45+CD123+ cells from moribund animals by flow cytometry and performed RNA-sequencing (**Figure 14**).

We also have begun to analyze gene expression changes in cells relapsing after SL401 (tagraxofusp) compared to vehicle alone. And for comparison we are analyzing gene expression changes after azacitidine alone or the combination. Volcano plots of log2 fold change in gene expression versus -log10 P value are shown below. Each dot represents a gene. We will use gene set enrichment analysis to determine pathways affected in resistance to each treatment and how they compare across treatments.

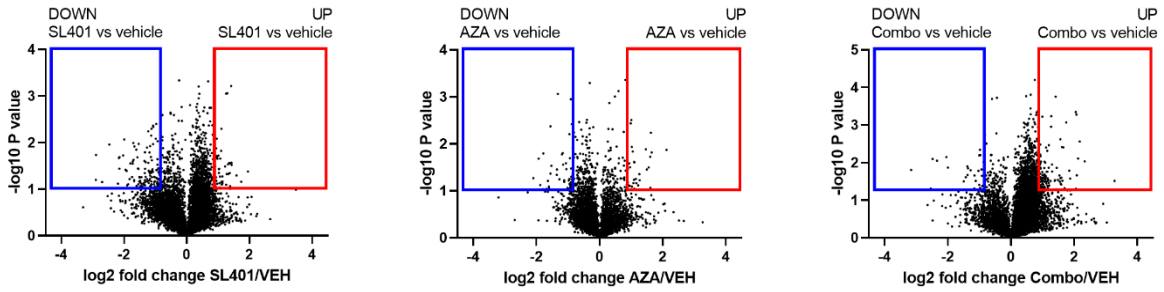


Figure 14. Volcano plots of RNA-sequencing gene expression versus P value by t test comparison between PDXs (n=8 total, 2 per treatment group) that progressed after receiving the indicated treatments in vivo.

Another activity has been to develop and pilot post-hoc centralized batch analysis of CD123 level on the surface of leukemias from patients. This is relevant to understanding if CD123 level correlates with up front response to tagraxofusp and other CD123-targeting agents and if the level of CD123 changes during, after therapy, or at the time of resistance to therapy. To that end, we developed an assay in which we can compare CD123 cell surface expression level across primary human leukemia specimens that have been previously cryopreserved. The goal was to determine how to normalize CD123 expression between samples frozen and/or analyzed on different days. We studied >10 human myeloid leukemias from patients that were banked prior to receiving tagraxofusp and azacitidine or tagraxofusp, azacitidine, and venetoclax. CD123 level was normalized to no staining, to isotype only, and to negative cells in the stained bone marrow specimen, and as a positive control to the CAL1 BPDCN cell line that is uniformly high for CD123.

Pilot data are shown below and showed stability over repeated measurements at different times. This suggests that we will be able to measure CD123 on the AML and BPDCN blasts that we intend to sort for RNA-seq and other tumor measurements in future study activity.

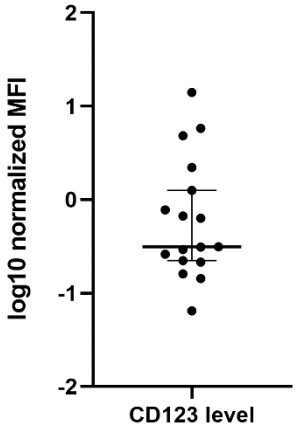


Figure 15. Log10 CD123 mean fluorescence intensity on the surface of AML blasts measured by flow cytometry and normalized to negative staining cells in the patient marrow specimen and to the CAL1 BPDCN cell line. Log10 = 0 are cells that are equivalently CD123 positive as the CAL1 cell line.

We also analyzed bone marrow and blood samples from patients with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS) who received TAGgraxofusp in combination with AZAcitidine or with AZA and VENetoclax. These samples are relevant to understand the mechanisms of

sensitivity and resistance to TAG in combination therapy to compare with our existing data of TAG monotherapy in myeloid cells. The results from studying MDS and AML cells will be relevant to the data we will obtain later in this project by studying BPDCN cells in a clinical trial (see Specific Aim 2). Another objective of these experiments is to study leukemia cells from patients for comparison with the patient derived xenograft cells (above) we are analyzing in the lab in parallel to determine if they harbor the same markers of sensitivity and resistance to therapy.

We performed flow cytometry on 43 samples, measuring CD123 expression level on the AML or MDS blasts as well as sorting blasts for RNA purification. 26 samples were from patients at baseline (prior to treatment) and 17 were post therapy. We obtained sufficient quality and quantity of RNA from 34 samples (>10 ng and RIN 6 or higher) that we sent for paired-end 100 bp RNA sequencing. We sorted patients as responder vs non-responder and performed DEseq for differential gene expression (**Figure 16**). We have begun to analyze these data in comparison to the BPDCN PDX data above (Figure 1), to see if diphthamide genes are dysregulated and which other pathways may be different between responders and non-responders at baseline (such as CD123, see below). Interestingly, among the genes most differentially expressed at baseline is CCL2, or MCP1 (monocyte chemoattractant protein 1), which is a proinflammatory monocyte/macrophage chemokine that has been linked to leukemia biology and infiltrating innate immune cells as well as being a dynamic marker after hypomethylating agent treatment.

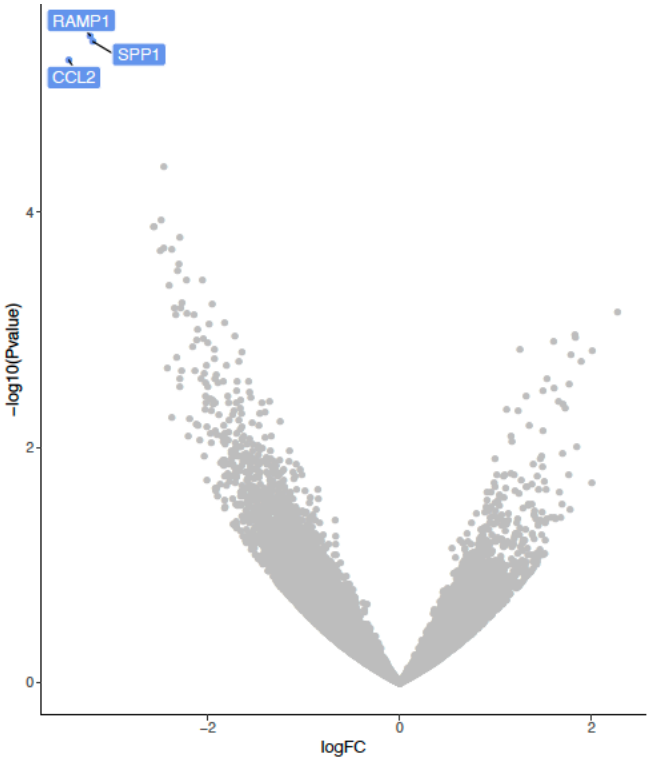


Figure 16. Volcano plot of genes with lower (negative) or higher (positive) expression in AML/MDS that responded to TAG/AZA vs those that did not.

We also tested additional leukemias using the flow quantitation assay normalized to CD123 expression on the CAL1 BPDCN cell line, where 10 = CAL1 CD123 MFI and 1 is CAL1 unstained

MFI. All patient leukemia blasts are quantitated relative to this range. This ensures reproducibility between different time points of analysis and from samples collected at different centers. Analysis of patient specimens demonstrated a range of CD123 level at baseline (all patients were required to have CD123 positivity on the blasts at study entry but there was no cut off for level required) (**Figure 17**). Pre and post treatment analyses showed that the majority of samples had similar CD123 levels after treatment as compared to prior to treatment, suggesting that treatment did not select for blasts with CD123 expression alterations. However, there were some that increased or decreased significantly, which we are following up now with additional validation. We are currently analyzing these data in the context of disease type, therapy received, and whether the patient responded to treatment.

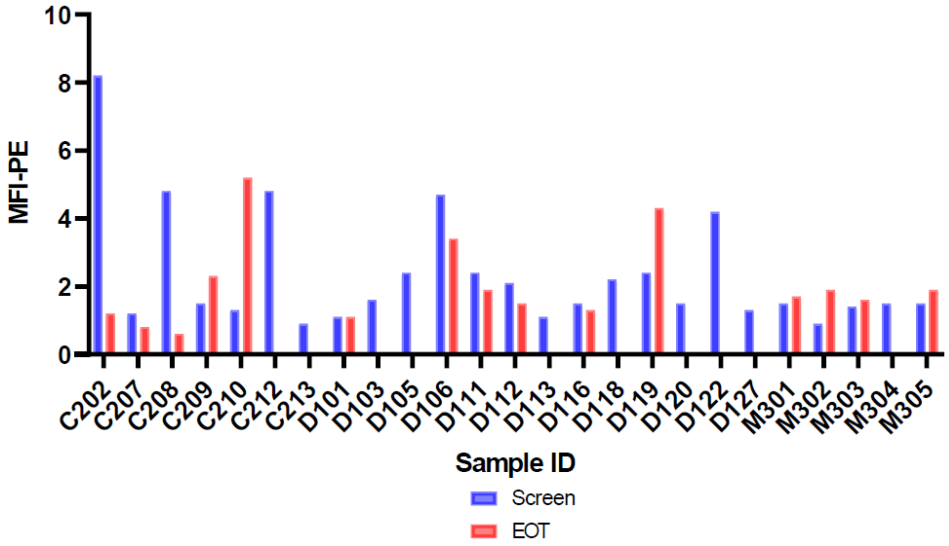


Figure 17. Normalized CD123 cell surface expression in leukemic blasts from patients with AML or MDS who received TAG/AZA or TAG/AZA/VEN. S

Finally, in this area of work, we simultaneously analyzed CD123 level and normal mature pDC infiltration in the bone marrow of AML and MDS patients that either responded or did not respond to TAG/AZA and for which we had adequate banked viably cryopreserved samples. pDC-AML is a newly recognized entity of AMLs, enriched for harboring somatic RUNX1 mutations, that have an expanded pDC population and they may be more sensitive to TAG than other AMLs. We found a trend toward higher CD123 on leukemias that responded, as might be expected for a CD123-targeting drug. Of great interest, pDC infiltration also was associated with response to therapy. This may link certain AMLs to the high response rate seen in BPDCN with TAG and may help us better target both diseases.

