

Combining IMGN632, a Novel CD123-Targeting Antibody Drug Conjugate with Azacitidine and Venetoclax facilitates Apoptosis *in vitro* and Prolongs Survival *in vivo* in AML Models

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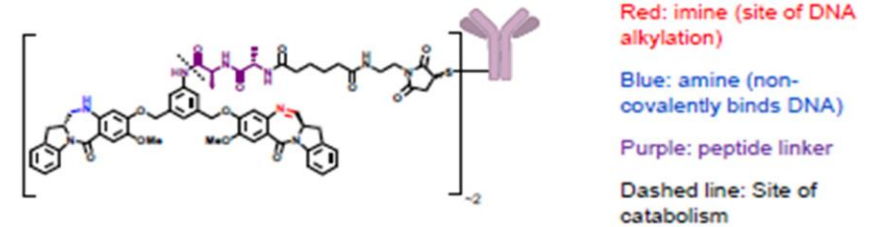
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Background

- IMGN632, a novel CD123-targeting ADC, demonstrates a favorable safety profile and clinical activity as a monotherapy in patients with relapsed/refractory AML and BPDCN (NCT03386513).
- We previously reported that the combination of IMGN632 with venetoclax (VEN, a BCL-2 inhibitor) induced additive or synergistic anti-leukemia effects in AML cell lines (EHA 2019, abstract #PF201)
- Given the FDA approval of the venetoclax and azacitidine (AZA, a hypomethylating agent) combination establishing this regimen as standard-of-care for elderly unfit AML patients, we further characterized efficacy of the triple combination of IMGN632, VEN and AZA in AML models *in vitro* and *in vivo* and the mechanism of synergy.

IMGN632

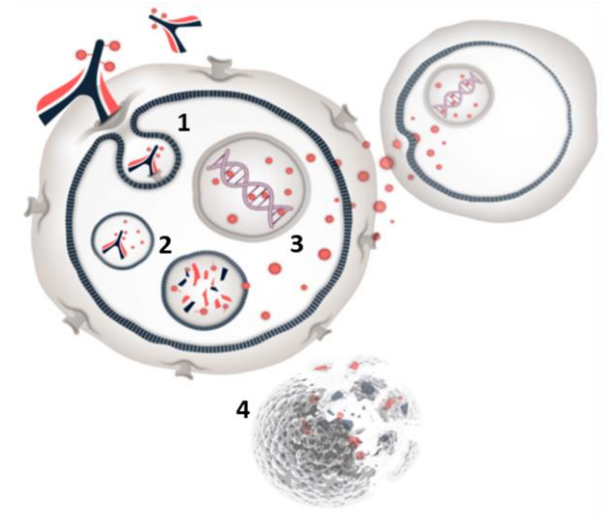
- CD123-targeting Antibody Drug Conjugate
- Composed of a humanized IgG1 antibody with high affinity to CD123.
- Highly potent payload, DGN549, alkylates DNA without cross-linking.
- Linker is a peptide cleaved intracellularly and is stable in circulation.
- Conjugation is site-specific via engineered cysteines.
- Two payload molecules per antibody.



Kovtun, Yelena. ASH 2016.

Antibody Drug conjugates (ADCs) with DNA alkylating payloads induce cell killing by:

1. Binding of ADC to the target and inducing internalization and uptake by the lysosomes.
2. The cytotoxic payload is released
3. Payload alkylates DNA
4. Induction of apoptotic cell death



Kovtun, Yelena. ASH 2016.

Methods

Cell Viability

- MOLM-13 and MV4-11 cells were treated with IMGN632 for 48h and VEN+AZA for 24 h
- Cell Viability was assessed using the luminescence-based CellTiter Glo® Luminescent cell viability Assay or CTG Assay.

Apoptosis

- MOLM-13 and MV4-11 cells were treated with IMGN632 for 48 h and VEN+AZA for 24 h. Cells were stained with Annexin V-FITC and DAPI to determine the degree of apoptosis by flow cytometry.

