Actimab-A (Lintuzumab-Ac225) has potent mutation agnostic antileukemic activity in preclinical models of AML

Amanda S. Chin[1], Sumit Mukherjee[1], Rubin Jiao[2], Kevin J.H. Allen[2], Jason Li[1], Karina Peregrina[1], Dina Yang[1], Debbie Lewis[1], Madhuri Vusirikala[1], Avinash Desai[1], Le-Cun Xu[1], Mackenzie Malo[2], Monideepa Roy[1], Denis Beckford-Vera[1], Ekaterina Dadachova[2], Adeela Kamal[1]

[1] Actinium Pharmaceuticals, Inc., New York, NY, USA [2] University of Saskatchewan, Saskatoon, SK, Canada

Abstract #594

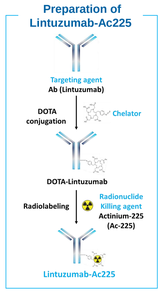
BACKGROUND

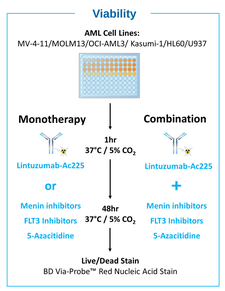
Acute myeloid leukemia (AML) is a complex and aggressive blood cancer with a poor prognosis, driven by genetic mutations that complicate treatment and often lead to relapse. Actimab-A (Lintuzumab-Ac225, Lint-Ac225) is a promising investigational antibody radio-conjugate that targets CD33. The payload, actinium-225 (Ac-225) is an alpha particle-emitting radionuclide that causes lethal DNA damage. In clinical trials, Lint-Ac225 had positive responses when combined with CLAG-M chemotherapy in relapsed/refractory AML patients, including those with high-risk features like venetoclax resistance and TP53 mutations. Here we tested the hypothesis that Lint-Ac225 has anti-leukemic activity in AML cells regardless of mutations (FLT3, NPM1, TP53 and KMT2A rearrangement). We also explored its potential as a single agent or in combination with other standard of care (SOC) therapies to enhance the treatment response for AML.

Antigen / Mutation	AML Prevalence	Standard of Care (SOC)	Reference
CD33	95%		1
KMT2A rearrangement	3 - 10%	Revumenib, Ziftomenib	2
FLT3	30%	Gilteritinib, Quizartinib	3
NPM1	30%	Revumenib, Ziftomenib	4
TP53	5 - 10%	5-Azacitidine	5

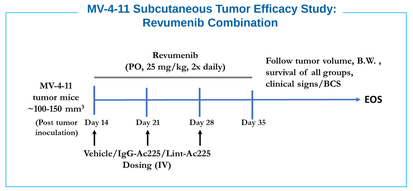
Table 1. The reported percentage of CD33 expression and mutations found in AML.

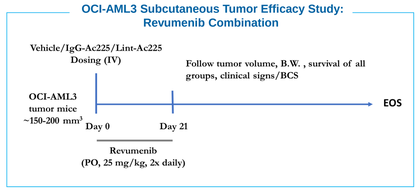
METHODS

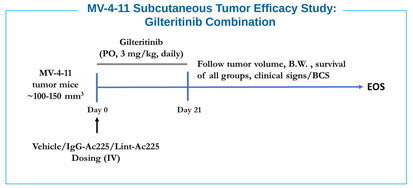


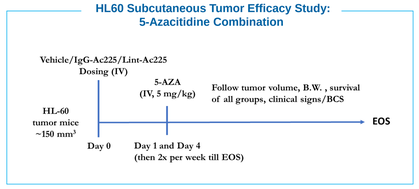


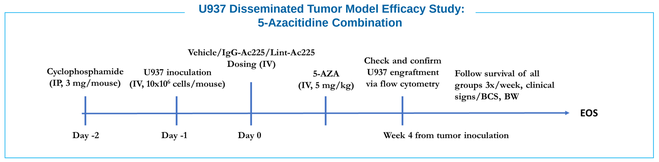
AML Cell Line Panel				
AML Cell Line	KMT2A	FLT3	NPM1	TP53
MV-4-11	MLL-AFF1	ITD		WT
MOLM-13	MLL-AF9	ITD		WT
OCI-AML3			NPM1	WT
Kasumi-1				R248Q
HL60				Deletion
U937				V173Wfs*59





