Abstract # 2664

LAM-003, a Novel Oral Heat Shock Protein 90 Inhibitor for Treatment of Acute Myeloid Leukemia, Including Wild-Type and FMS-like Tyrosine Kinase 3 (FLT3)-Mutant Disease

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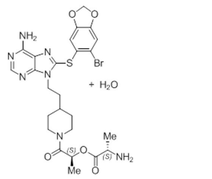
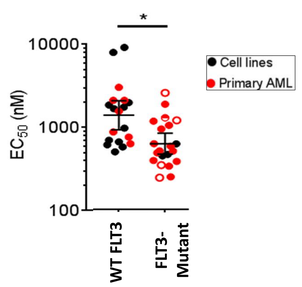
Introduction

- Acute myeloid leukemia (AML) is a heterogeneous disease characterized by abnormal proliferation and accumulation of myeloid cells in the bone marrow
- Mutation of the FLT3 receptor is one of the most common genetic alterations, occurring in ~30% of all patients with AML
- FLT3 inhibitors that target the constitutively active mutant receptor have recently been approved (e.g., midostaurin, gilteritinib)
- However, not all patients respond, and those who do inevitably relapse due to acquisition of secondary FLT3 mutations, up-regulation of other molecular pathways, or the influence of the bone marrow microenvironment
- In vitro studies demonstrate that inhibiting heat shock protein 90 (HSP90), a major chaperone protein, is effective in reducing AML blast viability
- We describe nonclinical studies of LAM-003, a novel, orally bioavailable HSP90 inhibitor prodrug, for the treatment of AML.

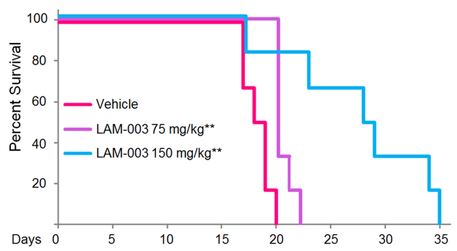
LAM-003 Displays Potent Activity in Wild-Type (WT) AML and in FLT3-Mutant AML



Conversion of pro-drug to active metabolite

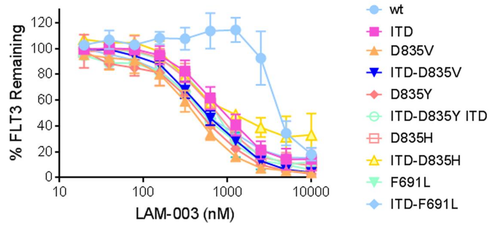


LAM-003 exhibits anti-leukemic activity in vitro.
Dot plot of average EC_{50} values in AML cell lines harboring WT FLT3 (WT) (n = 12) or FLT3-ITD (n = 3) or primary blasts (FLT3 WT, n = 7; FLT3-ITD and/or D835 mutation, n = 18) treated with LAM-003 for 72 hours. Viability was determined used CellTiter-Glo. Cell lines were tested in duplicate a minimum of 2 independent times, and primary samples were tested once, in duplicate. The geometric mean ± 95% confidence interval is shown. Open circles are primary samples harboring D835 mutations, half circles are primary samples harboring FLT3-ITD and D835 mutation.
*P = 0.05

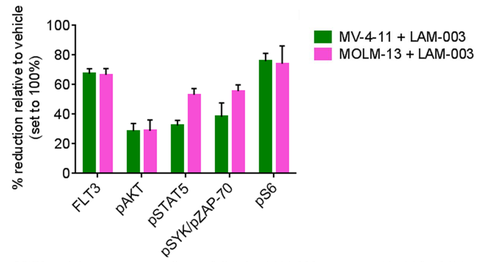


LAM-003 increases survival of mice in a MOLM-13 (FLT3-ITD) systemic AML model. Mice were inoculated with 1x10^7 MOLM-13 cells via tail vein injection for tumor development. 3 days post-inoculation, mice were randomized into 3 groups (n=6 animals per group) and dosed orally, once daily, with vehicle, or LAM-003 at 75 or 150 mg/kg. Significance between groups was determined using the Log-rank (Mantel-Cox) test. **P = 0.005 for LAM-003 75 mg/kg; **P = 0.008 for LAM-003 150 mg/kg.