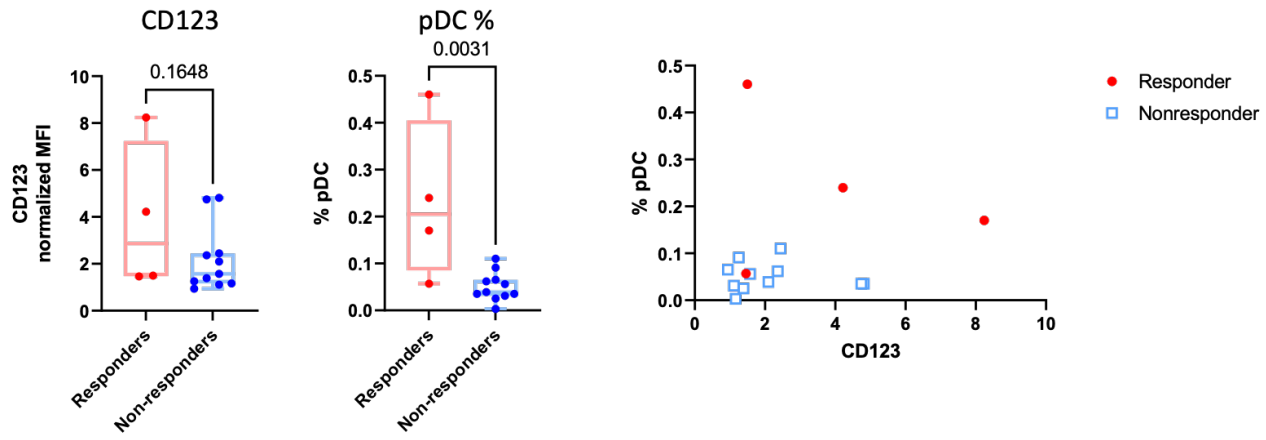


Figure 18. CD123 level on blasts and pDC infiltration at baseline in AML/MDS that responded vs did not to the TAG/AZA doublet.

Next, we performed experiments to ask the relative contribution of baseline CD123 expression level on the cell surface and ADP ribosylation activity of tagraxofusp (TAG) in permeabilized cells, each to AML and BPDCN cell sensitivity to TAG. These experiments are important because from our prior data we don't know whether diphthamide synthesis pathway activity, which is required to generate the target for diphtheria toxin or TAG in the cell, is important for up front sensitivity to TAG or only most relevant in acquired resistance. The graphs below (**Figure 19**) plot, on the left: CD123 expression on the x axis (relative to isotype control) and TAG -log IC50 on the y axis (higher equals more TAG sensitive). And, on the right: ADP ribosylation activity of TAG in permeabilized cells versus TAG -log IC50, each for 13 AML and BPDCN cell lines. What we observe is that overall, there is a trend toward increased TAG sensitivity in the higher CD123-expressing cells. This is most clear when we focus on the CD123 low to moderate expressing cells (bottom row), which suggests that above a certain CD123 level, there is maximal TAG response and additional surface CD123 may not improve response. In the low-moderate expressing cells there is a clear correlation between higher CD123 and TAG sensitivity. In contrast, we see no clear correlation between baseline TAG ADP-ribosylation activity in permeabilized cells and TAG cytotoxicity. This suggests that up front profiling of ADP-ribosylation activity is not a good biomarker for TAG sensitivity and that it may be most relevant as an acquired escape mechanism as we found previously in our cell line and PDX models of acquired resistance to TAG and in patients who received TAG.

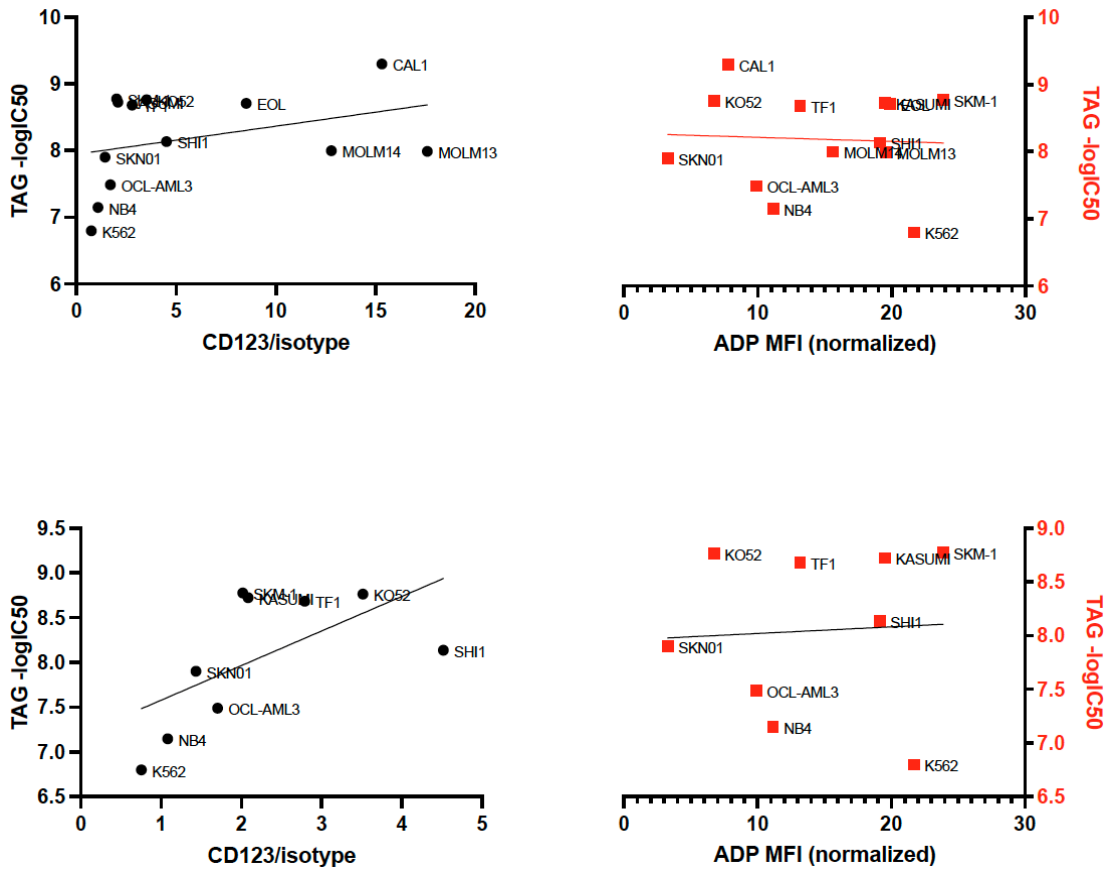


Figure 19. Thirteen AML and BPDCN cell lines analyzed for the correlation between CD123 expression (left) or ADP ribosylation activity of TAG (right) on TAG cytotoxicity (y-axis). The top two row is for all 13 cell lines, and the bottom row is for those with lower CD123 expression (below 5-fold CD123 staining normalized to isotype).

In a second major area of work, we developed a genomewide CRISPR interference interrogation of genetic determinants of sensitivity and resistance to tagraxofusp and venetoclax/azacitidine in BPDCN cells. We elected to use CRISPR interference with dead dCas9-KRAB as opposed to wild-type Cas9 because the knockdown efficiency with CRISPRi is equal or better than traditional cutting CRISPR knockout yet there is no activation of the DNA damage response because there are no double stranded breaks generated. We generated CAL-1 BPDCN cells stably expressing dCas9-KRAB and verified dCas9 expression by western blot and CRISPRi activity by transduction with a CD81 sgRNA (**Figure 20**). CAL1 dCas9-KRAB cells had very efficient and specific knockdown of CD81 cell surface expression.

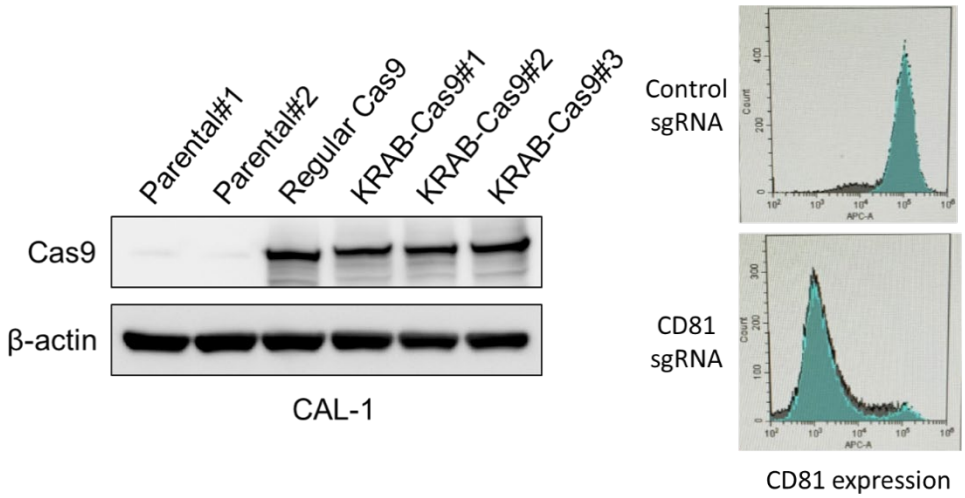


Figure 20. (Left) Western blot for Cas9 in parental CAL-1 BPDCN cells or CAL-1 cells stably expressing Cas9 for traditional "cutting" CRISPR knockout or dCas9-KRAB for CRISPR interference. (Right) dCas9-KRAB expressing CAL-1 cells transduced with a control sgRNA or a CD81-targeting sgRNA followed by cell surface staining and flow cytometry for CD81 showing high efficiency knockdown.