Western blot analysis

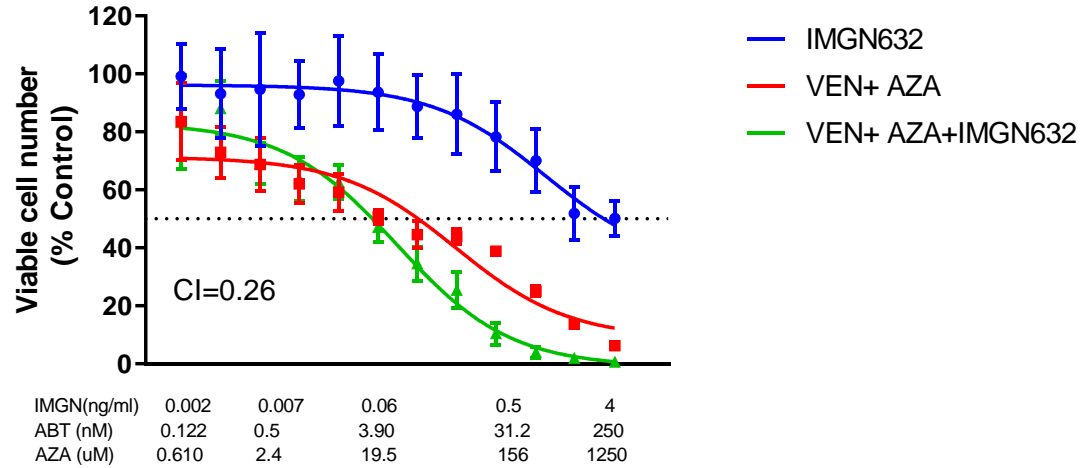
- AML cells were treated with IMGN632 for 48 h and VEN+AZA for 24 h and whole cell lysates were collected after treatment and subject to western blot analysis.

In Vivo studies

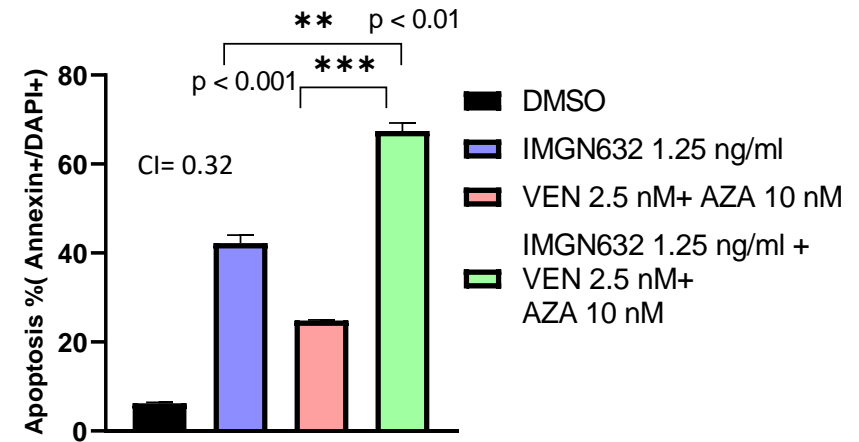
- NSG mice were injected with AML PDX cells and upon engraftment in peripheral blood, randomized mice into four cohorts to receive vehicle, IMGN632 (0.120 mg/kg once a week x 3 weeks), VEN (100 mg/kg, 5 days/week x 3 weeks) with AZA (2.5 mg/kg, daily x 5 days) or the triple combination.
- The endpoints included circulating leukemia burden monitored by hCD45/hCD123 flow cytometry of serial peripheral blood (PB) samples and mice survival.

Triple combination (IMGN632 + VEN + AZA) reduced cell viability and induced apoptosis in MOLM-13 and MV4-11 AML cells

