IMGN779, a CD33-Targeted Antibody-Drug Conjugate (ADC) with a Novel DNA-Alkylating Effector Molecule, Induces DNA Damage, Cell Cycle Arrest, and Apoptosis in AML Cells.

Abstract
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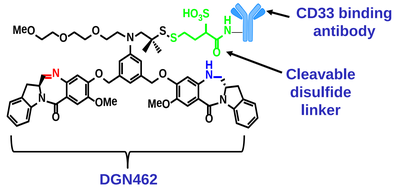
INTRODUCTION

- IMGN779 is a CD33-targeting ADC created for the treatment of acute myeloid leukemia (AML) and other CD33-positive malignancies.
- CD33 is broadly expressed on leukemic blasts of patients with AML, making it a promising target for this malignancy[1]. IMGN779 is highly active in AML cell lines and patient samples *in vitro* and causes complete regression of tumors in AML xenograft models *in vivo* with a favorable therapeutic index[2,3].
- To investigate the mechanism of action of DGN462 and of IMGN779, we used AML cell lines and primary patient AML samples to evaluate:
  - DNA binding
  - DNA alkylation versus crosslinking
  - Cell cycle effects
  - DNA damage signaling, apoptosis, and cell death

IMGN779 profile

- IMGN779 is comprised of a humanized anti-CD33 antibody, Z4681A, to which approximately three DGN462 molecules per antibody are conjugated using a cleavable disulfide linker.
- DGN462 is a member of the novel IGN class of DNA-acting cytotoxic agents and consists of an indolino-benzodiazepine dimer containing a mono-imine moiety.

Structure of IMGN779



DGN462 covalently binds DNA without crosslinking

- (A) DGN462 covalently adducts to DNA through its electrophilic imine moiety.
- (B) DGN462 does not crosslink DNA .

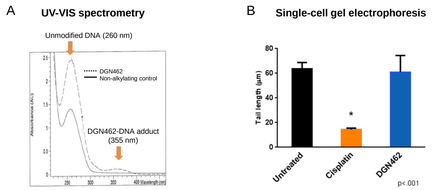
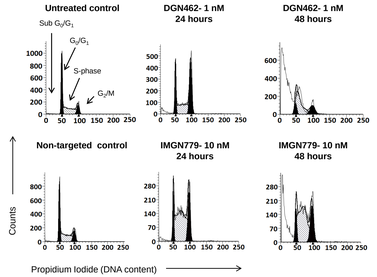


Figure A. EOL-1 AML cells were treated with 10 uM DGN462 or DGN495, a DNA binding but non-alkylating control, for 5 hours. Extracted genomic DNA was analyzed by UV-VIS.

Figure B. UV damaged lymphocytes treated with 50 nM DGN462 or 1.5 mg/mL cisplatin (a crosslinking agent) were run on an agarose gel in a modified comet assay. A decrease in tail length indicates drug-induced crosslinking of cellular DNA.

DGN462 and IMGN779 induce cell cycle arrest

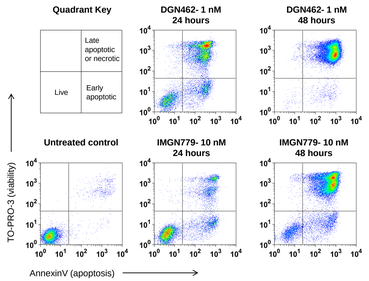
- Exposure to DGN462 or IMGN779 causes cell cycle arrest in G_2/M phase, S-phase accumulation, and appearance of a sub G_0/G_1 population.



HL60 AML cells (~21,000 CD33 receptors per cell) were exposed continuously to DGN462, IMGN779, or non-targeted control ADC (chKTI-sulfo-SPB-DGN462). EOL-1 and MV-4-11 cells showed similar results.

DGN462 and IMGN779 induce apoptosis and cell death

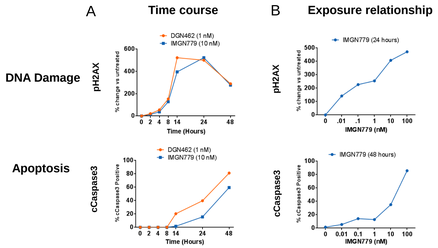
- Exposure to DGN462 or IMGN779 causes apoptosis and cell death.



MV-4-11 AML cells (~18,000 CD33 receptors per cell) were exposed continuously to DGN462 or IMGN779 for the indicated time periods. Similar results were obtained using EOL-1 and HL60 cell lines.

DGN462 and IMGN779 induce DNA damage response and apoptosis signaling

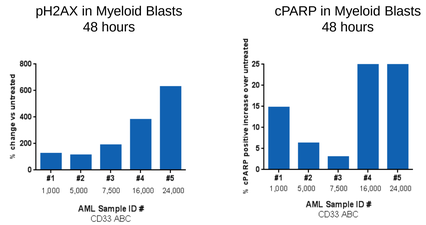
- (A) Phosphorylated H2AX and cleaved Caspase-3, markers of DNA damage and apoptosis, respectively, were induced in a time dependent manner following exposure to either DGN462 or IMGN779.
- (B) Marker changes were directly related to IMGN779 exposure.



MV-4-11 AML cells (~18,000 CD33 receptors per cell) were exposed to DGN462 or IMGN779 for 48 hours. Similar results were seen using EOL-1 and HL60 cells.

IMGN779 treatment induces a DNA damage response and apoptosis in patient-derived AML cells

- Induction of phosphorylated H2AX (DNA damage) and cleaved PARP (apoptosis) was detected in the myeloid blasts of 5 AML patient samples.
- In contrast, normal lymphocytes did not show a significant induction of either marker (not shown).
- IMGN779 targets CD33+ cell populations in AML patient white blood cells.



Primary AML patient samples were untreated or continuously exposed to 10 nM of IMGN779. Myeloid blasts and lymphocytes were gated using CD45/SSC. CD33 antigen expression (antigens bound per cell, ABC) was determined using BD Quantibrite beads (BD Biosciences).

CONCLUSIONS

IMGN779 is an ADC consisting of a humanized anti-CD33 antibody conjugated to DGN462, a novel DNA-alkylating IGN.

- DGN462 covalently binds to cellular DNA, without crosslinking, demonstrating it is a DNA-alkylating agent.
- DGN462 and IMGN779 are highly potent against AML cell lines *in vitro* and in primary patient AML samples, acting mechanistically via induction of DNA damage, cell cycle arrest, and apoptosis.
- IMGN779 is advancing to clinical testing for the treatment of relapsed/ refractory CD33+ AML. Pharmacodynamic studies utilizing phosphorylated H2AX as a biomarker will be implemented to verify the mechanism of this novel DNA alkylating payload.

References:

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2. Whiteman, et al. The Antibody-Drug Conjugate (ADC) IMGN779 Is Highly Active in Vitro and in Vivo Against Acute Myeloid Leukemia (AML) With FLT3-ITD Mutations. Abstract 2321, 56th Annual Meeting of the American Society of Hematology, Dec 6-9, 2014.
3. Whiteman, et al. IMGN779: A CD33-targeted antibody-drug conjugate (ADC) utilizing a novel DNA alkylator, DGN462, is highly active in vitro against primary patient AML cells and in vivo against AML xenografts in mice. 19th Congress- European Hematology Association, Jun 12-15, 2014

Conflict of Interest Statements: Krystal Watkins, Russell M. Walker, Nathan Fishkin, Charlene Audette, Yelena Kovtun, Angela Romanelli (Employment, ImmunoGen, Inc.).

57th Annual Meeting of the American Society of Hematology. Dec 5-7, 2015.