Abstract
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The Antibody-Drug Conjugate (ADC) IMGN779 Is Highly Active In Vitro and In Vivo Against Acute Myeloid Leukemia (AML) With FLT3-ITD Mutations

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INTRODUCTION

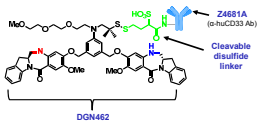
- IMGN779 is a CD33-targeted ADC utilizing DGN462, a novel DNA-alkylating agent consisting of an indolino-benzodiazepine dimer containing a mono-imine moiety.
- AML with mutations in FMS-like tyrosine kinase 3 (FLT3) are associated with poor prognosis; the most common FLT3 mutation is the internal tandem duplication (FLT3-ITD). Patients with FLT3-ITD AML have a worse prognosis than those with wild-type (WT) FLT3, with an increased rate of relapse and a shorter duration of response to induction chemotherapy.
- To investigate the activity of IMGN779 in FLT3-ITD AML, we evaluated the in vitro potency of IMGN779 against cell lines and primary patient AML cells with FLT3-ITD mutations, and the antitumor activity against both FLT3-ITD and FLT3 WT AML xenograft models.

IMGN779 Profile

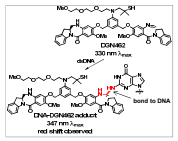
IMGN779 consists of a humanized anti-huCD33 antibody with approximately three DGN462 molecules attached per antibody using a disulfide linker. Toxicology studies in mice demonstrated:

- IMGN779 does not cause delayed toxicity - the mono-imine DGN462 effector molecule alkylates DNA without DNA-crosslinking. Delayed toxicity has been observed with ADCs employing DNA-crosslinking effector molecules.
- The cleavable disulfide linker avoids liver toxicity, the DLT observed with non-cleavable linker formats.

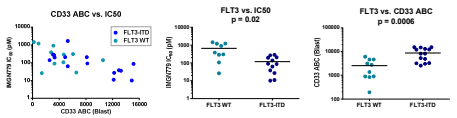
Structure of IMGN779



DGN462 Mechanism of Action

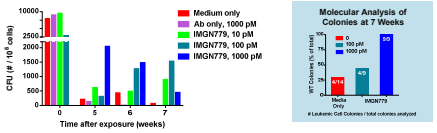


Patient AML Cells Are Highly Sensitive to IMGN779 In Vitro: FLT3-ITD Cells Are More Sensitive, Have Higher CD33 Expression



- CD33 expression level on primary patient AML blasts was determined by flow cytometry; cells were stained with a phycoerythrin (PE)-conjugated anti-CD33 antibody (BD Biosciences) and compared with a BD Quantibrite bead calibration curve to determine antigens per cell (ABC) on AML blasts (CD45/SSC).
- Primary patient AML samples were exposed to various concentrations of IMGN779 for 24 hours. Short-term liquid culture (STLC) assay to measure colony forming units after 10-14 days was performed to assess the cytotoxicity toward AML progenitor cells and determine IMGN779 IC_{50} values.

IMGN779 Causes a Dose-Dependent Decrease of Leukemic Colony Formation and Increase In Normal HSC Colonies In Long-Term FLT3-ITD/NPM1+ LSC Cultures



- Colony formation after long-term culture of AML cells demonstrates the activity of IMGN779 on leukemic stem cells (LSCs). Since AML progenitor cells are extinct by 5 weeks, colonies observed are stem cell-derived. Dose-dependent decrease in CFU immediately post exposure was observed.
- After 5 weeks, there was a dose-dependent increase in colony number. Colonies were analyzed at 7 weeks for the presence of FLT3-ITD and NPM1 molecular markers. The absence of AML molecular markers indicates wild-type (WT) colonies derived from normal hematopoietic stem cells (HSCs).

