2639

SYK Inhibitors Entospletinib and Lanraplenib Show Potent Antileukemic Activity in Combination With Targeted Agents

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Background

Spleen tyrosine kinase (SYK) acts as a key integrator of cellular signaling from surface contact and receptor tyrosine kinase (RTK) receptors containing an immunoreceptor tyrosine-based activation motif (ITAM).[1,2] In acute myeloid leukemia (AML), SYK serves as a relay to an oncogenic transcriptional regulatory network (TRN) linked to *HOXA9* and *MEIS1,* helping to suppress myeloid progenitor maturation and promote proliferation.[3]

The selective, oral SYK inhibitor entospletinib (ENTO) has demonstrated clinical activity in *HOXA9/MEIS1*-driven AML with acceptable tolerability when combined with intensive induction chemotherapy.[4]

Lanraplenib (LANRA) is a next-generation SYK inhibitor with similar potency, enhanced selectivity, and more favorable pharmacologic properties that is currently being evaluated in combination with gilteritinib in patients with relapsed or refractory (R/R) *FLT3*-mutated AML (NCT05028751).

Results

ENTO exhibits antiproliferative activity in *NPM1*m AML with associated *HOXA9/MEIS1* dysregulation in ex vivo drug sensitivity studies.[4] We hypothesized that SYK inhibition could synergize with other targeted therapies to enhance antileukemic activity at different nodes in this pathway. To address this hypothesis, the *FLT3* internal tandem duplication (ITD)/*MLL*–rearranged cell lines, *MOLM13* and *MV411*, were treated with increasing doses of either LANRA or ENTO in combination with a small molecule *MLL*-menin inhibitor (SNDX-5613). Synergistic antiproliferative effects were observed across a broad range of concentrations. No combinatorial or single-agent SNDX-5613 activity was seen in the KASUMI-1 AML cell line, which lacks an *MLL* rearrangement. Phenotypic studies using flow cytometry were performed to assess the mechanistic response for SYK inhibition with LANRA in combination with SNDX-5613. The effects of LANRA on differentiation and apoptosis were mild as a single agent compared to SNDX-5613. In contrast, the combination of LANRA and SNDX-5613 enhanced the apoptotic and differentiation effects of the single agents, suggesting a more complete blockade of the *HOXA9/MEIS1* transcriptional program through synergistic inhibition by orthogonal mechanisms. Further, treatment of leukemic cells with either ENTO or LANRA inhibited SYK autophosphorylation in a dose-dependent manner.

We also explored the potential for synergistic activity with gilteritinib (*FLT3* inhibitor) and venetoclax (*BCL2* inhibitor) using patient-derived AML isolates ex vivo based on the potential for SYK signaling cross talk with *FLT3* itself and survival pathways. We found strong synergistic antiproliferative activity for the combination of LANRA with either gilteritinib or venetoclax. Patient-derived xenograft (PDX) studies also demonstrated deeper reductions in leukemic burden in the peripheral blood (PB), liver, and bone marrow (BM) after 28 days of treatment. In a follow-up PDX study assessing overall survival (OS) using an optimized dosing regimen, the combination of LANRA and gilteritinib significantly extends OS (62 days) compared to either single agent (31 days for LANRA or 55 days for gilteritinib). The median OS for the vehicle group was 27 days.

SYK Mechanistic Model in AML

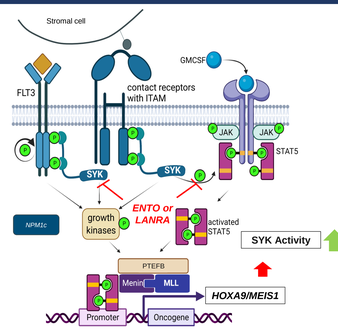
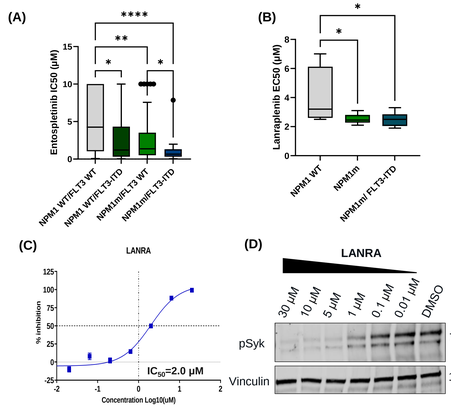


Figure 1.
- SYK integrates paracrine and contact signaling by reinforcing RTKs like *FLT3*.
- This cascades downstream to the HOXA9/MEIS1 TRN through signal transducer and activator of transcription (STAT) and kinase transduction.[2]
- Inhibition of upstream RTKs (ie, gilteritinib) can synergize with inhibition of SYK to potentiate antileukemic effects.
- LANRA shows strong antileukemic activity in combination with gilteritinib and other targeted agents in preclinical models.

We hypothesize that stromal signaling strongly contributes to the maintenance of the *HOXA9/MEIS1* oncogenic program and survival of AML.

