Antileukemic Activity of CD33-Directed Mutation-Agnostic Lintuzumab-Ac225 in *KMT2A* Mutant AML

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INTRODUCTION

Acute myeloid leukemia (AML) is a highly heterogeneous hematologic malignancy with poor prognosis. Menin is critical for leukemogenesis in AML patient subsets driven by the rearrangement of the lysine methyltransferase 2A (*KMT2A*) gene (also known as mixed lineage leukemia, *MLL*) that can impair transcriptional networks. Small molecule inhibitors of menin-KMT2A have shown promise in treating eligible patients, but most patients eventually relapse.

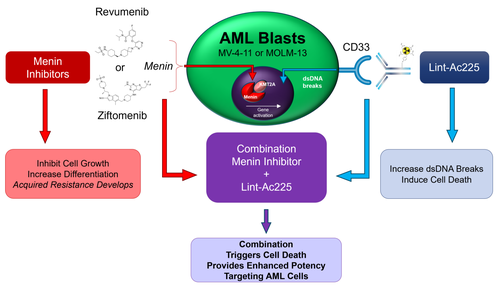
Combining KMT2A inhibition with an AML targeted radionuclide could leverage radiation-induced DNA damage to mitigate incomplete responses. We have shown that leukemic cells are broadly targeted by lintuzumab-Ac225, a CD33-directed monoclonal antibody conjugated with the alpha particle-emitting radionuclide actinium-225. In clinical trials, the addition of lintuzumab-Ac225 to chemotherapy regimen CLAG-M has shown substantial improvement in clinical outcomes in heavily pretreated relapsed/refractory AML patients, including venetoclax failures and those harboring TP53 mutations.

To investigate if AML patients with *KMT2A* rearrangement may benefit from lintuzumab-Ac225, we examined the anti-leukemic response in *KMT2A* mutant preclinical models.

AIMS

In this study, we evaluated the cytotoxic and anti-leukemic activity of lintuzumab-Ac225 in the *KMT2A* mutant AML cell line MV-4-11 as a single agent or in combination with menin inhibitors revumenib or ziftomenib.

Proposed AML mutation agnostic mechanism of Lintuzumab-Ac225



Model of lintuzumab-Ac225 mechanism in *KMT2A* mutant AML. The response to CD33 targeted radiotherapy and menin inhibitors is illustrated.

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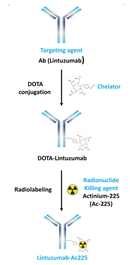
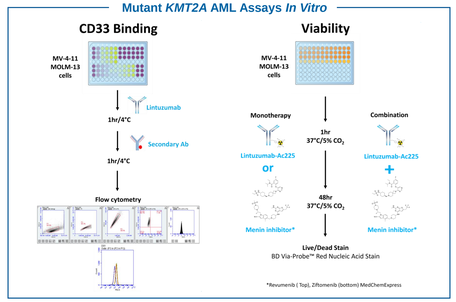
2. Papaemmanuil E, et al. Genomic classification and prognosis in acute myeloid leukemia. *N Engl J Med* 2016; 374: 2209-21
3. Winters AC and Bernt KM. MLL-rearranged leukemias- an update on science and clinical approaches. *Front Pediatr* 2017; 5: 4
4. Skopek R, et al. Choosing the right cell line for acute myeloid leukemia (AML) research. *Int J Mol Sci* 2023; 24: 5377

METHODS

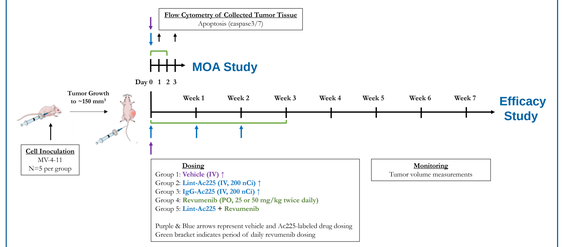
Preparation of Lintuzumab-Ac225



Mutant *KMT2A* AML Assays *In Vitro*



MOA and Efficacy Evaluation *In Vivo*



RESULTS

Lintuzumab-Ac225 binds to CD33 in *KMT2A* mutant AML

A.

Antigen	AML %	Reference
CD33	95%	1
Mutant KMT2A	3-10%	2, 3

B.

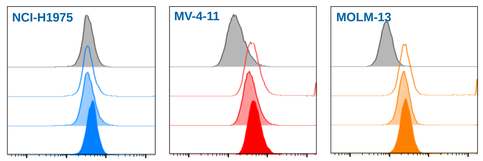


Figure 1. Lintuzumab binds to CD33 expressed in *KMT2A* mutant AML. (A) The reported percentage of CD33 expression and *KMT2A* rearranged human AML. (B) Flow cytometry histograms of lintuzumab (Lint), Lint-DOTA, and Lint-Ac225 binding in CD33 positive and *KMT2A* mutant harboring MV-4-11 (MLL-AF4) and MOLM-13 (MLL-AF9) AML cells (4). NCI-H1975 cells served as negative control to demonstrate CD33 binding specificity.

