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

PF201

Sharlene Adams[1], Qi Zhang[2], Rebecca McCarthy[1], Lee J. Flaherty[1], Vinitha M. Kuruvilla[2], Krystal Watkins[1], Callum M. Sloss[1], Angela Romanelli[1], Patrick A. Zweidler-McKay[1], and Marina Konopleva[2]

[1]ImmunoGen, Inc., Waltham, MA, USA; and [2]MD Anderson Cancer Center, Houston, TX, USA

INTRODUCTION

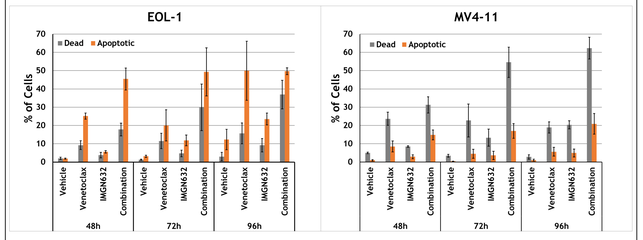
- IMGN632 is a CD123-targeting antibody-drug conjugate composed of a humanized anti-CD123 antibody, which binds to a unique IL-3 receptor epitope, and a novel DNA-alkylating IGN payload. IMGN632's IGN payload mono-alkylates DNA, inducing DNA damage and apoptosis, leading to cell death[1].
- IMGN632 is currently in Phase I dose escalation (NTC03386513) in patients with relapsed/refractory AML (acute myeloid leukemia) or BPDCN (blastic plasmacytoid dendritic cell neoplasm), demonstrating responses across a wide range of doses[2].
- Venetoclax, a BCL-2 inhibitor which lowers the apoptotic threshold in AML cells, was recently approved for elderly AML patients in combination with azacitidine.
- Combining these potentially synergistic anti-leukemic mechanisms in pre-clinical AML models, we hypothesized that BCL-2 inhibition would synergize with the pro-apoptotic effects of DNA damage induced by IMGN632.

Methods

In Vitro Studies: EOL-1 and MV4-11 AML cell lines were treated in triplicate with either vehicle control, IMGN632 (IC50 x5), venetoclax (EOL-1: IC50 x2, MV4-11: IC50), or IMGN632 plus venetoclax for 48, 72 and 96 hours. The percentage of dead cells (by fixable viability dye) and the percentage of apoptotic live cells (by fluorophore-conjugated, anti-active Caspase 3 monoclonal antibody) were quantified by flow cytometry. Additionally, EOL-1, KG-1, Molm-13, and MV4-11 were evaluated for IMGN632 and venetoclax cytotoxicity after 96 h (or 120 h, KG-1) using the WST-8 reagent, with each chemotherapeutic tested as a single-agent treatment and in combination. Each combination assay was repeated for at least three biореplicate runs in total. Data output were the average "surviving fraction" values. Combination Indices (CIs) were calculated in CalcuSyn by comparing the expected (additive) fraction affected value for a combination dose pairing to its observed fraction affected value. A CI in between 1.1 and 0.9 represent "additive" combination effects, while a CI in between 0.0 and 0.9 represent "synergistic" combination effects.

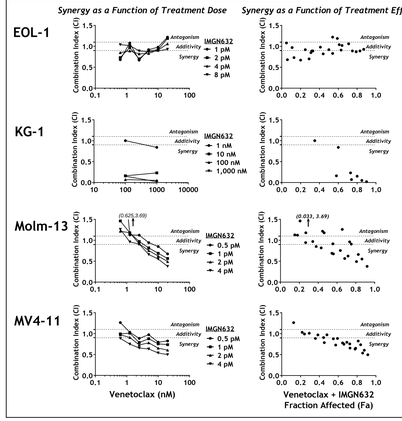
In Vivo Studies: Disseminated (Molm-13) and subcutaneous (sc; EOL-1, KG-1) AML CDX models and disseminated AML PDX models (3912018, 4203126) were established in immunodeficient mice. Mice were treated with: vehicle control; venetoclax daily for 28 days (Molm-13, EOL-1, KG-1) or for 22 days (PDX); IMGN632 weekly for 3 weeks; or the combination of both drugs. Treatment began after AML cell engraftment as follows: day 10 (EOL-1), day 7 (Molm-13), day 21 (MV4-11), day 48 (3912018), and day 41 (4023126) . Mice were terminated for weight loss >20%, clinical signs or, for sc models, for tumor volume ≥ 1000 mm^3. Median survival was determined for each group. Tumor growth delay (T/C) was calculated as [(median survival, Treated) – (median survival, Control)] and % Increased Lifespan was calculated as [(T-C)/ C] x 100%.

