IMGN632: AN ANTIBODY-DRUG CONJUGATE (ADC) OF A CD123-TARGETING ANTIBODY WITH A NOVEL DNA-ALKYLATING PAYLOAD IS HIGHLY ACTIVE AND PROLONGS SURVIVAL IN AML XENOGRAFT MODELS

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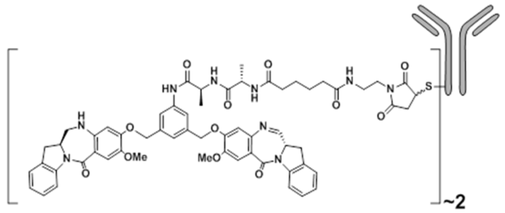
BACKGROUND

Targeted delivery of cytotoxic molecules by ADCs that recognize cancer-specific antigens is a promising therapeutic approach. CD123, the alpha subunit of the IL3 receptor, is an attractive cancer target implicated in AML cell survival and proliferation. CD123 is universally expressed on AML blasts, is differentially expressed on AML stem cells relative to normal hematopoietic cells, and is associated with aggressive disease. Here, we report the preclinical evaluation, in AML xenograft models, of IMGN632, a novel conjugate of a humanized anti-CD123 antibody with a novel IGN mono-imine payload that alkylates DNA without cross-linking. Mono-imine was selected over di-imine due to superior selective toxicity against AML progenitors *vs*. normal progenitors, and superior tolerability in mice.

Methods

Unique anti-CD123 antibodies were generated in mice through immunization with a human CD123-expressing cell line. Following antibody selection and humanization in the IgG1 format, IMGN632 was produced by conjugating a novel peptide-linked, DNA-alkylating, mono-imine payload at engineered cysteine residues, resulting in an ADC with a drug: antibody ratio of ~2. The *in vitro* cytotoxicity of IMGN632 or a non-targeted control ADC was evaluated on AML cell lines after continuous exposure for up to 7 days, and the IC50 was determined for each conjugate. Antitumor *in vivo* activity of IMGN632, or a non-targeted control ADC, was assessed in immunodeficient mice bearing disseminated or subcutaneous human AML xenografts. The maximum tolerated dose (MTD) of IMGN632 was determined by administering single IV injections of IMGN632 to CD-1 mice. ADC doses are expressed as mg/kg or µg/kg by antibody. Azacitidine and cytarabine are dosed at 75% of MTD in mice.

IMGN632 is an ADC combining an anti-CD123 antibody and a novel DGN549 IGN payload



- Antibody, G4723
  - Binds to CD123 with a sub-nMol affinity
- IGN Payload, DGN549
  - IMGN-generated single strand DNA alkylating mono-imine
- Linkage
  - Peptide, stable in circulation, cleaved intracellularly
  - Site-specific conjugation via engineered cysteines

IMGN632 is potent and CD123-specific against AML cell lines *in vitro*

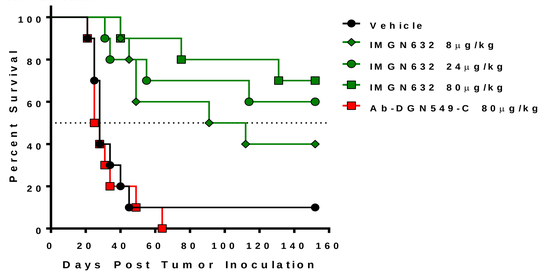
- Cell viability was assessed with WST-8 reagent after continuous up to 7 day incubation with ADCs

Cell Line	Poor Prognostic Factor	IMGN632 IC_{50} pMol	Control ADC IC_{50} pMol	Specificity Ratio IC_{50} Control ADC / IC_{50} IMGN632
CD123 negative cell lines				
Namalwa		10,000	10,000	1
K562		8,000	8,000	1
Cell lines with CD123 resembling AML patient levels				
EOL-1		2	2,000	1,000
Molm-13	FLT3-ITD	0.5	1,000	2,000
MV4-11	FLT3-ITD	1	700	700
KASUMI-3	P53 and MDR1	3	121	40