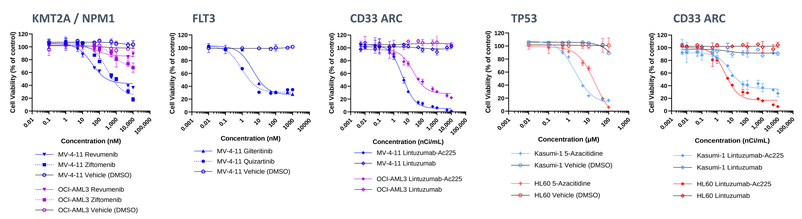






RESULTS

AML Cell Line Cytotoxicity by Lint-Ac225 or SOC Monotherapies



AML Cell Line	Menin Inhibitors				FLT3 Inhibitors				Targeting CD33	
	Revumenib (nM)		Ziftomenib (nM)		Gilteritinib (nM)		Quizartinib (nM)		Lint-Ac225 (nCi/mL)	
	IC25	IC50	IC25	IC50	IC25	IC50	IC25	IC50	IC25	IC50
MV-4-11	7.1	35.7	78.4	294.3	2.6	4.4	0.6	1	1.4	4
OCI-AML3	~1,000	~10,000	~1,000	~10,000					3.8	22.4

AML Cell Line	Chemo Agent		Targeting CD33	
	5-Azacitidine (μM)		Lint-Ac225 (nCi/mL)	
	IC25	IC50	IC25	IC50
Kasumi-1	1	2.2	2.2	7.3
HL60	8.6	21.1	0.5	3.5

Figure 1. Monotherapy Lint-Ac225 and SOC therapies reduce the viability of AML 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 IC50 and IC25 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.

Enhanced Cytotoxicity with Lint-Ac225 and SOC Combinations

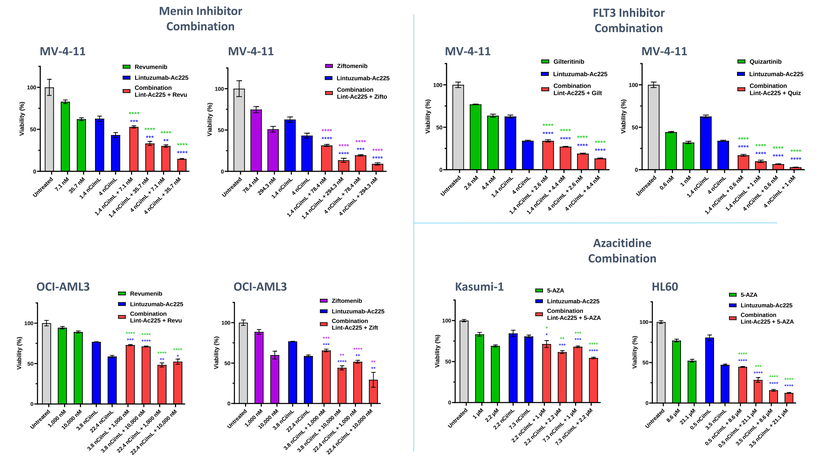


Figure 2. Combination of Lint-Ac225 with menin inhibitors (revumenib or ziftomenib), FLT3 inhibitors (gilteritinib or quizartinib), or azacitidine (5-AZA) potentiates cytotoxicity. Dosing of each agent was selected based on the monotherapy data findings. After incubating cells with Lint-Ac225 for 1 hour, inhibitors were added, and AML cell viability was measured after 48 hours. Each sample was tested in triplicate. Multiple t-test statistical analysis comparing combination therapy to each monotherapy (asterisk colors match the compared monotherapy). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, error bars represent SD.

Lint-Ac225 Mechanism of Action

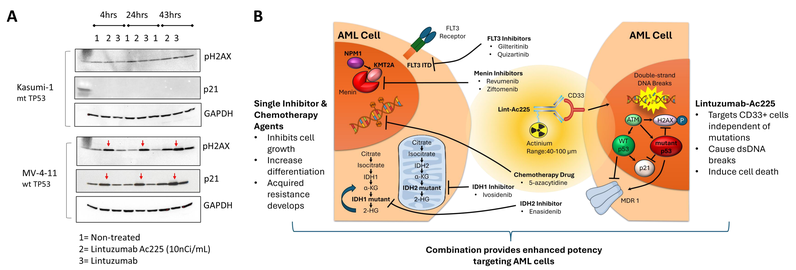


Figure 3: DNA stress and damage signaling in response to Lint-Ac225. (A) Lint-Ac225 induces phosphorylation of H2AX (pH2AX) and p21 protein levels in the wild type TP53 MV-4-11 cells but not mutant TP53 Kasumi-1 cells by western blot. (B) Illustration of Lint-Ac225 mechanism of action and the effects of combining with various inhibitors to address AML mutations.

