Plasma Pharmacokinetics and Tumor Accumulation in Mice of IMGN779, an Antibody-Drug Conjugate for Acute Myeloid Leukemia

Abstract **4504**

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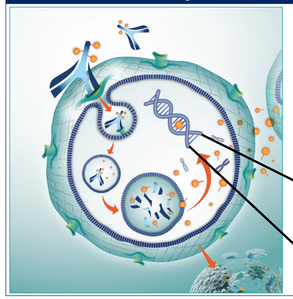
INTRODUCTION

IMGN779 is antibody-drug conjugate (ADC) in pre-clinical development for the treatment of acute myeloid leukemia (AML). IMGN779 is highly active *in vitro* against AML cell lines and primary patient AML cells and causes complete regression of AML xenograft tumors at non-toxic doses *in vivo*[1,2].

To understand the fate of IMGN779 upon dosing and cellular uptake, we performed:

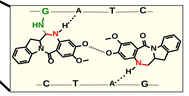
- *in vitro* catabolism studies on cultures of ADC-treated AML cells
- *in vivo* distribution studies in mice, including:
  - Plasma clearance
  - AML xenograft tumor accumulation

IMGN779 Composition and Mechanism of Action



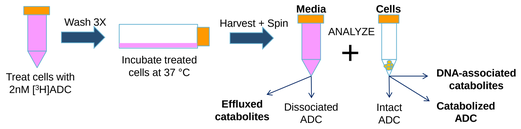
IMGN779 is composed of:

- Z4681A Antibody
  - Humanized monoclonal antibody targets CD33 receptors
- Sulfo-SPDB Linker
  - Charged, disulfide-cleavable
- DGN462 Cytotoxic Agent
  - DNA-alkylating payload, ~3 per Ab



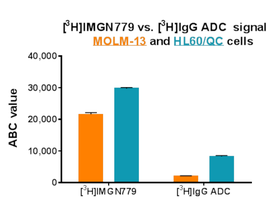
in vitro Cell Treatment Scheme

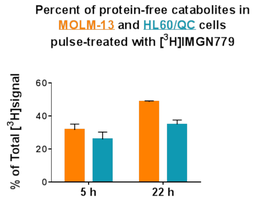
Cultures of AML cells were pulse-treated with 2 nM [^{3}H]IMGN779 or non-targeting IgG-sulfo-SPDB-[^{3}H]DGN462 control, washed extensively, and incubated at 37 °C.



IMGN779 Demonstrates CD33 Binding and Time-Dependent Catabolism *in vitro*

AML cells were treated with [^{3}H]ADC as described and resulting signal was converted to Antibody Bound per Cell (ABC). After 5 or 22 h incubation, cells and media were acetone-precipitated and analyzed for [^{3}H] signal, representing protein-free catabolized IMGN779.

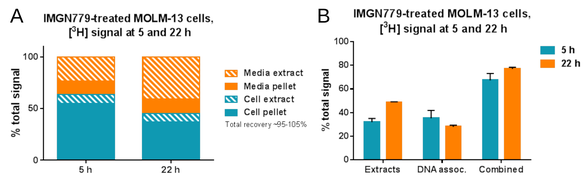




- MOLM-13 and HL60/QC express ~20,000 CD33 receptors per cell
- IMGN779 is efficiently catabolized, with protein-free catabolites effluxed from the cell, in a time-dependent manner

IMGN779 is Degraded Within Cells, With Catabolites Both Effluxed From Cells and Associated With DNA

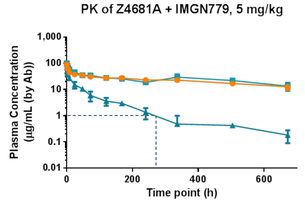
A) Cells and Media were separated upon harvest, acetone-precipitated and analyzed separately for [^{3}H]signal. Media extract represents effluxed catabolites, Media pellet represents dissociated IMGN779, Cell extract represents retained soluble catabolites, Cell pellet represents precipitable IMGN779 **and** DNA-associated species. B) In a separate set of replicates, genomic DNA was isolated from cells and analyzed for [^{3}H]DGN462 species. Total soluble catabolite values (media + cell extracts) are plotted with DNA-associated values.

