soho2023
ELEVENTH ANNUAL MEETING

ANTILEUKEMIC ACTIVITY OF LINTUZUMAB-AC225 IN PRECLINICAL MODEL OF *FLT3* MUTANT AML

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INTRODUCTION

Lintuzumab-Ac225, an anti-CD33 antibody conjugated with actinium-225, has demonstrated promising therapeutic responses in patients with acute myeloid leukemia (AML), including in combination trials with chemotherapy regimen CLAG-M (NCT03441048). We believe the potent alpha particle emission associated with actinium-225 radionuclide is agnostic to genetic abnormalities commonly found in relapsed/refractory AML patients, including FMS-like tyrosine kinase-3 (*FLT3*) mutations. Although FLT3 inhibitors suppress the growth of leukemic blasts in patients, resistance mechanisms emerge. We hypothesized CD33-targeted radiotherapy would be an effective approach for targeting *FLT3* mutant AML in the context of FLT3 inhibition.

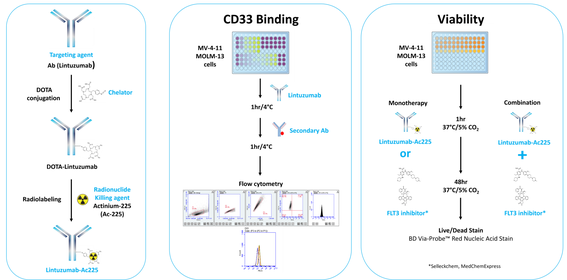
AIM

To evaluate the antileukemic activity of lintuzumab-Ac225 in *FLT3* mutant AML as a single agent and in combination with the FLT3-targeted inhibitors gilteritinib and midostaurin.

METHOD

Preparation of Lintuzumab-Ac225

Mutant *FLT3* AML Assays in vitro



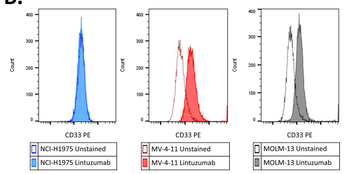
RESULTS

CD33 expression and mutant *FLT3* incidence in AML

A. Human AML

Antigen	AML %	Reference
CD33	95%	1
Mutant *FLT3*	~30%	2

B.



C.

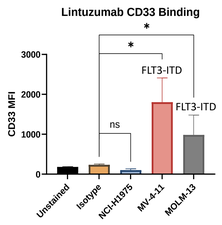
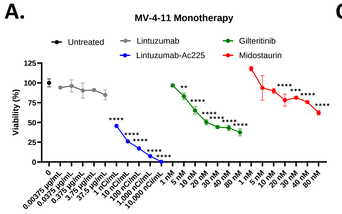


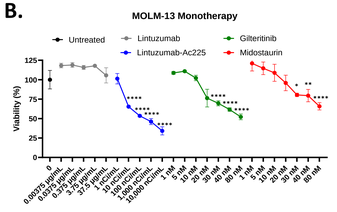
Figure 1. Lintuzumab binds to CD33 expressed in mutant *FLT3* AML. (A) The reported percentage of CD33 expression and mutant *FLT3* incidence (*FLT3-ITD, FLT3-TKD*) in human AML. (B) Flow cytometry histograms and (C) Mean fluorescent intensity (MFI) of lintuzumab staining in CD33 positive and *FLT3-ITD* mutant harboring MV-4-11 and MOLM-13 AML cells (3). NCI-H1975 cells served as negative control to demonstrate CD33 binding specificity. One-way ANOVA statistical analysis: *p<0.05, ns=not significant, error bars represent SD.

Lintuzumab-Ac225 has potent anti-AML activity

A.



B.



C.

Drug	MV-4-11 IC_{50}	MOLM-13 IC_{50}
Lint-Ac225 (nCi/mL)	0.74	8.00
Gilteritinib (nM)	6.34	51.14
Midostaurin (nM)	11.27	77.37

Figure 2. Lintuzumab-Ac225 (Lint-Ac225) and FLT3 inhibitors reduce the viability of *FLT3-ITD* AML cells. The dose response to each monotherapy is shown in (A) MV-4-11 and (B) MOLM-13 cells after 48hrs of drug treatment. Unlabeled lintuzumab was used at mass equivalent concentrations to the lint-Ac225 doses. Each sample was tested in triplicate. One-way ANOVA was used to determine significance of single therapy relative to untreated control, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, error bars represent SD. (C) The viability IC_{50} values calculated by nonlinear regression to fit log(inhibitor) vs. response curve in GraphPad Prism.

Lintuzumab-Ac225 combined with FLT3 inhibitors enhances AML cell death

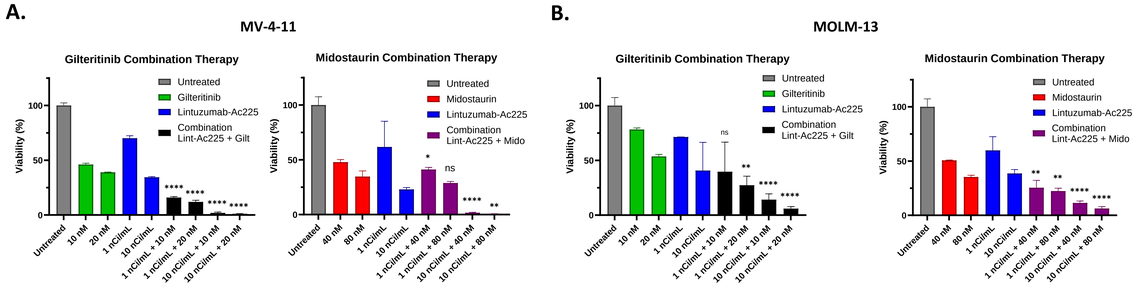


Figure 3. Lintuzumab-Ac225 (Lint-Ac225) potentiates cytotoxicity in combination with gilteritinib (Gilt) or midostaurin (Mido). Dosing of each agent was selected based on the monotherapy data findings. After incubating cells with lintuzumab-Ac225 for 1hr, gilteritinib or midostaurin was added and viability 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 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.

Proposed mechanism of action of Lintuzumab-Ac225 targeting CD33 in mutant AML

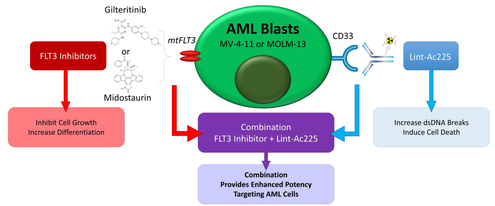


Figure 4. Model of lintuzumab-Ac225 mechanism in *FLT3* mutant AML. The response to mutant *FLT3* inhibitors and lintuzumab-Ac225 is illustrated.

CONCLUSIONS

- Lintuzumab-Ac225 has potent anti-leukemic activity against *FLT3* mutant AML and can significantly improve the effect of FLT3 inhibitors in combination
- These results support evaluating lintuzumab-Ac225 combination studies with FLT3 inhibitors in the clinical setting

REFERENCES

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CONTACT INFORMATION

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