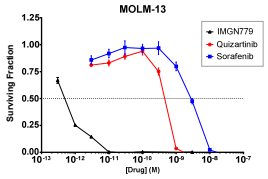
AML Cell Lines Are Highly Sensitive to IMGN779 In Vitro: FLT3-ITD AML Lines Are Among the Most Sensitive

Cell line	CD33 ABC	IMGN779 IC_{50} (pM)	FLT3 status
MV4-11	17757	2	FLT3-ITD
OCI-AML5	22417	3	WT
MOLM-13	44354	5	FLT3-ITD
EOL-1	7864	10	WT
HL60/QC	21000	16	WT
THP1	23557	20	WT
OCI-M1	55353	20	WT
HEL 92.1.7	39353	40	WT
TF1	66212	243	WT
OCI-AML3	1532	300	WT
TF1-α	34703	1000	WT
KG-1	6801	3000	WT
KASUMI-1	3727	3000	WT

Subset of results from a panel of 42 AML cell lines tested.
FLT3 status from http://cancer.sanger.ac.uk/cell_lines, http://www.broadinstitute.org/ccle, and ImmunoGen RNA-Seq testing

- Continuous exposure cytotoxicity for 4–7 days, WST-8 viability assay
- IMGN779 median IC_{50} = 70 pM (2 to 3000 pM range)
- CD33 expression ranged from ~1,000 – 66,000 antigens per cell
- The two FLT3-ITD AML cell lines were highly sensitive

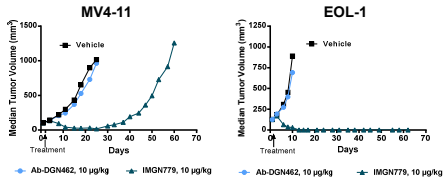
IMGN779 Is More Potent than FLT3 Inhibitors Quizartinib and Sorafenib Against FLT3-ITD AML Cell Line



Agent	IC_{50} (pM)
IMGN779	0.5
Quizartinib	500
Sorafenib	3000

- MOLM-13 FLT3-ITD AML cells were exposed continuously for 4 days to varying concentrations of IMGN779, quizartinib (FLT3 inhibitor) or sorafenib (multi-kinase inhibitor). Surviving fraction of cells was determined using a WST-8 viability assay

IMGN779 Is Highly Active and CD33-Specific Against MV4-11 (FLT3-ITD) and EOL-1 (FLT3 WT) AML Xenografts in Mice



Tumor Model	Treatment	T/C	PR	CR	Result
MV4-11 (FLT3-ITD)	IMGN779	1%	6/6	3/6	Highly Active
	Ab-DGN462	95%	0/6	0/6	Inactive
EOL-1 (FLT3 WT)	IMGN779	3%	6/6	5/6	Highly Active
	Ab-DGN462	78%	0/6	0/6	Inactive

CONCLUSIONS

- IMGN779 is highly active in vitro against primary patient AML samples, with increased potency observed against cells with FLT3-ITD mutations.
- The differential expression of CD33 on LSC compared to HSCs makes CD33 an attractive target for treatment of AML, with the potential to eliminate LSCs and, thus, minimal residual disease in FLT3-ITD AML.
- The activity of IMGN779 against AML cell lines *in vitro* and in xenograft models in vivo demonstrates that IMGN779 is active independent of FLT3 mutational status, and has high potency compared to FLT3 inhibitors in the clinic.
- These results support the advancement of IMGN779 as a potential treatment for AML, including FLT3-ITD AML.

Conflict of Interest Statements: Kathleen Whiteman, Russell Walker, Krystal Watkins, Yelena Kovtun, Lauren Harvey, Alan Wilhelm, Holly Johnson-Modafferi (Employment, ImmunoGen, Inc.); Paul Noordhuis, Gerrit J. Schuurhuis, Gert J. Ossenkoppele (Research funding, ImmunoGen, Inc.); Robert J. Lutz (Employment and equity ownership, ImmunoGen, Inc.).

56th Annual Meeting of the American Society of Hematology. Dec. 6 - 9, 2014