IMGN779, A Next Generation CD33-Targeting ADC, Combines Effectively With Cytarabine in Acute Myeloid Leukemia (AML) Preclinical Models, Resulting in Increased DNA Damage Response, Cell Cycle Arrest and Apoptosis *In Vitro*, and Prolonged Survival *In Vivo*

1357

Sharlene Adams, Krystal Watkins, Lee J. Flaherty, Rebecca McCarthy, Alan Wilhelm, Leanne Lanieri, Callum M. Sloss and Angela Romanelli
ImmunoGen, Inc., Waltham, Massachusetts, United States

BACKGROUND

IMGN779, a CD33-targeting ADC comprised of an indolino-benzodiazepine mono-imine DNA-alkylating payload (DGN462) coupled at lysine residues by a cleavable N-succinimidyl 4-(2-pyridylditho)-2-sulfobutanoate (s-SPDB) linker to a novel huCD33-targeting antibody (Z4681A), is currently undergoing clinical evaluation in adult patients with relapsed/refractory CD33-positive acute myeloid leukemia (NCT02674763).

Cytarabine (ara-c) has been an important drug for the treatment of patients with AML for over 30 years and is included in the most common remission induction regimens.

With improved patient outcomes in mind, we set out to evaluate the mechanism and antileukemia efficacy of the combination of IMGN779 and cytarabine using *in vitro* and *in vivo* human AML preclinical models.

Methods

In vitro cytotoxicity was assessed at 96 hours in MV4-11 and Molm-13 cell lines using a WST-8-based cell viability assay (Dojindo Molecular Technologies). MV4-11 data are shown. Molm-13 results were similar.

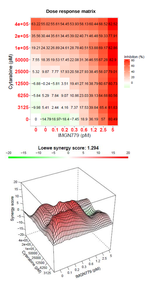
Apoptosis and DNA damage responses were measured at 48 hours by flow cytometry (Annexin V, Thermo Fisher Scientific; cCaspase3, Cell Signaling; cPARP, BD Biosciences) and immunoblotting methods (Cell Signaling antibodies). MV4-11 data are shown. Molm-13 results were similar.

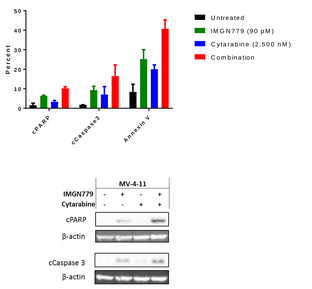
Cell cycle analysis carried out by flow cytometry techniques measuring the fluorescence of propidium iodide added to methanol-fixed AML cell lines (Thermo Fisher Scientific). MV4-11 data are shown. Molm-13 results were similar.

In vitro combinations of free IGN payload and either an MDM2 antagonist or a CHK1/2 inhibitor were assessed for impact on cell viability at 72 hours using the ATP-Lite proliferation assay (PerkinElmer) across a panel of 12 human AML cell lines. Synergy scores were calculated using proprietary software from Horizon Discovery.

IMGN779 and cytarabine were evaluated *in vivo* in disseminated (Molm-13, MV4-11) and subcutaneous (SC; EOL-1) AML xenograft models (6 mice/ group). A suboptimal IMGN779 dose was selected for each model based on prior single-agent dose-finding studies (data not shown). Assessment was terminated for weight loss >20%, clinical signs, or when EOL-1 SC tumor volume reached approximately 1000 mm^3. Disseminated model median survival (days) was determined for each group, and tumor growth delay (T-C) was calculated as median survival of drug treated group (T) - median survival of vehicle treated group (C). Percent Increased Life Span (%ILS) was calculated as [(T-C)/C] x 100%. For EOL-1, median tumor volume was determined per group; and %T/C = (median volume, treated group)/(median volume, vehicle group)x100%. Partial regression was a reduction in SC tumor volume of $\geq$ 50%. Complete response = 0 tumor volume.

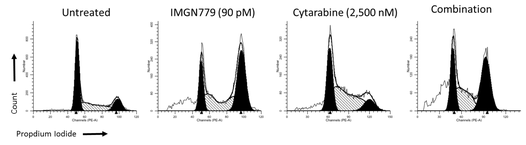
Combination of IMGN779 and cytarabine increases apoptosis and cell death in AML cells line *in vitro*



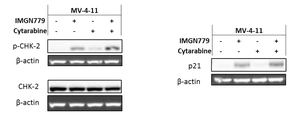


- Combination of IMGN779 and cytarabine induced additive or synergistic reductions in cell viability across a broad range of concentrations
- Combination of IMGN779 and cytarabine induced greater levels of apoptosis than either single agent alone

Combination of IMGN779 and cytarabine inhibits cell cycle progression in AML cell lines *in vitro*

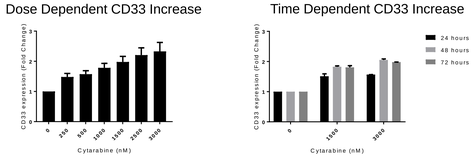


- IMGN779 arrests cells in the G2/M phase of the cell cycle while cytarabine induces accumulation of cells in S phase
- The combination of IMGN779 + cytarabine arrests cells in both S and G2/M phases of the cell cycle and increases the Sub-G0/G1 population due to the increased levels of apoptosis