IMGN632 is highly active and CD123-specific in Molm-13 disseminated AML xenografts, resulting in a TI of ≥ 1000

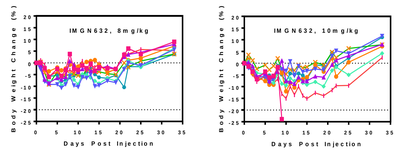
Molm-13 disseminated xenograft (FLT3-ITD):

- Nude mice injected IV with Molm-13 cells on Day 0 were randomized into study groups on Day 6 and were injected with 400 mg/kg of non-targeted human IgG1 antibody to block Fc receptors on the Molm-13 cells. On Day 7, the mice received a single IV injection of either vehicle or an ADC. On days 4 and 9 post-ADC dosing, the mice were injected with 100 mg/kg human IgG1 to maintain Fc receptor blockade on the Molm-13 cells.



Treatment Group	Median Survival Time (Days)	T-C (Days)	% ILS	Result
Vehicle	28	---	---	---
IMGN632 (8 µg/kg)	101	73	262	Highly Active
IMGN632 (24 µg/kg)	> 152	> 124	> 443	Highly Active
IMGN632 (80 µg/kg)	> 152	> 124	> 443	Highly Active
Ab-DGN549-C (80 µg/kg)	26.5	0	0	Inactive

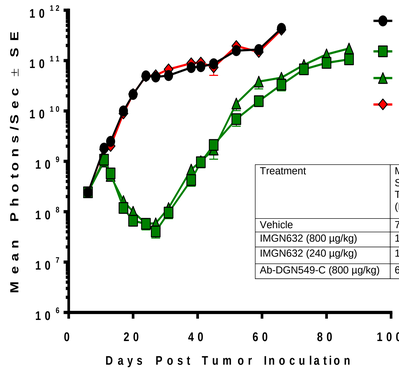
- Tumor Growth Delay (T-C), where T = median survival of the ADC-treated group, and C = median survival of the vehicle-treated group.
- % Increased Life Span (% ILS) = (T-C)/C X100%
- Therapeutic Index (TI) = MTD / Active dose in xenograft model



IMGN632 is highly active in disseminated AML xenografts with poor prognostic factors, reducing tumor burden and prolonging survival

Kasumi-3-Luc disseminated xenograft (p53, MDR1+):

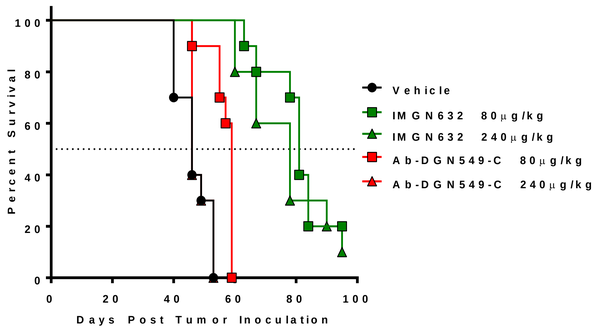
- NSG mice injected IV with Kasumi-3-Luciferase cells on Day 0 were randomized Day 6 based on tumor burden, as quantified with bio-imager, and were injected with 400 mg/kg of non-targeted human IgG1 antibody to block Fc receptors on the Kasumi-3 cells. On Days 7 and 41, the mice received single IV injections of either vehicle or an ADC. At 5 and 10 days post-ADC dosing, the mice were injected with 100 mg/kg human IgG1 to maintain the Fc receptor blockade on the Kasumi-3 cells.



Treatment	Median Survival Time (Days)	T-C (Days)	%ILS	Result
Vehicle	70	---	---	---
IMGN632 (800 µg/kg)	115	45	64	Highly Active
IMGN632 (240 µg/kg)	105	35	50	Highly Active
Ab-DGN549-C (800 µg/kg)	65.5	0	0	Inactive

MV4-11 disseminated xenograft (FLT3-ITD):

- NOD/SCID mice were pre-treated with 150 mg/kg cyclophosphamide on days -3 and -2, followed by injected IV with MV4-11 cells on Day 0. On Day 6 post-MV4-11 inoculation, mice were randomized into study groups and received an injection of 400 mg/kg of non-targeted human IgG1 antibody to block Fc receptors on the MV4-11 cells. On Day 7, the mice received a single IV injection of either vehicle or an ADC.