Sensitivity to SYK Inhibition Correlates Strongly With the Presence of *NPM1* and/or *FLT3*-ITD Mutation in AML Patient Bone Marrow Samples



DMSO=dimethyl sulfoxide; EC_{50}=half maximal effective concentration; GMCSF=granulocyte macrophage colony stimulating factor; IC_{50}=half maximal inhibitory concentration; JAK=Janus kinase; PTEFB=positive transcription elongation factor B.

Figure 2. (A) A subset analysis of 152 BM samples from AML patients as part of the Beat AML program.[5] BM isolates were treated with ENTO in a drug sensitivity screen as part of the Beat AML Program. The specimens were assessed using whole exome sequencing for mutations, RNA-seq for gene expression, and ex vivo drug sensitivity using an MTT assay.

(B) Primary leukapheresis-derived AML cells were cultured for 6 days using vehicle (0.1% DMSO) or LANRA across a range of dilutions (10–0.001μM). On day 6, cell viability was measured by the addition of CellTiterGlo® reagent and relative luminescence units (RLUs) were recorded on a plate reader.

(C) The *FLT3*-ITD mutant cell line MV4-11 was treated with LANRA across a range of dilutions (20–0.002 μM). On day 5 cell viability was measured by CellTiterGlo®.

(D) The *FLT3*-ITD mutant cell line MV4-11 was treated with LANRA across a range of dilutions (30–0.01 μM). Whole cell lysates were resolved by SDS-PAGE and immunoblotted with an antibody against phosphorylated SYK (Tyr 525/526 from Cell Signaling Technologies #2711).

*P<0.05, **P<0.001, ****P<0.0001.

ENTO or LANRA Shows Synergy With Menin Inhibition in *MLLr/FLT3*-ITD Models

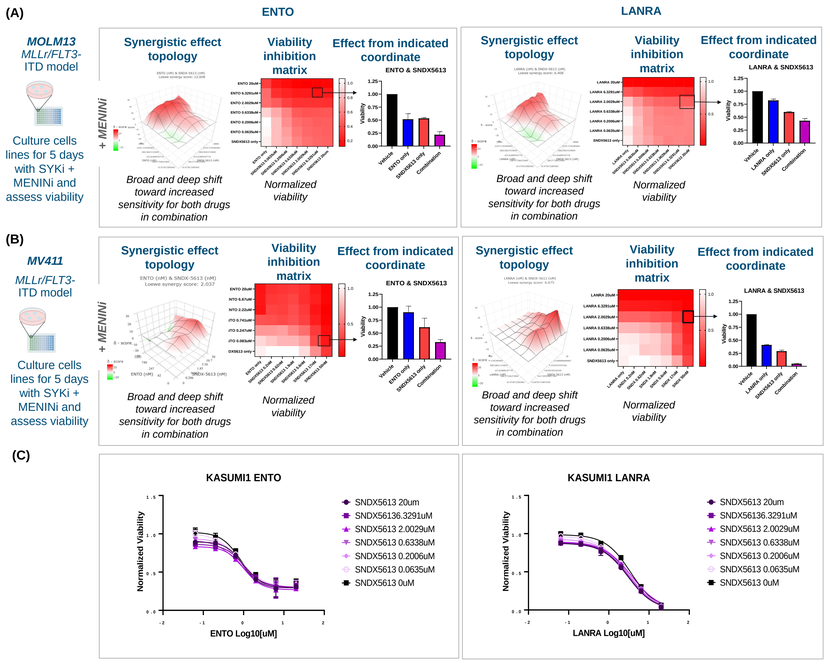


Figure 3. (A) *MLLr/FLT3*-ITD mutant AML cell lines *MOLM13* or **(B)** *MV411* were treated with either LANRA or ENTO in combination with the menin inhibitor SNDX-5613 at the indicated doses. The antileukemic effects of the combination were assessed using CellTiterGlo® as readout for cell viability. Both ENTO and LANRA showed broad and robust synergistic effects with SNDX-5613 at multiple doses exceeding a Loewe synergy score of 10. **(C)** *FLT3/MLL* wildtype cell line, KASUMI1 (*AML1-ETO*, *C-KIT*-mut) treated as indicated in **(A)** and **(B)** displaying sensitivity to both SYK inhibitors as single agents but not SNDX-5613 single agent or in combination with either SYK inhibitor.

LANRA and SNDX-5613 Trigger Robust Differentiation and Apoptosis Response in Combination

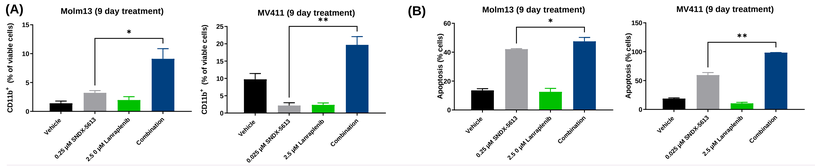


Figure 4. *FLT3*-ITD mutant AML cell lines *MOLM13* or *MV411* were treated with either LANRA or ENTO in combination with the menin inhibitor SNDX-5613 at the indicated doses. The antileukemic effects of the combination were assessed by flow cytometry measuring **(A)** the percent of CD11b-positive cells as a marker of differentiation or **(B)** AnnexinV/PI staining as a marker of apoptosis. LANRA enhanced the differentiation and apoptotic effects of SNDX-5613 single agent.
*P<0.05, **P<0.001.