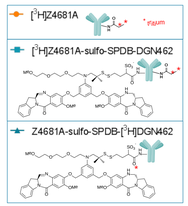


- Protein-free catabolites increase in a time-dependent manner
  - ~50% of IMGN779 signal by 22 h.
- DNA-associated [^{3}H]DGN462 species add to the total catabolite level
  - ~80% of IMGN779 signal by 22 h.

IMGN779 Demonstrates Antibody-Driven Clearance and Release of DGN462 Over Time

Liquid Scintillation Counting (LSC) was used to determine the plasma concentration of samples at various time points after a single 5 mg/kg IV injection of radiolabeled reagent.





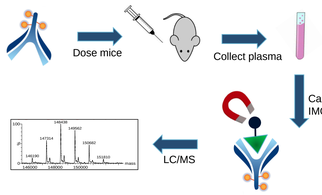
- Therapeutic concentration (1 µg/mL, 6 nM) retained past 10 d

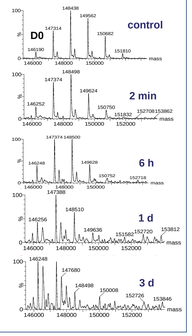
Sample	$t_{1/2}$ (days)	C_{max} (µg/mL)	$AUC_{0-\infty}$ (hr*µg/mL)	CL (mL/hr/kg)	V_{ss} (mL/kg)
[^{3}H]Z4681A	18.8	93.0	23,754	0.2	135.0
[^{3}H]Z4681A-sulfo-SPDB-DGN462	17.9	100.4	25,668	0.2	119.6
Z4681A-sulfo-SPDB-[^{3}H]DGN462	4.5	70.7	1,936	2.6	275.7

LC/MS of Affinity-Captured IMGN779 Confirms Release of DGN462 Over Time

Plasma from mice treated with 5 mg/kg IMGN779 was affinity-captured using purified CD33 Fc and analyzed by LC/MS

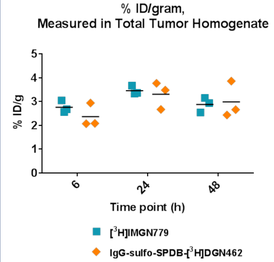
- The Mass-Distribution Profile of captured IMGN779 shows a decrease in higher-conjugated species over time

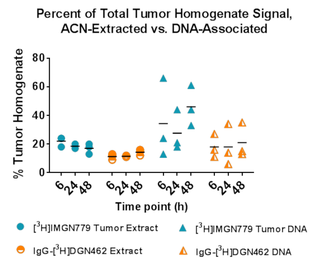




IMGN779 Demonstrates CD33-targeted Tumor Localization in HL60/QC Xenografts

Mice bearing HL60/QC tumors were dosed with 5 mg/kg [^{3}H]IMGN779 or control [^{3}H]ADC; Tumors were harvested at 6, 24, or 48 h, homogenized and counted by LSC either directly, after acetonitrile (ACN) precipitation, or DNA-isolation.





- Similar %ID/g levels of targeting and non-targeting ADC are found in total tumor homogenate at all time points
- Catabolism is CD33-specific: higher levels of protein-free and DNA-adducted DGN462 species with IMGN779 vs. control ADC

CONCLUSIONS

IMGN779 demonstrates CD33 target-mediated generation of DGN462 catabolites, and also exhibits DNA-modification consistent with the mechanism of action of its effector molecule, DGN462.

- *in vitro* studies demonstrate time-dependent generation of IMGN779 catabolites found within cells, adducted to DNA and effluxed to media
- IMGN779 demonstrates Ab-driven clearance and release of DGN462 over time *in vivo*
- IMGN779 undergoes CD33-specific catabolism *in vivo*

References:

1. 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
2. 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. Abstract P802, 19th Congress- European Hematology Association, Jun 12-15, 2014

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