We optimized treatment with TAG or VEN/AZA in CAL-1 dCas9-KRAB cells at the large scale necessary for performing the CRISPRi screen with >500 cell representation per sgRNA (there are approximately 110,000 test and control sgRNAs in the Broad Institute Dolcetto human CRISPRi lentiviral library that includes 6 sgRNAs per gene. We have identified and are testing a novel plasticware vessel (HYPERFlask, Corning) that is optimized for oxygen delivery to large volume suspension or adherent cell cultures, which ensures adequate growth conditions in high volume (500 mL+) cultures that are necessary for performing the CRISPRi screen in ~10^8 cells with multiple replicates and treatment conditions. We are performing pilot testing of the drug concentrations of TAG and AZA/VEN that will be used in large volume screening conditions to efficiently identify enriched and depleted sgRNAs by DNA sequencing and bioinformatic deconvolution.

Next, we completed a genomewide CRISPR interference screen in CAL1 BPDCN cells in the presence of TAG or venetoclax plus azacitidine. We used the Dolcetto human CRISPRi sgRNA library, set A and set B, which together provides average 6 independent sgRNAs targeting the promoter of nearly all genes (~18,600) in the genome for knockdown. We optimized drug treatment so that after 72 hours, drug treated cells would have 20% of the cell number as in vehicle treated cells (**Figure 21**). This 80% reduction in cell number has been shown by the Genome Perturbation Platform at the Broad Institute to maximize guide dropout/enrichment, but not be confounded by outgrowth rate differences determined by guides that have no effect on drug sensitivity. This was the timepoint for DNA collection for library generation and next generation sequencing to decovolute sgRNA representation to quantitate enrichment/depletion. This screen is therefore distinct from others previously done to study venetoclax resistance for three main reasons: 1) rather than allow outgrowth of resistant cells over weeks as was done previously, we are assessing the up-front contributors to sensitivity and resistance; 2) combining venetoclax with azacitidine in a CRISPR screen has not been reported to our knowledge; 3) we used BPDCN cells. We performed the screen in sufficient cell numbers to achieve >500 cells/sgRNA representation. PCR amplified sgRNA barcodes were

sequenced at the Broad Institute and the data are now in analysis and validation of single guides that scored as top hits.

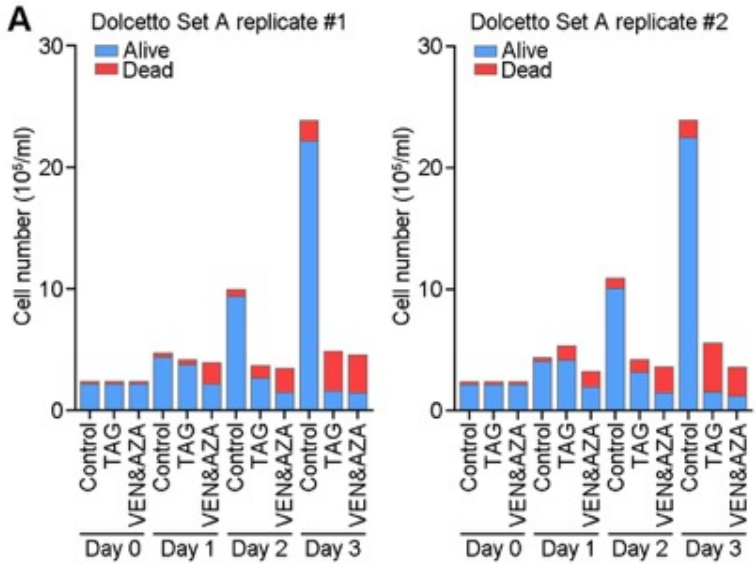


Figure 21. Cell numbers harvested from the CRISPRi screen in CAL1 BPDCN cells treated with vehicle, tagraxofusp, or VEN/AZA.

Finally, we began expanding three BPDCN PDXs for follow up in vivo experiments to overlay with the CRISPR screen and per the SOW. Three independent (from 3 different patients) BPDCN PDXs with classic characteristics were chosen for their consistent expression of standard BPDCN markers CD4, CD56, CD123, and TCL1 and we selected those with high BCL2 expression by RNA-seq, which is how we initially began to study BCL2 sensitivity in the disease. When these animals develop overt BPDCN in the peripheral blood (>2% human CD45/CD123 cells) we will harvest bone marrow and spleen BPDCN cells for transplantation into fresh recipients, in which we will perform drug treatment (TAG, VEN/AZA, or TAG/VEN/AZA).

Specific Aim 2 (Lane)

The overall goal of this task is to obtain approval, activate, and enroll patients with BPDCN on a clinical trial testing the triplet combination of tagraxofusp, azacitidine, and venetoclax. We have made good progress on this task during the funding period. Our SOW goal was to open the trial to enrollment in Year 2, with Year 1 being assigned to regulatory work, which we achieved.

In this period, we completed the protocol amendment to open a dedicated expansion cohort for patients with BPDCN on a phase 1-2 trial of TAG/AZA/VEN. It has been reviewed by the drug supplier and by the FDA. The protocol has been submitted and approved by the Dana-Farber SRC and IRB. It was then reviewed at the outside sites including MD Anderson. It was then submitted to HRPO for review. No project funding was spent on the trial until HRPO provided approval. HRPO has now fully approved the trial at all sites, and therefore has completed review.

In this period, the clinical trial testing TAG/AZA or TAG/AZA/VEN for patients with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS) completed dose escalation and the

recommended phase 2 dose (RP2D) was determined for the TAG/AZA/VEN triplet (this was in progress before this DoD project). This allowed us to submit for IRB approval the expansion cohort for patients with BPDCN using the RPD2 of TAG/AZA/VEN, which will be AZAcitidine 75 mg/m2 on days 1-7, VENetoclax 400 mg daily on days 1-21, and TAG 12 ug/kg/day on days 4-6. We are now enrolling patients with relapsed/refractory BPDCN on this triplet.

Specific Aim 3 (Lane)

We performed an experiment to begin to understand if BPDCN cells with ZRSR2 mutations are more sensitive to splicing modulator drugs. We saw no difference in the sensitivity to the splicing modulator E7107 in CAL1 cells with and without ZRSR2 mutation induced by CRISPR/Cas9. Small sample numbers for primary BPDCNs of each splicing factor genotype and lack of established protocols for prolonged culture conditions amenable to drug treatment experiments ex vivo, even for PDXs, limit our ability to ask this question in a statistically powered manner in patient cells. We note that none of the other published ZRSR2-focused studies (in context of MDS or AML samples and model systems) have shown differential sensitivity to splicing modulators, in contrast to what was reported with SRSF2 and SF3B1 mutant cells. Of note, the H3Biosciences trial of the E7107 derivative H3B-8800 in patients with MDS has recently changed its eligibility criteria to be restricted to patients harboring SF3B1 mutations, i.e., they no longer include patients with SRSF2, U2AF1, or ZRSR2 mutations, possibly because the available drugs target the SF3B complex and have been reported to be most active in SF3B1-mutated cells and patients. It is possible that other splicing modulators would be effective in ZRSR2 mutant pDCs/BPDCN, or E7107 or its derivatives will be effective in vivo or in combinations, which can be explored in future work periods.

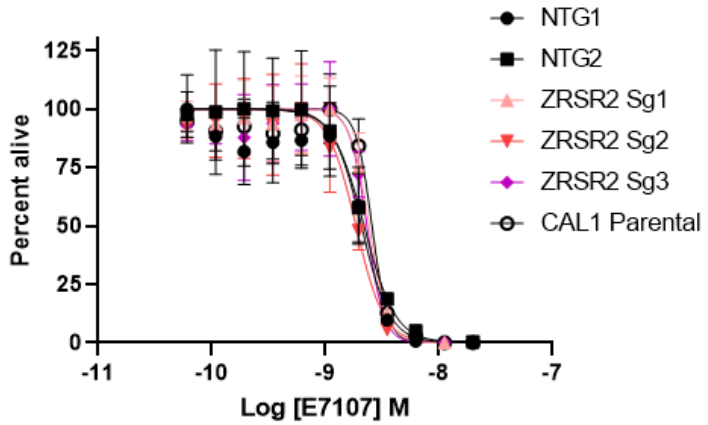


Figure 22. Sensitivity to the splicing modulator E7107 in parental CAL1 cells and Cas9-expressing CAL1 cells harboring control of three independent ZRSR2-targeting sgRNAs.

Specific Aim 3 (Abdel-Wahab)

Specific Aim 3
Major Task 2: Determine aberrant splicing events generated by mutant *ZRSR2* and *SRSF2* in BPDCN which promote transformation through a positive enrichment CRISPR screen.