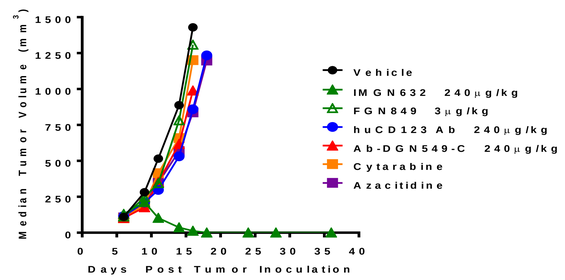


Treatment Group	Median Survival Time (Days)	T-C (Days)	% ILS	Result
Vehicle	46	---	---	---
IMGN632 (80 µg/kg)	81	35	76	Highly Active
IMGN632 (240 µg/kg)	78	32	70	Highly Active
Ab-DGN549-C (80 µg/kg)	59	13	28	Minimally Active
Ab-DGN549-C (240 µg/kg)	46	0	0	Inactive

IMGN632 demonstrates high activity in EOL-1 AML xenografts resistant to cytarabine and azacitidine

EOL-1 subcutaneous xenograft:

- Nude mice injected subcutaneously with EOL-1 cells on Day 0 were randomized on Day 6 into study groups by tumor volume and were injected with 400 mg/kg of non-targeted human IgG1 antibody to block Fc receptors on EOL-1 cells. At 24 h post-Fc receptor block, mice received single IV injections of either vehicle, an ADC free drug or naked antibody. On the same day as ADC treatment, other groups of mice began IP treatment with either cytarabine or azacitidine. On days 4 and 9 post-ADC dosing, the mice were injected with 100 mg/kg human IgG1 to maintain the Fc receptor blockade on the EOL-1 cells.



Treatment Group	% T/C (Day 16)	PR	CR	Result
Vehicle	---	0/8	0/8	---
IMGN632 (240 µg/kg)	1	8/8	8/8	Highly Active
FGN849 (3 µg/kg, free drug)	92	0/8	0/8	Inactive
huCD123 antibody (240 µg/kg)	60	0/8	0/8	Inactive
Ab-DGN549-C (240 µg/kg)	69	0/8	0/8	Inactive
Cytarabine; 75 mg/kg, qd x 5	84	0/8	0/8	Inactive
Azacitidine; 3.75 mg/kg, q3d x 5	59	0/8	0/8	Inactive

% T/C (% Tumor Growth Inhibition) = (Median tumor volume of test article-treated group)/ (Median tumor volume of vehicle-treated group) x 100%.

CONCLUSIONS

- **IMGN632 exhibits potent, CD123-specific *in vitro* activity against AML cell lines, including those with markers of poor prognosis.**
- **IMGN632 is well tolerated in mice at doses ≤ 8 mg/kg, and is highly active *in vivo* against Molm-13, Kasumi-3 and MV4-11 disseminated xenografts, resulting in prolonged survival and reduced tumor burden.**
- **IMGN632 achieves a high TI in the AML xenograft models.**
- **IMGN632 is highly active against EOL-1 subcutaneous xenografts, resulting in tumor regression and prolonged tumor-free survival in this cytarabine- and azacitidine-resistant model.**
- **These findings support advancing IMGN632 into clinical trials.**

Dr. Yelena Kovtun will be presenting additional IMGN632 preclinical data on Monday, Dec. 5, at 11:45 AM, at Oral Session # 616. Abstract # 768. Title: A CD123-Targeting Antibody-Drug-Conjugate (ADC), IMGN632, Designed to Eradicate Acute Myeloid Leukemia (AML) Cells While Sparing Normal Bone Marrow Cells

Conflict of Interest Statement: Sharlene Adams, Alan Wilhelm, Lauren Harvey, Chen Bai, Nicholas Yoder, Yelena Kovtun, Thomas Chittenden and Jan Pinkas (Employment, ImmunoGen, Inc.).

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