Lintuzumab-Ac225 has potent AML cell killing activity in *KMT2A* harboring cells

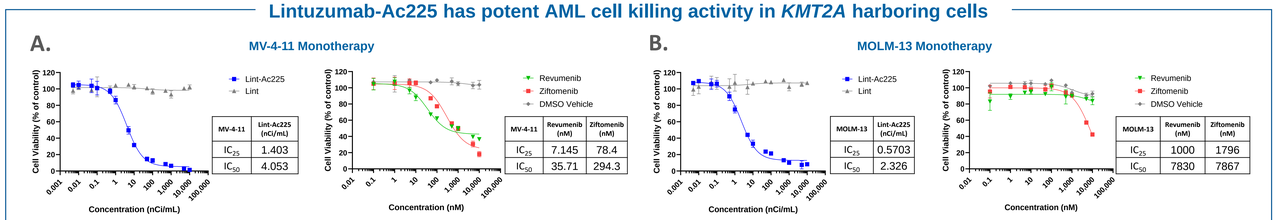


Figure 2. Lintuzumab-Ac225 (Lint-Ac225) and menin inhibitors reduce the viability of AML cells. The dose response to each monotherapy is shown in (A) MV-4-11 and (B) MOLM-13 cells after 48 hrs of drug treatment. Unlabeled lintuzumab (Lint) was used at mass equivalent concentrations to the Lint-Ac225 doses. Each sample was tested in triplicate. The viability IC_{50} and IC_{25} values were calculated by nonlinear regression to fit log (inhibitor) vs. response curve in GraphPad Prism. Control represents untreated cells. One representative of multiple experiments is shown.

Lintuzumab-Ac225 enhances AML cell death when combined with Menin inhibitors

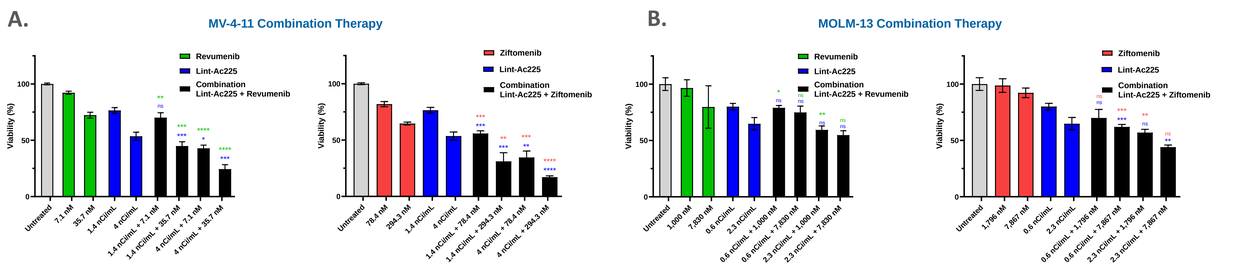
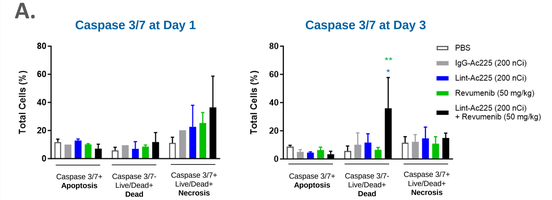


Figure 3. Combination of lintuzumab-Ac225 (Lint-Ac225) with revumenib or ziftomenib potentiates cytotoxicity. Dosing of each agent was selected based on the monotherapy data findings. After incubating cells with Lint-Ac225 for 1hr, revumenib or ziftomenib was added and viability measured after 48hrs treatment in (A) MV-4-11 and (B) MOLM-13 cells. Each sample was tested in triplicate. Multiple t test statistical analysis comparing combination therapy to each monotherapy (blue asterisk is Lint-Ac225, green asterisk is revumenib, red asterisk is ziftomenib) was performed with GraphPad Prism. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns=not significant, error bars represent SD; One representative of multiple experiments is shown.

RESULTS

Lintuzumab-Ac225 potentiates cell death in tumor-bearing mice when combined with Revumenib

A.



B.

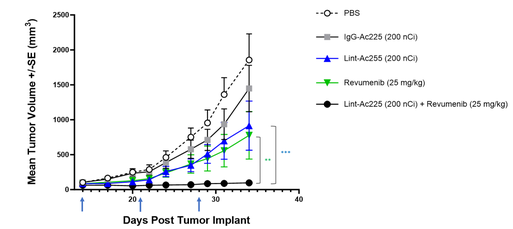


Figure 4. In vivo biological activity of Lint-Ac225 in female nude mice bearing human MV-4-11 AML xenografts. See methods section for study designs and dosing regimens. (A) Cell death analysis was performed on AML tumors collected on day 1 and day 3 post drug treatment by flow cytometry using the CellEvent Caspase 3/7 Detection Reagent. The combination of Lint-Ac225 and revumenib triggered a fast increase in cell-death and necrosis signal relative to single agent therapy. Statistical analysis using ANOVA in GraphPad prism comparing combination to Lint-Ac225 *p=0.0404 or revumenib **p=0.0087. (B) AML growth inhibition was observed in mice receiving either Lint-Ac225 or revumenib monotherapy. Anti-AML efficacy was significantly potentiated and prolonged by the drug combination. Statistical analysis was performed using ANOVA in GraphPad prism (****p<0.0001 Lint-Ac225 or revumenib vs PBS, ***p=0.0004 combo vs Lint-Ac225, **p=0.0024 combo vs revumenib). Blue arrows indicate Lint-Ac225 dosing; revumenib dosed for 3 weeks.

CONCLUSIONS

- Lintuzumab-Ac225 has potent anti-leukemic activity in AML cells harboring *KMT2A* genetic aberrations
- Combination of CD33-targeted radionuclide therapy with menin inhibitor significantly improves AML control, demonstrating that targeted radiotherapy approaches can augment menin-targeted therapy
- These findings, together with our clinical experience with lintuzumab-Ac225, warrant further evaluation as a mutation-agnostic therapeutic with backbone regimen potential in AML

CONTACT INFORMATION

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