In this quarter, we have made significant progress in addressing Aim 3, Subtask 4 "Evaluation of molecular basis for the individual splicing events found in screen as well as analysis of effects on protein expression in BPDCN samples." The progress we have made is as follows:

Mapping direct RNA binding targets of ZRSR2: To understand the mechanistic basis for the relationship between ZRSR2 mutations in BPDCN and alterations in splicing of minor introns, we attempted to identify direct binding targets of ZRSR2 on RNA. We therefore performed anti-ZRSR2 eCLIP-seq[31] (enhanced UV crosslinking immunoprecipitation followed by next-generation sequencing) in human myeloid leukemia (K562) cells (**Fig. 23A**). This revealed that ~80% of ZRSR2 binding sites mapped to exons, with a highly significant enrichment for ZRSR2 binding to minor intron-containing genes whose minor intron was responsive versus non-responsive to *ZRSR2* mutations (**Fig. 23A-B**; p-value < $2.2e^{-16}$ with an odds ratio in the range (95% CI): 2.1-2.5). ZRSR2 binding was specifically enriched in minor introns, consistent with our analyses of the effects of ZRSR2 loss on minor intron retention (**Fig. 23C**). Finally, ZRSR2-bound mRNAs are enriched for mRNAs encoding RNA regulatory proteins as well as genes with known involvement in leukemia and protein processing and translation (**Fig. 23D**). Overall, these analyses identify that minor intron-containing genes whose splicing is regulated by ZRSR2 are direct binding targets of ZRSR2.

Both the RNA-seq and eCLIP-seq analyses above identified that only approximately one-third of U12-type intron-containing genes are sensitive to loss of ZRSR2. In order to understand the specificity of ZRSR2 for regulation of the splicing of minor introns and why only a portion of minor introns are regulated by ZRSR2, we next evaluated the sequence features of introns which were retained upon ZRSR2 loss.

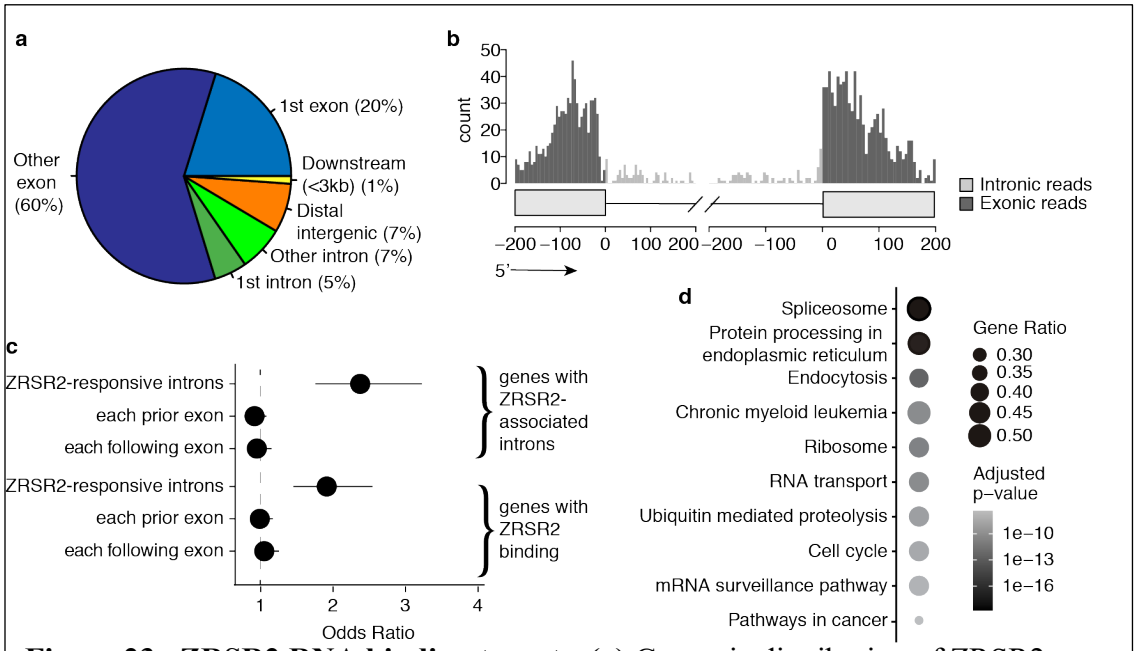


Figure 23 . ZRSR2 RNA binding targets. (a) Genomic distribution of ZRSR2 eCLIP-seq (enhanced UV crosslinking immunoprecipitation followed by next-generation sequencing) peaks. **(b)** Metaplot of ZRSR2 eCLIP sequencing reads at ZRSR2-regulated minor introns. **(c)** Fisher's exact test analysis evaluating the enrichment of ZRSR2 within responsive introns and each flanking exon by eCLIP-seq in genes with ZRSR2-responsive introns or those with ZRSR2 binding. **(d)** Gene ontology analysis of ZRSR2-bound genes by eCLIP-seq.

Characteristics of ZRSR2 regulated introns: While branchpoints within U2-type introns are highly constrained in their location, branchpoints within U12-type introns exhibit a bimodal distribution, such that half of U12-type introns have branchpoints similar in location to U2-type branchpoints while half of U12-type branchpoints occur in closer proximity (within 20 nucleotides (nt)) of the 3' splice site (3'ss)[32] (**Fig. 24A**). To test whether this bimodality was relevant to ZRSR2 responsiveness, we augmented our previously published branchpoint annotation by querying available RNA-seq data from cohorts within The Cancer Genome Atlas (TCGA) to search for lariat-derived reads which span the 5' splice site-branchpoint junction within minor introns. Such reads are extremely rare due to typically rapid lariat degradation—hence the need for an extremely large-scale analysis—but allow for inference of branchpoint location with nucleotide-level resolution. Using this large U12-type branchpoint annotation, we discovered that introns that respond to ZRSR2 loss had branchpoints that were significantly more proximal to the 3'ss than did non-responsive introns (two-sided Kolmogorov-Smirnov test $p<2.2e-16$; **Fig. 24B**). In contrast, non-responsive U12-type introns exhibited no such spatially restricted enrichment, suggesting that branchpoint location influences U12-type intron susceptibility to retention in the absence of ZRSR2[32]. We therefore examined the branchpoint more closely as a potential determinant of response to ZRSR2 loss. This revealed that ZRSR2-responsive introns prefer adenosine nucleotides as branchpoints (**Fig. 24C**; $p=1.5x10^{-5}$ by two-sided binomial proportion test); have more branchpoints per intron compared to ZRSR2 non-responsive introns (**Fig. 24D**; $p=0.03$ by two-sided *t*-test); and have branchpoints that more closely match the U12 snRNA consensus sequence (**Fig. 24E**; $p=5.2e10^{-16}$ by Wilcoxon rank sum test). ZRSR2-responsive minor introns additionally have less-defined polypyrimidine tracts and a reduced preference for G at the +1 position compared to non-responsive introns (**Fig. 24F-G**). Overall, these data identify that U12-type introns fall into two classes: those which are resistant to ZRSR2 loss and those which respond strongly to ZRSR2 loss. Responsive introns are typically characterized by a 3'ss-proximal, adenosine branchpoint that is surrounded by nucleotides that closely resemble the U12 snRNA consensus, as well as having a weak or absent polypyrimidine tract.