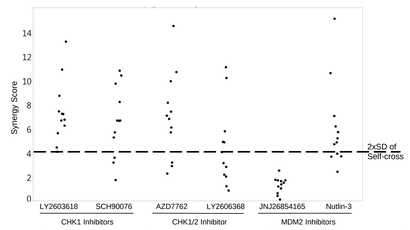
- The combination of IMGN779 + cytarabine increases p-CHK2 expression
- The combination of IMGN779 + cytarabine increases cyclin-dependent kinase inhibitor p21
- CHK1 and P53 were both phosphorylated in response to either treatment (data not shown)

Cytarabine increases CD33 expression in AML cell lines *in vitro*



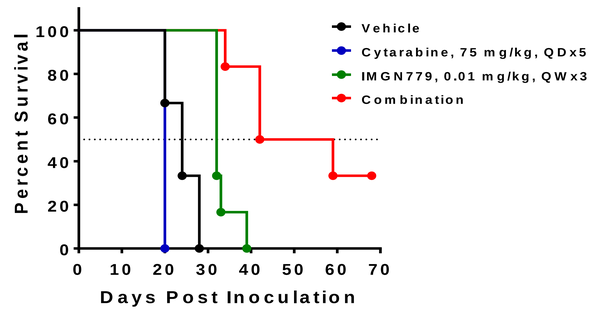
- Surface expression of CD33 increases in a time- and dose-dependent manner with cytarabine exposure

Free payload of IMGN779, DGN462, displays strong synergism with MDM2 and CHK1/2 inhibitors



- The IGN free payload DGN462 shows synergism with CHK1, CHK2, and MDM2 inhibitors in 12 AML cell lines confirming the DNA damage based MoA of the IMGN779 payload

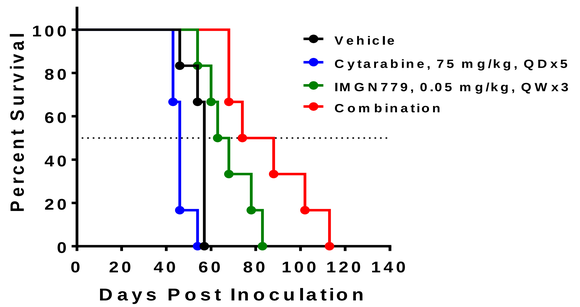
IMGN779 combines with cytarabine *in vivo* to increase survival in Molm-13 disseminated AML xenograft model



Treatment	Median Survival Time (Days)	Tumor Growth Delay (Days)	% Increased Life Span	Result
Vehicle	24	---	---	---
Cytarabine	20	0	0	Inactive
IMGN779 (0.2 µg/kg, DGN462)	32	8	33	Minimally Active
Combination	50.5	26.5	110	Highly Active

Treatments began day 7.

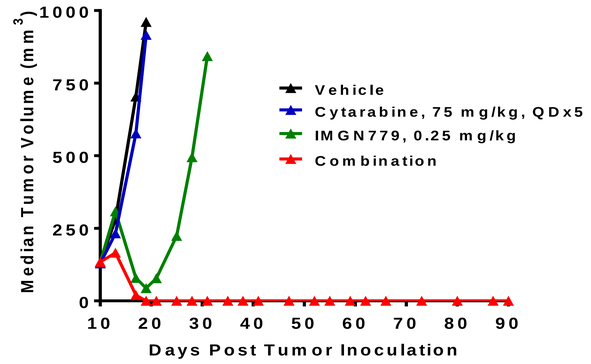
IMGN779 combines with cytarabine *in vivo* to increase survival in MV4-11 disseminated AML xenograft model



Treatment	Median Survival Time (Days)	Tumor Growth Delay (Days)	% Increased Life Span	Result
Vehicle	57	---	---	---
Cytarabine	46	0	0	Inactive
IMGN779 (1 µg/kg, DGN462)	65.5	8.5	15	Inactive
Combination	81	24	42	Active

Cytarabine treatment began day 24, and IMGN779 treatment began day 21.

IMGN779 combines with cytarabine to increase tumor-free survivors in EOL-1 subcutaneous AML xenograft model



Treatment	%T/C (Day 19)	Partial Response (PR)	Complete Response (End of Study)	Result
Vehicle	---	0/6	0/6	---
Cytarabine	95	0/6	0/6	Inactive
IMGN779 (5 µg/kg, DGN462)	5	2/6	2/6	Highly Active
Combination	0	6/6	4/6	Highly Active

Treatments began day 10; single dose of IMGN779.

CONCLUSIONS

- The combination of IMGN779 and cytarabine increased DNA damage response, cell cycle arrest and apoptosis *in vitro*, when compared to single agents.
- Cytarabine increased cell surface CD33 levels on AML cells, indicating a potential novel mechanism for potentiating IMGN779 uptake and efficacy.
- The combination of IMGN779 and cytarabine leads to increased survival and greater numbers of complete responses in *in vivo* preclinical AML models.
- These results support testing IMGN779 in combination with cytarabine and cytarabine containing regimens in clinical trials.

IMGN779 Clinical Poster, #1312: IMGN779, a Next-Generation CD33-Targeting Antibody-Drug Conjugate (ADC) Demonstrates Initial Antileukemia Activity in Patients with Relapsed or Refractory Acute Myeloid Leukemia; Saturday, December 9, 5:30-7:30 PM, Hall A2

Clinical Testing: NCT02674763: Open-label Study of IMGN779 in Adult Patients With Relapsed/Refractory CD33-positive Acute Myeloid Leukemia.

ASH, Dec. 9 to 12, Atlanta, GA

Conflict of Interest Statement: Sharlene Adams, Krystal Watkins, Lee J. Flaherty, Rebecca McCarthy, Alan Wilhelm, Leanne Lanieri, Callum M. Sloss and Angela Romanelli (Employment, ImmunoGen, Inc.).