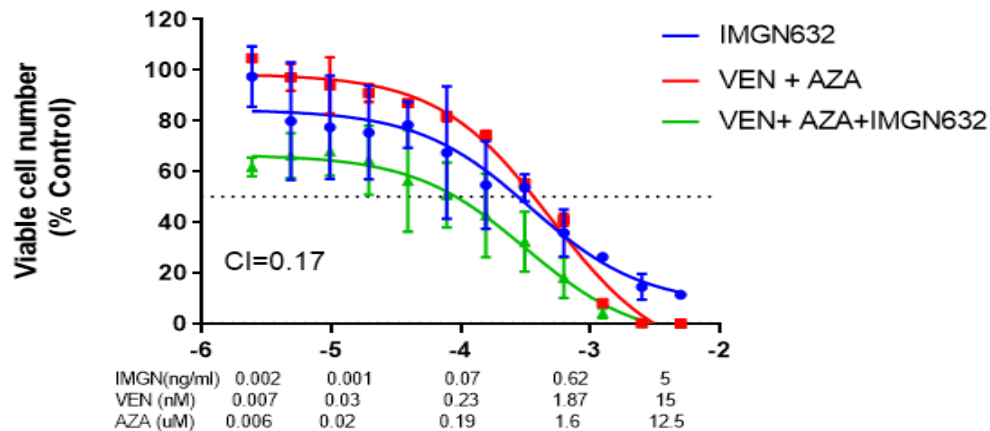
MOLM-13



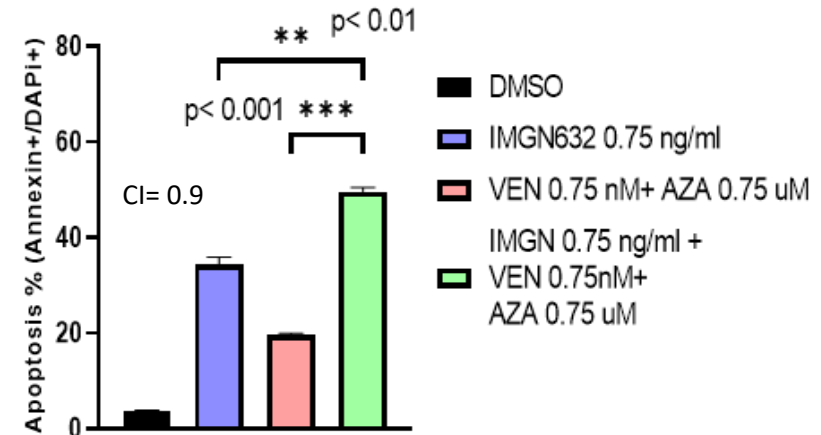
MOLM-13



MV4-11

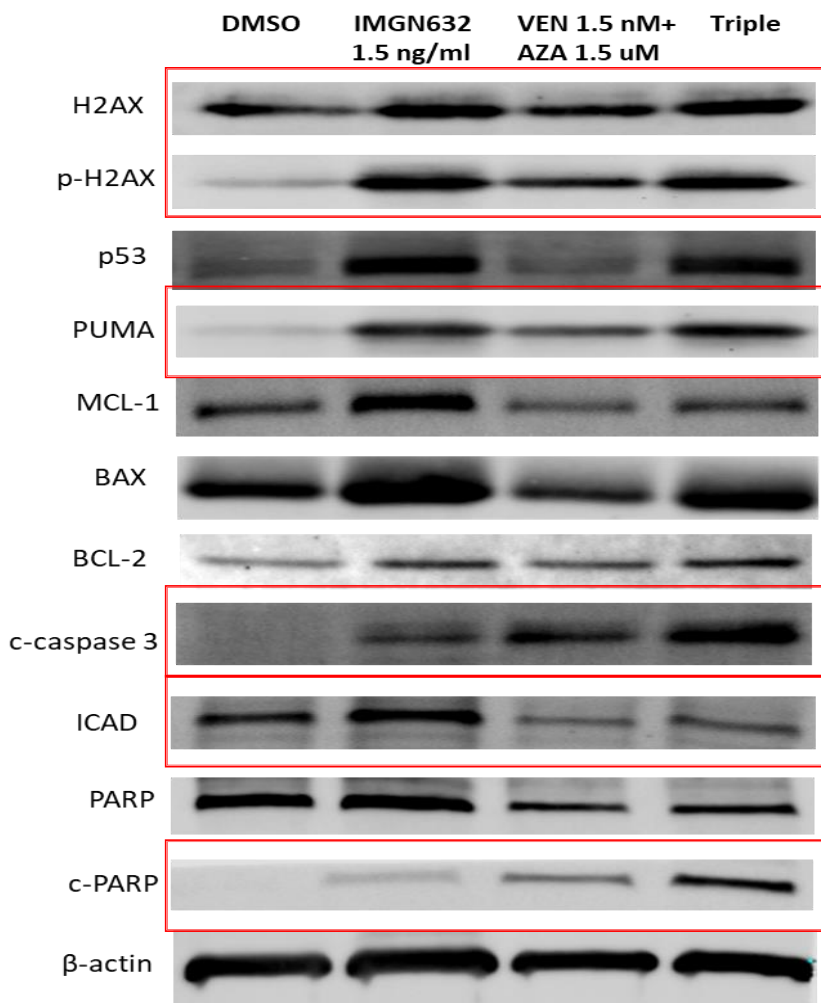


MV4-11

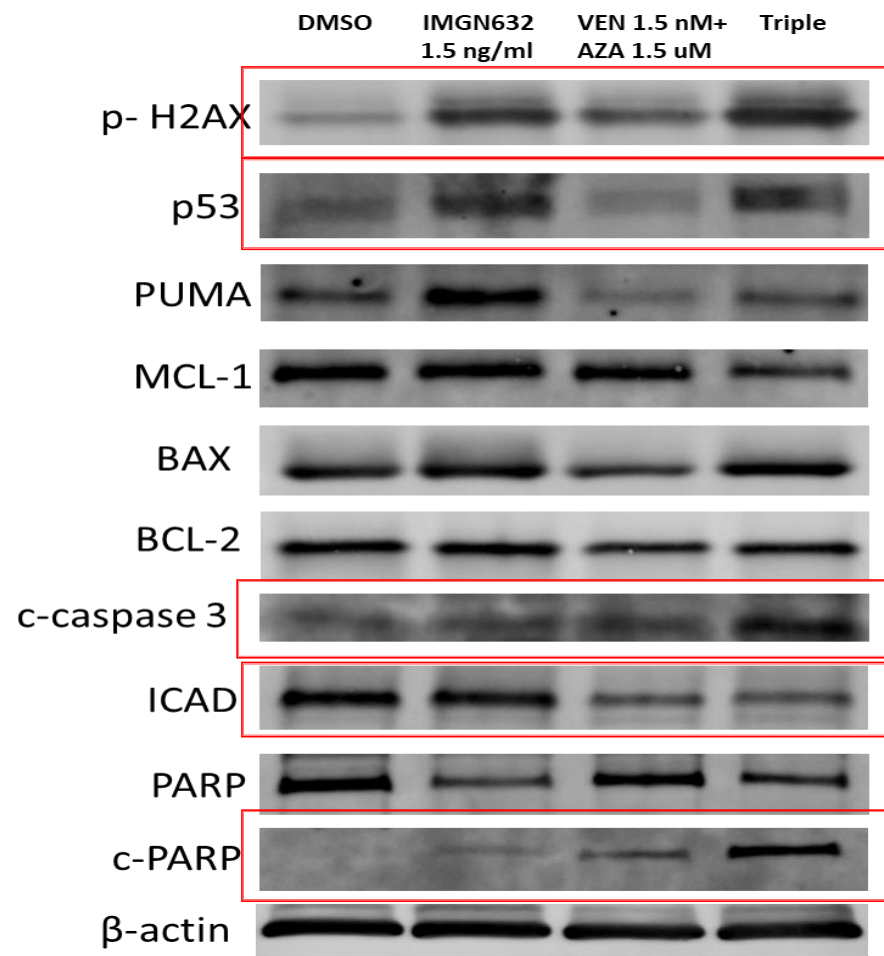


Triple combination induced upregulation of pro-apoptotic proteins and induced DNA damage in MOLM-13 and MV4-11 cells.

MV4-11 cells treated with
IMGN632 for 48h and VEN+AZA for 24h



MOLM-13 cells treated with
IMGN632 for 48h and VEN+AZA for 6h



Summary

- Combining IMGN632 with VEN and AZA induced synergistic cell death in AML cell lines and significantly improved survival in AML PDX models compared with AZA+VEN doublet or IMGN632 monotherapy
- The triple combination induced the expression of pro-apoptotic proteins: cleaved-PARP, cleaved-caspase-3, and PUMA, decreased levels of MCL-1 and ICAD, and induced apoptosis.
- IMGN632 is active in p53 knockdown cells and the triple combination induced increased DNA damage and apoptosis compared with single agent IMGN632 or VEN + AZA combination
- These data support the addition of a CD123-targeted ADC with a novel DNA-damaging payload to the standard of care, AZA+VEN in AML patients