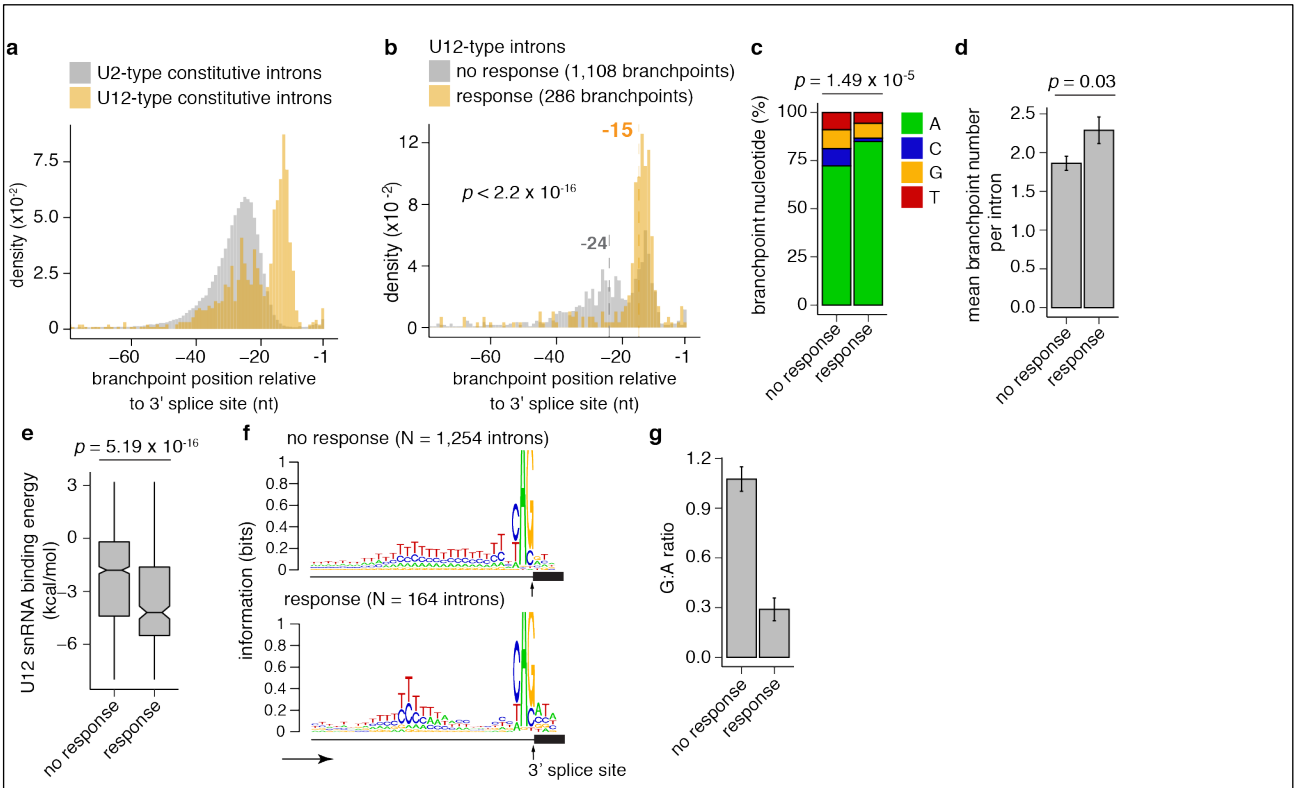


Figure 24. Features of ZRSR2-responsive introns (a) Histogram of the locations of branchpoints relative to the 3' splice site (3'ss) in U2- versus U12-type constitutive introns. **(b)** Histogram of the locations of branchpoints relative to the 3'ss for ZRSR2 non-responsive versus responsive minor introns (*p*-value estimated by a two-sided Kolmogorov-Smirnov test). **(c)** Branchpoint nucleotide preference for ZRSR2 non-responsive versus responsive minor introns (*p*-value estimated with a two-sided binomial proportion test for a difference in fraction of adenine branchpoints). **(d)** Mean number of branchpoints within ZRSR2 non-responsive versus responsive minor introns (*p*-value estimated by a two-sided *t*-test). Error bars represent $\pm$ 1 standard error of the mean (sd/sqrt(n)). **(e)** U12 snRNA binding energy for branchpoint motifs in ZRSR2 non-responsive versus responsive minor introns (*p*-value estimated by a two-sided Mann-Whitney *U* test). **(f)** Sequence logo plots of the 3'ss of ZRSR2-responsive versus non-responsive introns (upward arrow indicates site of 3'ss). As shown, ZRSR2-responsive introns have weaker/less-defined polypyrimidine tracts. **(g)** G:A ratio at the +1 position (relative to the 3'ss). Error bars represent $\pm$ 1 s.d. estimated by bootstrapping (10k iterations). P-value = 0 by two-sided Mann-Whitney U test.

Given the wide-spread mis-splicing of mRNAs induced by mutations in SRSF2 and ZRSR2, we computationally investigated whether the highly ste reotyped patterns of mis-splicing induced by recurrent spliceosomal mutations could potentially give rise to shared neoantigens. We used large-scale RNA-seq analyses to identify recurrently mis-spliced isoforms in BPDCN and myeloid leukemia patient samples with (n = 120, 137, 56, and 38 for *SF3B1*, *SRSF2*, *U2AF1*, and *ZRSR2*) or without (WT; n = 910) spliceosomal mutations; restricted to mis-spliced isoforms that were not detectably expressed in healthy bone marrow, peripheral blood, or a panel of 16 solid tissues; translated each isoform in silico and split into 8-12-mer peptides; restricted to 8-12-mers that arose uniquely from the mis-spliced isoform; and restricted to predicted high-affinity binders to MHC class I binding with NetMHCpan 4.0. This analysis revealed 37, 42, 60, and 125 candidate splicing-derived neoepitopes for the common allele HLA-A*02:01 in myeloid leukemias with *SF3B1*, *SRSF2*, *U2AF1*,

and *ZRSR2* mutations (**Figure 25**), with similar numbers for other common MHC class I alleles. We then experimentally evaluated the ability of a subset of these predicted neoepitopes to be presented by MHC class I (HLA-A2) with a peptide stabilization assay in TAP-deficient T2 cells. We observed a range of abilities of each predicted neoepitope to stabilize cell-surface MHC class I, in some cases notably more so than for positive control peptides, with the majority of predicted neoepitopes exhibiting some binding. These data support a hypothesis that widespread mis-splicing in BPDCN and myeloid leukemias bearing recurrent splicing factor mutations gives rise to potentially immunologically meaningful neopeptide production.

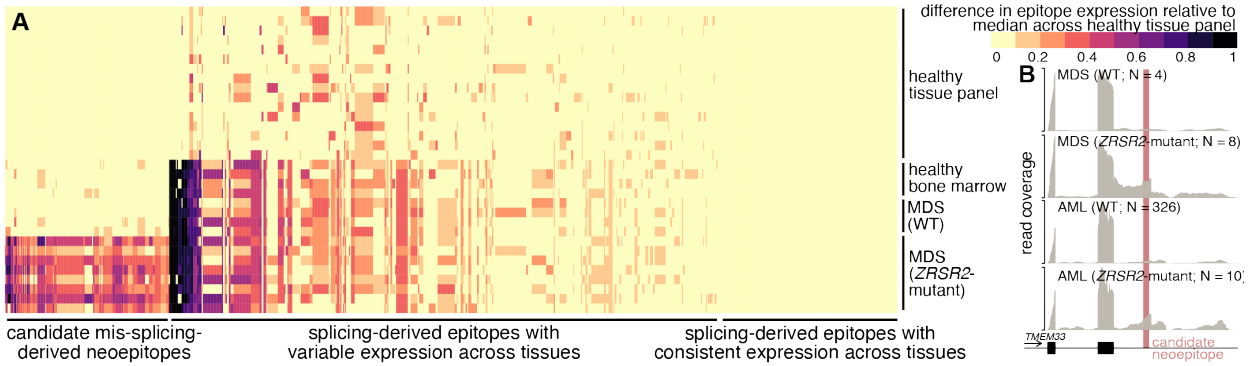


Figure 25. BPDCN and myeloid leukemia patient samples with recurrent spliceosomal mutations express mis-spliced mRNAs encoding predicted neoepitopes. (**A**) Heat map illustrating the expression levels of predicted splicing-derived neoepitopes in myeloid leukemia patient samples with or without mutations in the recurrently mutated spliceosomal gene *ZRSR2*. Neoepitopes defined as peptides that are uniquely encoded by mis-spliced isoforms expressed in *ZRSR2*-mutant, but not WT, samples; are predicted to bind HLA-A*02:01; and are not detectably expressed in 16 healthy human tissues, peripheral blood, or bone marrow. (**B**) RNA-seq coverage plots illustrating predicted neoepitopes arising from mis-splicing in *ZRSR2*-mutant myeloid leukemia patient samples. We characterized the mis-spliced transcriptome of *ZRSR2*-mutant leukemia in our recent study cited below.

Accomplishment: our collaborative group published a paper together in Cancer Discovery (see full citation below; Togami et al, 2021). In this work, we reported the high incidence of ZRSR2 and other splicing factor mutations in BPDCN and discovered that splicing mutations protect BPDCN from apoptosis in the setting of inflammation and DC activation. This is a very important result for all of the Aims in this project, as we now begin to understand the molecular mechanisms that promote pDC/BPDCN cell survival and apoptosis.

What opportunities for training and professional development has the project provided?

Nothing to report

How were the results disseminated to communities of interest?

(Konopleva)
We have presented following posters at the national meetings:
2020 ASH
Combining IMGN632, a Novel CD123-Targeting Antibody Drug Conjugate with Azacitidine and Venetoclax Facilitates Apoptosis *in Vitro* and Prolongs Survival *In Vivo* in AML Models
https://www.sciencedirect.com/science/article/pii/S0006497118720326

2020 SOHO
AML-367: IMGN632, a CD123-Targeting ADC Bearing a DNA-Alkylating IGN Payload, Combines Effectively as a Triplet Regimen with Azacitidine and Venetoclax In Vivo, Prolonging Survival in Preclinical Models of Human Acute Myeloid Leukemia (AML)
https://www.sciencedirect.com/science/article/pii/S2152265020307680

2019 ASH
IMGN632, a CD123-Alkylating ADC Bearing a DNA Alkylating IGN Payload, Combines Effectively with Azacitidine and Venetoclax In Vivo, Prolonging Survival in Preclinical Models of Human Acute Myeloid Leukemia (AML)
https://ashpublications.org/blood/article/134/Supplement_1/1375/427254/IMGN632-a-CD123-Alkylating-ADC-Bearing-a-DNA

2019 EHA

PF201 THE COMBINATION OF IMGN632, A CD123-TARGETING ADC, WITH VENETOCLAX ENHANCES ANTI-LEUKEMIC ACTIVITY IN VITRO AND PROLONGS SURVIVAL IN VIVO IN PRE-CLINICAL MODELS OF HUMAN AML
https://journals.lww.com/hemasphere/Abstract/2019/06001/PF201_THE_COMBINATION_OF_IMGN632,_A.101.aspx

2018 ASH
Pre-Clinical Efficacy of CD123-Targeting Antibody-Drug Conjugate IMGN632 in Blastic Plasmacytoid Dentritic Cell Neoplasm (BPDCN) Models
https://www.sciencedirect.com/science/article/pii/S0006497119400566

(Lane)
Conference abstract oral presentation:

Togami K, Chung SS, Madan V, Kenyon CM, Cabal-Hierro L, Taylor J, Kim SS, Griffin GK, Ghandi M, Li J, Li YY, Angelot-Delettre F, Biichle S, Seiler M, Buonamici S, Lovitch SB, Louissaint A, Moran EA, Jardin F, Piccaluga PP, Weinstock DM, Hammerman PS, Yang H, Konopleva M, Pemmaraju N, Garnache-Ottou F, Abdel-Wahab O, Koeffler P, Lane AA. Male-Biased Spliceosome Mutations in Blastic Plasmacytoid Dendritic Cell Neoplasm (BPDCN) Impair pDC Activation and Apoptosis. Oral presentation. American Society of Hematology Annual Meeting 2020. *Blood* (2020) 136 (Supplement 1): 13–14. https://doi.org/10.1182/blood-2020-137727

(Lane)
Published paper:

Togami K, Chung SS, Madan V, Booth CAG, Kenyon CM, Cabal-Hierro L, Taylor J, Kim SS, Griffin GK, Ghandi M, Li J, Li YY, Angelot-Delettre F, Biichle S, Seiler M, Buonamici S, Lovitch SB, Louissaint A, Moran EA, Jardin F, Piccaluga PP, Weinstock DM, Hammerman PS, Yang H, Konopleva M, Pemmaraju N, Garnache-Ottou F, Abdel-Wahab O, Koeffler P, Lane AA. Sex-biased *ZRSR2* mutations in myeloid malignancies impair plasmacytoid dendritic cell activation and apoptosis. Cancer Discovery. 2021; doi: 0.1158/2159-8290. CD-20-1513. Online ahead of print. PMID: 34615655

(Abdel-Wahab)
Conference abstract oral presentation:
2021 ASH
ZRSR2 Mutation Induced Minor Intron Retention Drives MDS and Diverse Cancer Predisposition Via Aberrant Splicing of *LZTR1*
https://ash.confex.com/ash/2020/webprogram/Paper136445.html

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

(Konopleva)
Aim 2, Sub-Aim 1:
- Continue efficacy tests of IMGN632/VEN/AZA in combination in BPDCN and AML primary samples.
- Study mechanisms of synergistic activity of IMGN632/VEN/AZA combination in BPDCN and AML cell lines. In particular: 1) test the effect of IMGN632/VEN/AZA treatment on activation of DNA damage response (DDR) pathways by confocal microscopy analysis of RAD51 foci (marker of homologous recombination, HR), 53BP1 foci (marker of non-homologous end joining, NHEJ); 2) western blot analysis of DDR markers: ATM/pATM(Ser1981)/CHK2/pCHK(Thr68), ATR/pATR (Ser428)/CHK1/pCHK1 (Ser345), BRCA1/2, PARP1, p53 effectors like p21. 3) Flow cytometry analysis of cell cycle and BrdU incorporation. We hypothesize that VEN may impair activation of DNA damage response triggered by IMGN632, thus leading to cell death.
- Continue studies on the role of p53 in the overall response of AML cells to IMGN632/VEN/AZA combination. In particular: test the effect of combination on the DDR and cell cycle in p53 wild-type and p53 KD AML cells.

Aim 2, Sub-Aim 3:
- We anticipate that the expansion phase at the RP2D of the interventional clinical trial cohort of the triplet TAG/AZA/VEN study to add patients with BPDCN will be approved by local IRBs. We will gather all regulatory documents and submit to HRPO for their initial review. Anticipate that we will still be undergoing the review process at IRB/HRPO for the next one or more reporting periods, but this is in line with our planned SOW, which had budgeted the first year for regulatory approvals for this study.

Aim 1 (Lane):

Continue with validation experiments from the genomewide CRISPRi experiment in Dolcetto library transduced CAL1 cells grown in the presence of vehicle alone or treated with tagraxofusp or azacitidine/venetoclax. We will prioritize top hits based on consistency across sgRNAs for each gene, across Dolectto set A and B (independent 3+ sgRNA/gene/set) and with two replicates. We will compare hits with published data on VEN RNAi/CRISPR screens, looking for ways that combination AZA/VEN may differ. We have numerous positive controls expected based on our own and others' prior work. For example, CD123 and DPH1 should be resistance mechanisms for TAG, and BAX/BAK and other mitochondrial genes for VEN.

Continue expansion of 3 BPDCN PDXs for in vivo drug treatment studies. We need enough BPDCN cells for 150 NSG mice to receive 1 million PDX cells each (50 million per PDX), so we are expanding each PDX in 5 NSG mice in this pre-round. If PDXs reach these numbers during this period, we will immediately transplant fresh (no freeze step) into new NSG mice and treat with TAG, AZA/VEN, TAG/AZA/VEN, or vehicle. We will harvest minimal residual disease cells 24 hours after the last day of dosing for RNA-seq, and then at the time of relapse for RNA-seq and protein analyses as appropriate based on CRISPR hits. We will monitor survival of each combination arm by Kaplan-Meier analysis.

Aim 2, Task 3 (Lane):

Enroll patients with relapsed/refractory BPDCN on the TAG/VEN/AZA triplet study.

The manufacturer of TAG, Stemline Therapeutics, has now agreed in principle to add a frontline BPDCN cohort to the clinical trial of TAG/AZA/VEN that currently is only written for relapsed or refractory BPDCN. We are currently working with our statisticians to amend the protocol to add a frontline BPDCN cohort. This will be submitted to FDA, IRB, and HRPO for review per guidelines during this year.

Aim 3, Task 3 (Abdel-Wahab):

Determine the effects of perturbing splicing *in vivo* in BPDCN PDX models wild-type or mutant for RNA splicing factors.

Aim 3, Task 4 (Abdel-Wahab):

Determine the effects of various drugs perturbing RNA splicing on splicing and gene expression in the above BPDCN models.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?
Nothing to Report

What was the impact on other disciplines?
Nothing to Report

What was the impact on technology transfer?
Nothing to Report

What was the impact on society beyond science and technology?
Nothing to Report

Changes in approach and reasons for change
Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them
Nothing to Report

Changes that had a significant impact on expenditures
Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Nothing to Report

Significant changes in use or care of human subjects
Nothing to Report

Significant changes in use or care of vertebrate animals
Nothing to Report

Significant changes in use of biohazards and/or select agents
Nothing to Report

5. PRODUCTS:

- **Publications, conference papers, and presentations**

  Journal publications.

Conference abstract oral presentation (Lane):

Togami K, Chung SS, Madan V, Kenyon CM, Cabal-Hierro L, Taylor J, Kim SS, Griffin GK, Ghandi M, Li J, Li YY, Angelot-Delettre F, Biichle S, Seiler M, Buonamici S, Lovitch SB, Louissaint A, Moran EA, Jardin F, Piccaluga PP, Weinstock DM, Hammerman PS, Yang H, Konopleva M, Pemmaraju N, Garnache-Ottou F, Abdel-Wahab O, Koeffler P, Lane AA. Male-Biased Spliceosome Mutations in Blastic Plasmacytoid Dendritic Cell Neoplasm (BPDCN) Impair pDC Activation and Apoptosis. Oral presentation. American Society of Hematology Annual Meeting 2020. *Blood* (2020) 136 (Supplement 1): 13–14. https://doi.org/10.1182/blood-2020-137727

Published paper (Lane):

Togami K, Chung SS, Madan V, Booth CAG, Kenyon CM, Cabal-Hierro L, Taylor J, Kim SS, Griffin GK, Ghandi M, Li J, Li YY, Angelot-Delettre F, Biichle S, Seiler M, Buonamici S, Lovitch SB, Louissaint A, Moran EA, Jardin F, Piccaluga PP, Weinstock DM, Hammerman PS, Yang H, Konopleva M, Pemmaraju N, Garnache-Ottou F, Abdel-Wahab O, Koeffler P, Lane AA. Sex-biased *ZRSR2* mutations in myeloid malignancies impair plasmacytoid dendritic cell activation and apoptosis. Cancer Discovery. 2021; doi: 0.1158/2159-8290. CD-20-1513. Online ahead of print. PMID: 34615655

Acknowledgement of federal support: Yes

Books or other non-periodical, one-time publications.
Nothing to Report

Other publications, conference papers and presentations.
Nothing to Report

- **Website(s) or other Internet site(s)**
  Nothing to Report

- **Technologies or techniques**
  Nothing to Report

- **Inventions, patent applications, and/or licenses**
  Nothing to Report

- **Other Products**

Nothing to Report

6. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

DFCI

Name:	Andrew Lane, MD, PhD
Project Role:	Site PI (DFCI)
Nearest person month worked:	0.6
Contribution to Project:	Dr. Lane supervised the project and worked on development of the clinical protocol.

Name:	Jada Garzon
Project Role:	Research associate
Nearest person month worked:	2.25
Contribution to Project:	Ms. Garzon performed sample processing, flow cytometry, and sorting.

Name:	Robert Bossong
Project Role:	Research associate
Nearest person month worked:	0.27
Contribution to Project:	Mr. Bossong performed sample processing and flow cytometry.

Name:	Katsuhiro Togami, MD, PhD
Project Role:	Postdoctoral fellow
Nearest person month worked:	2.25
Contribution to Project:	Dr. Togami performed benchwork and analysis of informatic data.

Name:	Qingyu Luo, PhD
Project Role:	Postdoctoral fellow
Nearest person month worked:	1.5
Contribution to Project:	Dr. Luo performed benchwork on BPDCN cell lines and animal work with patient derived xenografts.

MDACC

Name: Marina Konopleva, MD, PhD
Project Role: Site PI (MDACC)
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 1 (rounded for this quarter; 0.6 person-months per year)
Contribution to Project: Dr. Konopleva designed and supervised the project

Name: Naveen Pemmaraju, MD
Project Role: Site Co-I (MDACC)
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 1 (rounded for this quarter; 0.6 person-months per year)
Contribution to Project: Dr. Pemmaraju worked on development of the clinical protocol.

Name: Lina Han, MD, PhD
Project Role: Site Co-I (MDACC)
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 2.4 (rounded for this quarter; 2.4 person-months per year)
Contribution to Project: Dr. Han performed benchwork and analysis of CyTOF data.

Name: Xuelin Huang, PhD
Project Role: Site Co-I (MDACC)
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 1 (rounded for this quarter; 0.36 person-months per year)
Contribution to Project: Dr. Huang has supervised biostatistical analysis and assisted with protocol statistical design

Name: Graciela Nogueras Gonzalez
Project Role: Sr. Stat Analyst (MDACC)
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 1 (rounded for this quarter; 0.6 person-months per year)
Contribution to Project: Performed biostatistical analysis

Name: Qi Zhang, PhD
Project Role: Postdoctoral fellow
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 3 (rounded for this quarter; 3 person-months per year)
Contribution to Project: Dr. Zhang performed in vitro and in vivo experiments

Name: Anna Skwarska, PhD
Project Role: Instructor
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 6 (rounded for this quarter; 6 person-months per year)
Contribution to Project: Dr. Skwarska performed in vitro experiments

MSK

Name:	Omar Abdel-Wahab, MD
Project Role:	Site PI (MSK)
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1 (rounded for this quarter; 0.6 person-months per year)
Contribution to Project:	Dr. Abdel-Wahab designed and supervised the project.