The combination of IMGN632 and venetoclax increases the percentage of both apoptotic and dead AML cells *in vitro*



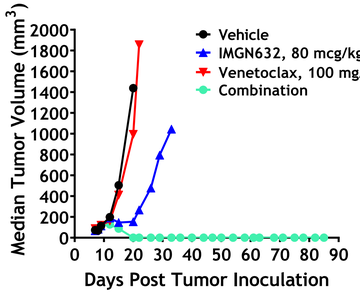
Dead cells (by viability dye): % of all cells; Apoptotic cells (by cleaved Caspase 3): % of live cells

IMGN632 plus venetoclax displays additive-to-synergistic *in vitro* combination indices across dynamic dose ranges



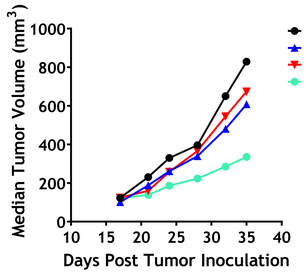
	Median CI (and Inter-Quartile Range)	Mean CI ± S.D.
EOL-1	0.90 (0.84 - 1.01)	0.91 ± 0.14
KG-1	0.15 (0.05 - 0.68)	0.31 ± 0.38
Molm-13	0.91 (0.66 - 1.17)	1.00 ± 0.64
MV4-11	0.79 (0.67 - 0.95)	0.81 ± 0.18

IMGN632 combines strongly with venetoclax in EOL-1 subcutaneous AML xenograft model



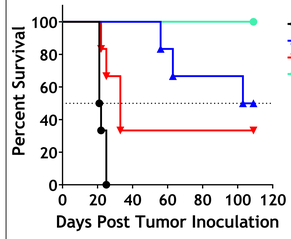
Group	% T/C (Day 18)	Result
Vehicle	------	------
IMGN632, 80 mcg/kg, single dose	11	Active
Venetoclax, 100 mg/kg, QDx28	69	Inactive
Combination	0	Highly Active

The IMGN632 plus venetoclax combination overcomes resistance in the KG-1 (p53, MDR1+) subcutaneous AML xenograft model



Group	% T/C (Day 35)	Result
Vehicle	------	------
IMGN632, 800 mcg/kg, QWx3	73	Inactive
Venetoclax, 100 mg/kg, QDx28	81	Inactive
Combination	40	Active

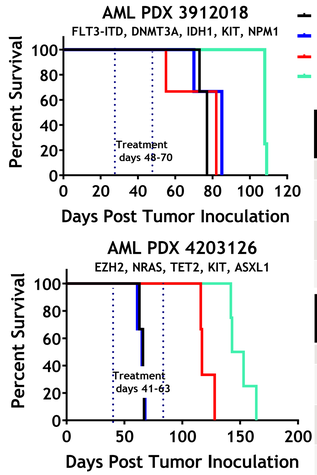
IMGN632 combines strongly with venetoclax in the Molm-13 (FLT3-ITD) disseminated AML xenograft model, prolonging survival



- Combination: No mice lost, day 109
- IMGN632: 3/6 mice lost, day 109

Group	Median Survival Time (Days	Tumor Growth Delay (Days)	% Increased Life Span	Result
Vehicle	21.5	------	------	------
IMGN632, 24 mcg/kg, QWx3	>109	>87.5	>407	Highly Active
Venetoclax, 100 mg/kg, QDx28	33	11.5	53.5	Highly Active
Combination	>109	>87.5	>407	Highly Active

IMGN632 combines strongly with venetoclax in AML PDX models, prolonging survival



3912018 Group	Median Survival Time (Days	Tumor Growth Delay (Days)	% Increased Life Span	Result
Vehicle	77	------	------	------
IMGN632, 24 mcg/kg, QWx3	85	8	10	Inactive
Venetoclax, 100 mg/kg, QDx22	82	5	6.5	Inactive
Combination	108	31	40	Active

4023126 Group	Median Survival Time (Days	Tumor Growth Delay (Days)	% Increased Life Span	Result
Vehicle	66	------	------	------
IMGN632, 24 mcg/kg, QWx3	65	0	0	Inactive
Venetoclax, 100 mg/kg, QDx22	117	51	77	Highly Active
Combination	148	82	124	Highly Active

CONCLUSIONS

- *In vitro*, the combination of IMGN632 and venetoclax increases the percentage of both apoptotic and dead AML cells.
- Median CI values across a dynamic range of IMGN632 and venetoclax *in vitro* treatments indicate additive-to-synergistic combinatorial cytotoxic effects in all four AML cell lines assessed.
- *In vivo*, the combination of IMGN632 and venetoclax prolongs survival and enhances anti-leukemic activity in subcutaneous and disseminated AML CDX models and in AML PDX models.
- These findings support testing the combination of IMGN632 and venetoclax (BCL-2 inhibition) in a clinical trial in AML patients.

References:
1. Kovtun et al., A CD123-targeting antibody-drug conjugate, IMGN632, designed to eradicate AML while sparing normal bone marrow cells. Blood Advances, 2018.
2. Daver et al., A Phase I, First-in-Human Study Evaluating the Safety and Preliminary Antileukemia Activity of IMGN632, a Novel CD123-Targeting Antibody-Drug Conjugate, in Patients with Relapsed/ Refractory Acute Myeloid Leukemia and Other CD123-Positive Hematologic Malignancies. Abstract 27, 60th Annual Meeting of the American Society of Hematology, Dec 1-4, 2018.