- The combination of IMGN632 and Venetoclax and/or Azacitidine is being tested in an ongoing Phase Ib/II clinical trial (NCT04086264).

Disclosures

- Dr. Konopleva reports grants and other from AbbVie, grants and other from Genentech, grants and other from F. Hoffman La-Roche, grants and other from Stemline Therapeutics, other from Amgen, grants and other from Forty-Seven, other from Kisoji, grants from Eli Lilly, grants from Cellectis, grants from Calithera, grants from Ablynx, grants from Agios, grants from Ascentage, grants from Astra Zeneca, other from Reata Pharmaceutical, grants from Rafael Pharmaceutical, grants from Sanofi, outside the submitted work; In addition, Dr. Konopleva has a patent US 7,795,305 B2 CDDO-compounds and combination therapies with royalties paid to Reata Pharm., a patent Combination Therapy with a mutant IDH1 Inhibitor and a BCL-2 licensed to Eli Lilly, and a patent 62/993,166 COMBINATION OF A MCL-1 INHIBITOR AND MIDOSTAURIN, USES AND PHARMACEUTICAL COMPOSITIONS THEREOF pending to Novartis
- Vinitha Kuruvilla and Qi Zhang are employees of M.D.Anderson Cancer center.
- Krystal Watkins, Callum M. Sloss, Patrick A. Zweidler-McKay, and Angela Romanelli are employees of ImmunoGen, Inc.