Name:	Sisi Chen, PhD
Project Role:	Postdoctoral fellow
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	3 (rounded for this quarter; 3 person-months per year)
Contribution to Project:	Dr. Chen performed in vitro and in vivo experiments.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Three grants for Dr. Konopleva have been completed that were listed on the active support in the original application:

Title: Targeting apoptosis in ALL with venetoclax and cytotoxic chemotherapy
Effort: 5%
Supporting Agency: Leukemia and Lymphoma Society
Grants Officer: Researchprograms@lls.org
Performance Period: 10/1/2016-9/30/2019
Funding Amount:
Project Goals: Perform initial clinical testing of the effectiveness of venetoclax in elderly B- and T-ALL patients in combination with low intensity conventional chemotherapy regimen to test the safety of the combination but also to get preliminary estimates of its effectiveness.
Specific aims: Aim 1: Test the safety and preliminary efficacy of venetoclax in elderly B- and T-ALL patients in combination with a low intensity conventional chemotherapy regimen. Aim 2: Test the ability of BH3 profiling that has previously demonstrated the ability to predict clinical response to venetoclax in prior clinical trials. In addition, we will measure the expression of BCL-2, BCL-X_L and MCL-1 in untreated elderly cases of ALL and in phenotypically defined leukemia subsets using the mass cytometry, CyTOF.

Title: Acute Myeloid Leukemia in the Immunosuppressed Microenvironment
Effort: 5%
Supporting Agency: CPRIT
Grants Officer: Patty Moore, PhD, Tel: 512-305-8491, Email: pmoore@cprit.texas.gov
Performance Period: 8/31/2016-8/30/2020
Funding Amount:
Project Goals: To test the hypothesis that AML-induced bone marrow hypoxia interferes with the adaptive immune surveillance.
Specific aims: Aim 1: Investigate the hypothesis that OxPhos causes hypoxia and HIF-1 stabilization in AML cells and reprogramming of leukemia BME. Aim 2: To test the hypothesis by intravital microscopy that OxPhos and CXCR4 inhibition affect hypoxia and leukemia cell homing in specialized perivascular and endosteal niches. Aim 3: Test the hypothesis that CXCR4 blockade enhances the therapeutic efficacy of chemotherapy, FLT3-ITD and PD-1 inhibitors in AML.

Title: Center for Precision Medicine in Leukemia: Center for Genomics and Pediatric and Adult ALL
Effort: 2% effort/0.24 calendar

Supporting Agency: NIH/NCI
Grants Officer: Rochelle M. Long, PhD. Email: longr@nigms.nih.gov
Performance Period: 9/1/2015-6/30/2020
Funding Amount:
Project Goals: We will be extensively involved as co-investigators in Dr. Evans Project 2 - Identify genomic determinants of drug sensitivity and resistance in children and adults with newly-diagnosed and relapsed ALL. We will contribute primary adult ALL samples (at diagnosis and/or at relapse) to these studies of in vitro drug sensitivity and also to the integrated omic profiling.

Eight new grants for Dr. Konopleva are now active:

Title: Inhibition of Bcl-xl by Targeted Degradation
Supporting Agency: NIH/NCI
Performance Period: 4/1/2020-3/31/2025
Project Number: R01CA241191
Project Goals: We will test the effects of Bcl-Ps on frequencies of phenotypically defined LICs, by CyTOF (Cytometry by Time-of-Flight).
Overlap: None

Title: Overexpression of CD200 is a Stem Cell- Specific Mechanism of Immune Evasion in AML
Supporting Agency: MDACC Institutional Research Grant
Performance Period: 8/1/2020-7/31/2022
Project Goals: These findings will indicate utility of CD200 as a novel immune checkpoint target for the development of therapeutic strategies against cancer stem cells.
Overlap: None

Title: Characterization of CD70 expression and CD70 CAR T efficacy in vitro and in vivo with AML PDX models
Supporting Agency: Allogene
Performance Period: 1/27/2021-1/26/2023
Project Goals: To study expression of CD70 on PDX models and primary patient AML blasts and LSC. To study in vitro cytotoxicity with PDX cells and CD70 CAR T cells. To study in vivo efficacy studies with PDX models.
Overlap: None

Title: Assessing BCL-XL inhibitors in models of Acute Myeloid Leukemia (AML)
Supporting Agency: AbbVie
Performance Period: 1/11/2021-1/10/2023
Project Goals: Test the BCL-2 family protein and B7H3 expression in AML cell lines by flow and WB. Test the efficacy of ABT-199, ABBV-155, and the corresponding selective BCL-XL inhibitor A-1331852 and ABT-263, in AML cell lines, and correlates the response with cell lines genomic background, as well as the BCL-XL and B7H3 protein expression level.
Overlap: None

Title: IMGN632 in BPDCN 1
Supporting Agency: ImmunoGen

Performance Period: 5/5/2019-11/30/2021
Project Goals: IMGN632 in BPDCN 1
Overlap: None

Title: Targeting hypomethylating agent failure in myelodysplastic syndrome
Supporting Agency: Cancer Prevention & Research Institute of Texas (CPRIT)
Name of PD/PI: Colla, Simona
Performance Period: 3/1/2019-2/28/2022
Project Goals: This proposal aims to: 1) functionally validate novel therapeutic approaches to overcome HMA failure in MDS; and 2) identify predictive biomarkers of response and/or resistance to venetoclax in the setting of clinical trials.
Project Number: RP190295
Overlap: None

Title: 2014-0490 LSC assessment Konopleva Lab AML ABT-199 trial
Supporting Agency: AbbVie
Performance Period: 6/1/2020-5/18/2022
Project Goals: Bone marrow aspirate specimens collected from patients on Venetoclax trial in combination with hypomethylating agents (M14-358) will be used for these analyses.
Overlap: None

Title: Targeting Metabolic Rewiring in CLL- Timofeeva
Supporting Agency: CLL Global Research Foundation
Name of PD/PI: Gandhi, Varsha
Performance Period: 3/1/2020-1/31/2022
Project Goals: We want to exploit metabolic nexus of CLL cells. For this we will test agents that target different aspects of metabolism in CLL by targeted small molecule inhibitors of glutaminase, oxidative phosphorylation, etc in CLL cells.

Two grants for Dr. Lane have been completed that were listed on the active support in the original application:

Title:	Therapeutic targeting of the acute myeloid leukemia stem cell
Supporting Agency:	Doris Duke Charitable Foundation (DDCF)
Performance Period:	07/01/17-06/30/20

Title:	Identifying vulnerabilities in hematologic malignancies driven by heterotrimeric G proteins
Supporting Agency:	Gabrielle's Angel Foundation for Cancer Research
Performance Period:	05/01/16-04/30/20

Two new grants for Dr. Lane are now active:

Title:	Blastic plasmacytoid dendritic cell neoplasm (BPDCN): understanding disease biology to improve therapy

Supporting Agency:	The Leukemia & Lymphoma Society
Performance Period:	10/01/21-09/30/26
Project Goals:	This award provides only salary support to promote Dr. Lane's overall research program that spans basic, translation, and clinical investigation into BPDCN.
Overlap**:**	None

Title:	Blastic Plasmacytoid Dendritic Cell Neoplasm (BPDCN) Genomics and Therapeutic Target Identification
Supporting Agency:	Internal funding from Harvard Medical School – Bertarelli Rare Cancers Fund
Performance Period:	12/01/20-11/30/23
Project Goals:	Characterize and evaluate the function of gene fusions in the context of BPDCN genomics; Evaluate BPDCN ontogeny and disease heterogeneity via single cell analyses; Define BPDCN-specific dependencies using functional genetics.
Overlap:	None

Seven new grants for Dr. Abdel-Wahab are now active:

Title:	Interrogating the minor spliceosome to understand and treat leukemia
Supporting Agency:	NCI
Performance Period:	7/3/2020 - 6/30/2025
Project Goals:	Leukemias are cancers in which the bone marrow produces too many abnormal blood cells at the expense of normal blood cells. Here we will determine how a commonly occurring change in a gene called ZRSR2, which encodes a protein that participates in the process of RNA splicing, gives rise to leukemia. We will determine how ZRSR2 mutations drive leukemia and find new ways to treat leukemias with ZRSR2 mutations.
Overlap:	None

Title:	Genetic and molecular basis for SRSF2 mutations in myelodysplasia
Supporting Agency:	NHLBI
Performance Period:	8/20/2020 - 7/31/2024
Project Goals:	Measure the therapeutic efficacy of drugs that inhibit RNA splicing, and test whether drug induced splicing inhibition specifically affects SRSF2 mutation responsive exons versus causing widespread splicing failure.
Overlap:	None

Title:	The Memorial Sloan Kettering Cancer Center SPORE in Leukemia (Administrative Core)
Supporting Agency:	NCI
Performance Period:	9/1/2020 - 8/31/2025
Project Goals:	There are currently no approved or effective therapies for most acute myeloid leukemia (AML) subtypes. Our proposed SPORE in Leukemia has the potential to develop practice-changing approaches for AML evaluation and management and directly lead to improved patient outcomes for AML.
Overlap:	None

Title:	The Memorial Sloan Kettering Cancer Center SPORE in Leukemia (Project 3: Therapeutic inhibition of splicing through inhibition of protein arginine methylation)