LAM-003 Inhibition of HSP90 Results in Degradation of Mutant FLT3 and Blocks Oncogenic Signaling in FLT3-Mutant AML Cells

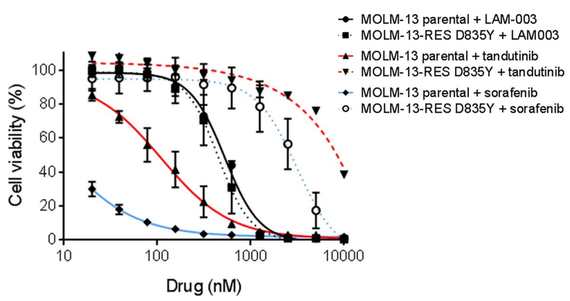


BA/F3 cells (murine cell line dependent on growth factor or expression of oncogene) were transfected with WT FLT3 or various FLT3 mutants. Cells were treated with increasing concentrations of LAM-003 for24h before being assayed for remaining cell surface expression of FLT3 (WT or mutant). Data shown is the mean ± SD from at least 2 independent experiments, each performed in duplicate.

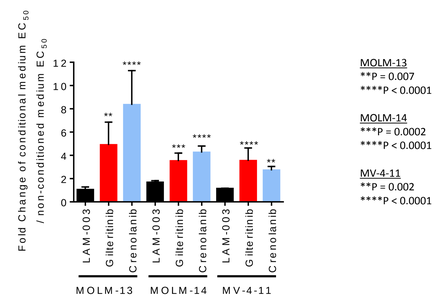


Reduction of FLT3 and downstream mediators following LAM-003 treatment (1 µM for 24h) of AML cell lines harboring FLT3-ITD mutations. Data shown is the mean ± SD from at least 2 independent experiments, each performed in duplicate.

LAM-003 Overcomes Conditions that Confer Resistance to FLT3 Inhibitors



LAM-003 overcomes mutations that confer FLT3i resistance. Parental MOLM-13 cells (solid lines) or MOLM13-RES cells expressing D835Y mutation (hatched lines) were treated with tandutinib, sorafenib or LAM-003 at the indicated concentrations for 72 hours before viability assayed using CellTiterGlo. Graph shown is the mean ± SD from at least 2 independent experiments, each performed in duplicate.



MOLM-13
**P = 0.007
****P < 0.0001

MOLM-14
***P = 0.0002
****P < 0.0001

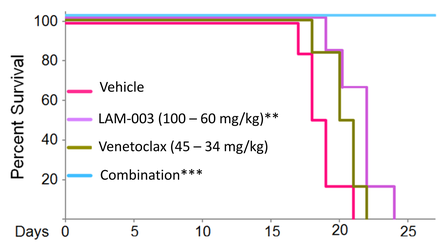
MV-4-11
**P = 0.002
****P < 0.0001

LAM-003 overcomes stromal factors that activate FLT3i resistance mechanisms. AML cell lines were treated with LAM-003, gilteritinib or crenolanib for 72 hours in either conditioned medium or non-conditioned medium before cell viability determined using CellTiterGlo. All comparisons were made to LAM-003 using 1-way ANOVA, Dunnett's multiple comparisons test. Data shown is the mean ± SD from at least 2 independent experiments, each performed in duplicate.

LAM-003 Displays Synergistic Activity with Venetoclax

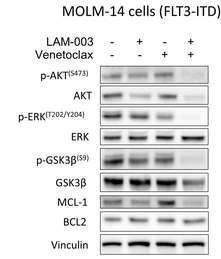
AML sample	Sample name	FLT3 Status	LAM-003 + Venetoclax Synergy (Combination Index Value)	BCL-2 levels (MFI)
Cell lines	MOLM-14	FLT3-ITD	0.6	43.2 ± 8
	MOLM-13	FLT3-ITD	0.3	40.5 ± 7
	MV-4-11	FLT3-ITD	0.7	41.7 ± 11
Primary Blasts	Y652	FLT3-ITD	0.24	ND
	Y588	FLT3-ITD	0.04	ND
Cell lines	OCI-AML-2	WT FLT3	0.59	59.5 ± 3
	OCI-AML-3	WT FLT3	0.35	44.5 ± 4
	ML-2	WT FLT3	0.55	44.7 ± 3
	ME-1	WT FLT3	0.59	98.5 ± 12
	TUR	WT FLT3	>1	4.4 ± 0
	MOLM-16	WT FLT3	0.92	2.2 ± 0
	U937	WT FLT3	0.9	10 ± 3