Combination of Lint-Ac225 with Menin or FLT3 Inhibitors Potentiates AML Cell Death In Vivo

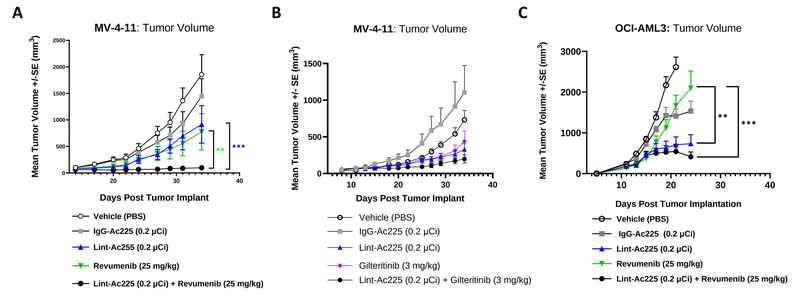
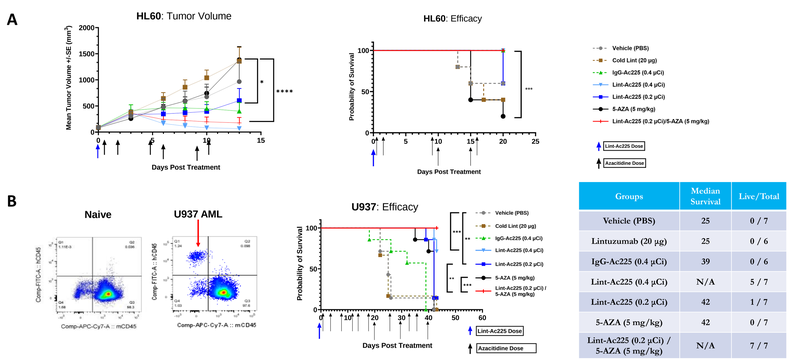


Figure 4. Anti-AML efficacy was significantly potentiated and prolonged by the drug combination in nude mice bearing human MV-4-11 and OCI-AML3 xenografts. Average tumor volume curve of each treatment group post-treatment with (A) combination of Lint-225Ac and revumenib, (B) combination of Lint-225Ac and gilteritinib, and (C) combination of Lint-225Ac and revumenib (in OCI-AML3) shows effective control of tumor growth in vivo.

Combination of Lint-Ac225 with Azacitidine Therapy Potentiates AML Cell Death In Vivo



Groups	Median Survival	Live/Total
Vehicle (PBS)	25	0 / 7
Lintuzumab (20 μg)	25	0 / 6
IgG-Ac225 (0.4 μCi)	39	0 / 6
Lint-Ac225 (0.4 μCi)	N/A	5 / 7
Lint-Ac225 (0.2 μCi)	42	1 / 7
5-AZA (5 mg/kg)	42	0 / 7
Lint-Ac225 (0.2 μCi) / 5-AZA (5 mg/kg)	N/A	7 / 7

Figure 5: Lint-Ac225 is efficacious as a monotherapy and in combination with azacitidine (5-AZA) in HL60 subcutaneous and U937 disseminated preclinical AML model. (A) HL60 average tumor volume curve and Kaplan-Meier plot showing % survival of each group post-treatment. (B) Survival (%) of the U937 disseminated model post-treatment. The black arrows indicate timings of 5-AZA dosing, and the blue arrow indicates the timing of Lint-Ac225 dosing. Statistical analysis of survival comparisons was performed using Log rank (Mentel-Cox) test in GraphPad Prism.

CONCLUSIONS

Actimab-A (Lintuzumab-Ac225) shows broad anti-leukemic activity in AML cell lines in a mutation (FLT3, NPM1, TP53 and KMT2A rearrangement) agnostic manner. It improves AML control in high-risk cases and enhances response durability when combined with standard of care treatments. These findings support its potential as a backbone therapy for relapsed/refractory AML, warranting further clinical evaluation.

REFERENCES

1. Khalidi HS, Medeiros LJ, Chang KL, Brynes RK, Slovak ML, et al. *Am J Clin Pathol* 1998; 109: 211-220
2. Papaemmanuil E, Gerstung M, Bullinger L, Gaidzik VI, et al. *N Engl J Med* 2016; 374: 2209-21
3. Skopek R, Palusinska M, Kaczor-Keller K, Pingwara R, et al. *Int J Mol Sci* 2023; 24: 5377
4. Falini B, Sciabolacci S, Falini L, Brunetti L, Martelli MP. *Leukemia* 2021; 35:3113-3126
5. Daver NG, Maiti A, Kadia TM, Vyas P, Majeti R, Wei AH, et al. *Cancer Discov* 2022; 11: 2516-2529