Supporting Agency:	NCI
Performance Period:	9/1/2020 - 8/31/2025
Project Goals:	There are currently no approved or effective therapies for most acute myeloid leukemia (AML) subtypes. Our proposed SPORE in Leukemia has the potential to develop practice-changing approaches for AML evaluation and management and directly lead to improved patient outcomes for AML.
Overlap:	None

Title:	The Memorial Sloan Kettering Cancer Center SPORE in Leukemia (Career Enhancement Program)
Supporting Agency:	NCI
Performance Period:	9/1/2020 - 8/31/2025
Project Goals:	There are currently no approved or effective therapies for most acute myeloid leukemia (AML) subtypes. Our proposed SPORE in Leukemia has the potential to develop practice-changing approaches for AML evaluation and management and directly lead to improved patient outcomes for AML.
Overlap:	None

Title:	Exploiting Vulnerabilities in RNA Splicing to Treat Hematologic Malignancies Project 3: Therapeutic targeting of spliceosomal mutant leukemia through RBM39 degradation
Supporting Agency:	Leukemia & Lymphoma Society
Performance Period:	10/1/2021 - 9/30/2026
Project Goals:	Omar Abdel-Wahab will be responsible for overseeing and completing the work described in Research Project 3 entitled “Therapeutic targeting of spliceosomal mutant leukemia through RBM39 degradation.” This will entail preclinical experiments aimed at determining the basis for the requirement of RBM39 in malignant versus normal hematopoietic cells and performing a phase II trial of E7820 monotherapy for patients with relapsed/refractory myeloid malignancies with mutations in RNA splicing factors.
Overlap:	None

Title:	Developing Novel Therapeutic Approaches for Classical and Variant Hairy Cell Leukemia
Supporting Agency:	Leukemia and Lymphoma Society HCL
Performance Period:	10/1/2021 - 9/30/2025
Project Goals:	Aim 1: Define a new chemotherapy-free treatment for HCL patients. Our proposal will conduct a randomized clinical trial comparing vemurafenib plus obinutuzumab against standard chemotherapy for HCL (cladribine plus the anti-CD20 antibody rituximab). Aim 2: Test new therapies for both HCLc and HCLv patients who become refractory to standard treatments. Our proposal will test a new oral therapy, ulixertinib, which targets ERK kinases and is aimed at overcoming resistance to vemurafenib in HCLc while also targeting survival pathways in HCLv. Aim 3: Develop new therapeutic modalities such as immunotherapy and selective protein degraders to treat HCL. We will develop novel T-cell immunotherapies for HCL as well as the use of a completely new form of targeted therapy which degrades the mutant BRAF protein. Both of these therapeutic approaches are completely new strategies for the treatment of HCL and have the potential to be more effective and to further challenge the current standard treatments of this disease.
Overlap:	None

Six grants for Dr. Abdel-Wahab have been completed that were listed on the active support in the original application:

Title:	Mechanistic and Therapeutic Implications of Spliceosomal Gene Mutations in ER+ Breast Cancer
Supporting Agency:	Congressionally Directed Medical Research Programs
Performance Period:	8/15/2018 - 8/14/2021
Project Goals:	(1) Determine the mechanism by which the SF3B1 K700E mutation promotes hormone independent growth of breast cancer, (2) Examine the effect of SF3B1 K700E mutation on mammary tumorigenesis in vivo, (3) Determine the therapeutic benefit of modulating RNA splicing in genetically defined breast cancers with and without spliceosomal gene mutations.
Overlap:	None

Title:	Identification of transcriptional determinants of asparaginase sensitivity in leukemias
Supporting Agency:	Starr Cancer Consortium
Performance Period:	1/1/2019 - 12/31/2020
Project Goals:	Aim 1: Determine the precise mechanism by which ZBTB1 enables ALLs to survive under asparagine depletion. Aim 2: Examine the clinical significance of ZBTB1 in ALL and other lymphoid malignancies. Aim 3: Map the amino acid dependencies of blood cancer cell lines using DNA-barcoding technology.
Overlap:	None

Title:	Determining the Contribution of MDS-associated RNA Splicing Factor Mutations to Altered DNA Methylation
Supporting Agency:	Edward P. Evans Foundation
Performance Period:	9/1/2018 - 8/31/2020
Project Goals:	As part of this collaboration, our lab will perform analyses to integrate DNA methylation and hydroxy-cytosine methylation data with RNA splicing data. We will also attempt to understand how alterations in DNA methylation alter splicing through focused studies of splicing and expression of potential regulators of DNA methylation. In addition, we will perform locus-specific biochemical experiments to understand how alterations in the epigenome impact splicing.
Overlap:	None

Title:	Understanding and manipulating pathologic splicing preferences and nonsense-mediated mRNA decay (NMD) in spliceosomal-mutant MDS
Supporting Agency:	Henry & Marilyn Taub Foundation
Performance Period:	1/15/2017 - 1/14/2021
Project Goals:	Aim I. Determine the precise mechanism by which mutant SRSF2 promotes NMD. Aim II. Utilize antisense techniques to restore expression of mRNAs pathologically degraded by NMD in spliceosomal-mutant MDS cells. Aim III. Determine the therapeutic potential of oligonucleotide decoys that exploit the

	RNAbinding preferences of WT versus mutant SRSF2 in SRSF2-mutant malignant cells.
Overlap:	None
Title:	Origins of BRAF-mutant hematologic malignancies and their therapeutic resistance
Supporting Agency:	NCI
Performance Period:	9/1/16 - 8/31/2021
Project Goals:	Aim 1. Determine the mechanistic basis for the divergent phenotypes of BRAFV600E mutant hematopoietic disorders. Aim 2. Determine whether recurrent mutations co-occurring with the BRAFV600E mutation HCL and SH alter the disease phenotype. Aim 3: Define mechanisms of vemurafenib resistance in HCL using patient samples and murine models of BRAFV600E-mutant hematopoietic malignancies.
Overlap:	None
Title:	Investigating and Targeting Diverse Kinase Alterations Driving Systemic Histiocytic Neoplasms
Supporting Agency:	Leukemia and Lymphoma Society
Performance Period:	7/1/2016 6/30/2021
Project Goals:	Aim 1: Determine the clinical activity of cobimetinib in adult systemic histiocytic disorder patients as measured by radiologic (RECIST) response criteria, metabolic (FDG-PET) response criteria, and longitudinal assessment of somatic mutation burden in cell-free DNA (cfDNA). Aim 2: Identify the biological importance of mutations outside of BRAFV600E on histiocytosis pathogenesis and the molecular determinants of response to MEK inhibition.
Overlap:	None

What other organizations were involved as partners?
Nothing to Report

7. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

8. APPENDICES:

W81XWH-20-1-0683: Identifying Novel Therapeutic Targets and Combination Strategies for Patients with BPDCN

PI: Marina Konopleva, MDACC, TX; Andrew Lane, DFCI, MA; Omar Abdel-Wahab, MSKCC, NY

Budget: $890,843 **Topic Area:** Blood Cancers **Mechanism:** Translational Team Science Award - Clinical Trial

Research Area(s): SCS Coding

Award Status: 30 September 2020 – 29 September 2024

Study Goals:

This project aims to investigate mechanisms of response and resistance to the most advanced targeted therapies in BPDCN, assess potential for combination treatment in a clinical trial, and explore novel mechanism-based therapies targeting recurrently mutated genes in BPDCN involved in RNA splicing. **We expect our aims to provide clinical data for the next front-line treatment strategy for BPDCN patients and the preclinical basis to intelligently select the next targeted therapy approach for clinical testing in BPDCN.**

Specific Aims:

Specific Aim 1

Major Task 1: Test the hypothesis that in vivo resistance to tagraxofusp in BPDCN cells is mediated by epigenetic silencing of diphthamide synthesis genes.

Major Task 2: Test the hypothesis that azacitidine cooperates with tagraxofusp in primary BPDCN cells in vivo via its effects on DNA methylation and expression of diphthamide synthesis genes.

Specific Aim 2

Major Task 1 Test the hypothesis that targeting of CD123 with tagraxofusp or IMGN-623 primes BPDCN cells for apoptosis when combined with BCL2 inhibitor venetoclax and AZA.

Major Task 2 Determine the efficacy of combining anti-CD123 therapy with AZA/venetoclax in vivo

Major Task 3 Conduct a Phase 1b/2 clinical trial combining azacitidine, tagraxofusp, and venetoclax in patients with relapsed/refractory BPDCN and frontline patients unfit for induction chemotherapy

Specific Aim 3

Major Task 1: Determine the effects of perturbing splicing in BPDCN models with or without mutations in *ZRSR2* or *SRSF2*.

Major Task 2: Determine aberrant splicing events generated by mutant *ZRSR2* and *SRSF2* in BPDCN which promote transformation through a positive enrichment CRISPR screen.

Key Accomplishments and Outcomes:

Publications: Togami K, Chung SS, Madan V, Booth CAG, Kenyon CM, Cabal-Hierro L, Taylor J, Kim SS, Griffin GK, Ghandi M, Li J, Li YY, Angelot-Delettre F, Biichle S, Seiler M, Buonamici S, Lovitch SB, Louissaint A, Moran EA, Jardin F, Piccaluga PP, Weinstock DM, Hammerman PS, Yang H, Konopleva M, Pemmaraju N, Garnache-Ottou F, Abdel-Wahab O, Koeffler P, Lane AA. Sex-biased *ZRSR2* mutations in myeloid malignancies impair plasmacytoid dendritic cell activation and apoptosis. Cancer Discovery. 2021; doi: 0.1158/2159-8290. CD-20-1513. Online ahead of print. PMID: 34615655

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