LAM-003 is synergistic in combination with venetoclax in WT FLT3 and FLT3-ITD mutant cells.
WT FLT3 (n=7), FLT3-ITD mutant cell lines (n = 3) or primary blasts (n = 2) were treated with 8 concentrations of LAM-003 alone, 8 concentrations of venetoclax alone or the combination of the two drugs (8x8) for 72 hours before viability assayed using CellTiter-Glo. Synergy was determined using the Chou-Talalay equation where combination index (CI) values <1 are synergistic, CI = 1 are additive and CI >1 are antagonistic. CI values show are from the average of 2 independent experiments, each performed in duplicate for cell lines, while the primary blasts were assayed once, in duplicate. CI values in green denote synergy, while those in red are not synergistic.
BCL-2 levels (MFI = mean fluorescence intensity) was determined in AML cell lines using flow cytometry. Cell lines were assayed in 2 independent experiments, each performed in duplicate. Data shown is the average MFI ± SD.
ND = not determined.

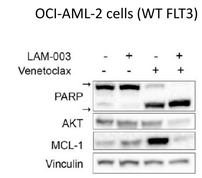


LAM-003 and venetoclax combination significantly improves animal survival in a MOLM-13 (FLT3-ITD) systemic AML model.
Mice were inoculated with 1x10^7 MOLM-13 cells via tail vein injection for tumor development. 3 days post-inoculation, mice were randomized into 4 groups (n=6 animals per group) and dosed orally, once daily, with vehicle, LAM-003 (100 – 60 mg/kg) alone, venetoclax (45 – 34 mg/kg) alone or the combination of the two. Significance between groups was determined using the Log-rank (Mantel-Cox) test. ** P = 0.008 for LAM-003 vs. vehicle; ***P = 0.0006 for drug combination vs vehicle.

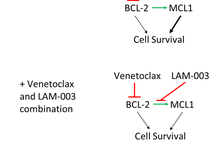
Mechanism of LAM-003 and venetoclax Synergy



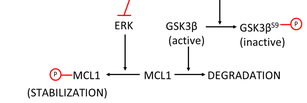
Western blot analysis of MOLM-14 cells treated with LAM-003 (1 µM), venetoclax (20 nM), or the combination for 24 hours. Lysates were probed with the indicated antibodies. Vinculin was used as a loading control. Representative blot shown from 2 independent experiments.



Western blot analysis of OCI-AML-2 cells (WT FLT3) treated with LAM-003 (625 nM), venetoclax (25 nM), or the combination for 24 hours. Lysates were probed with the indicated antibodies. Vinculin was used as a loading control. Representative blot shown from 2 independent experiments.



BCL-2 and MCL1 proteins are key in regulating AML cell survival. Upon BCL-2 inhibition, MCL1 levels increases as a compensatory mechanism and attenuates cell death. However, the addition of LAM-003 blocks the compensatory increase in MCL1 and cell survival is significantly compromised. Solid arrows denote 'active signaling' while dashed arrows denote compromised signaling



AKT and ERK are known positive regulators of MCL1 expression, by preventing degradation and increasing stabilization, respectively. Our Western blot studies suggest that the combination of LAM-003 and venetoclax inhibits both mechanisms. The combination degrades AKT and decreases AKT activity, resulting in active GSK3β (loss of inhibitory Ser9 phosphorylation) targeting MCL1 for degradation. In parallel, the combination treatment results in loss of ERK activity, in turn reducing MCL1 stabilization.

Conclusions

- LAM-003 displays antileukemic activity in both WT and FLT3-mutant AML, with preferential activity observed in FLT3-mutant cells
- LAM-003, through inhibition of HSP90, degrades FLT3-mutant receptor with mutations that confer resistance to FLT3 inhibitors
- LAM-003 disrupts stromal factor signaling that confer resistance to FLT3 inhibitors
- LAM-003 exhibits potent synergy with venetoclax in both WT and FLT3-ITD-mutant AML
- Based on these findings, a dose-ranging study is evaluating LAM-003 safety, pharmacokinetics, pharmacodynamics, and efficacy in patients with relapsed AML (NCT03426605)

Acknowledgements

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Disclosures

N.B., S.L., S.G., J.G., P.B., M.H., P.Y. and H.L. are employees at AI Therapeutics. T.X. is on the AI Therapeutics advisory board. J.R. is a Director of AI Therapeutics. L.M. is a consultant for AI Therapeutics. AI Therapeutics is the owner of LAM-003/LAM-003A patents.