LANRA Shows Synergistic Activity in an *FLT3*-ITD/*NPM1*m AML Patient Isolate in Combination With Gilteritinib or Venetoclax

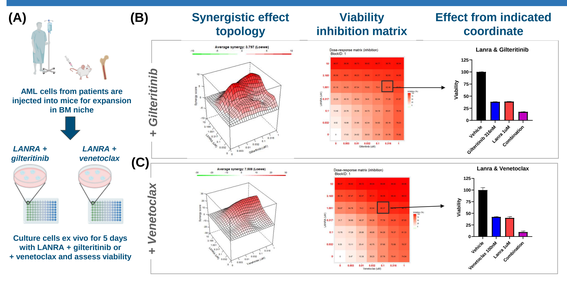


Figure 5. (A) *FLT3*-ITD/*NPM1*m AML model (AM7577) derived from primary AML isolate and expanded in mice were treated ex vivo with LANRA in combination with **(B)** gilteritinib or **(C)** venetoclax as indicated. The antileukemic effects of the combination were assessed using CellTiterGlo® as readout for cell viability. LANRA showed a broad and robust synergistic effect in combination with both gilteritinib or venetoclax exceeding a Loewe synergy score of 10 at multiple doses.

LANRA in Combination With Gilteritinib Shows Strong Antileukemic Activity in Vivo

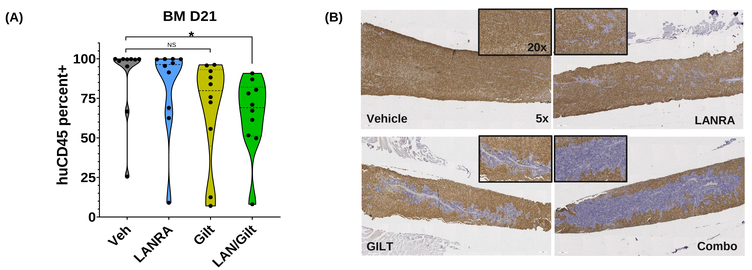
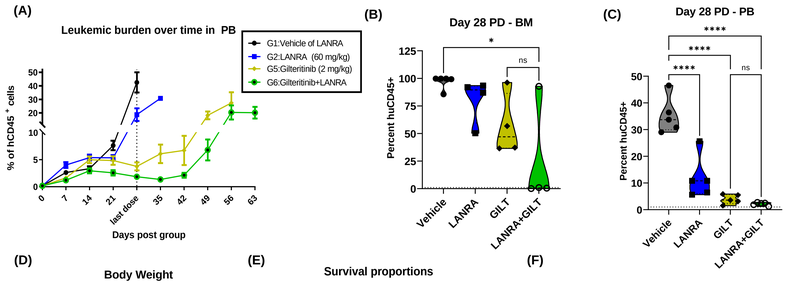


Figure 6. LANRA was studied alone and in combination with gilteritinib in a *FLT3*-ITD/*NPM1*m PDX. Mice were treated with **50 mg/kg** LANRA via oral gavage, twice daily, or with **3 mg/kg** gilteritinib via intraperitoneal injections, once daily, as single agents or in combination. **(A)** Leukemic burden was assessed by flow cytometry measuring human CD45 in BM at the end of study (day 21). **(B)** Representative image of BM section stained with an antihuman CD45 antibody by immunohistochemistry (IHC), demonstrating enhanced clearance of human leukemic cells from BM exposed to the combination of LANRA and gilteritinib.
*P<0.05. GILT=gilteritinib; LAN=LANRA; Veh=vehicle.

LANRA in Combination With Gilteritinib Extends OS in *FLT3*-ITD PDX Model



	Vehicle	LANRA	GILT	LANRA + GILT
Median OS	28	31	56	64
P-value	-	0.0822	<0.0001	0.0008

Figure 7. LANRA was studied alone and in combination with gilteritinib in an *FLT3*-ITD/*NPM1*m PDX. Mice were treated with **60 mg/kg** LANRA via oral gavage, twice daily, or with **2 mg/kg** gilteritinib via intraperitoneal injections, once daily, as single agents, or in combination. **(A)** Leukemic burden was assessed by flow cytometry measuring human CD45 in PB obtained from serial bleeds over time or **(B)** and **(C)** on day 28 in BM and PB respectively. **(D)** Body weights were recorded longitudinally up to day 30 of the study showing no significant loss in body weight during the dosing period or follow-up after the last dose. **(E)** Kaplan-Meier survival curve showing the probability of survival following treatment with each single agent or the combination. **(F)** Table summarizing the median OS in days for all treatment groups on study.
*P<0.05, ****P<0.0001.

Conclusions

- LANRA is currently undergoing early clinical development in combination with gilteritinib in patients with relapsed or refractory (R/R) *FLT3*-mutated AML (NCT05028751).
- Given its central role as a mediator of leukemogenic signaling, SYK inhibition with LANRA has the potential to synergize with other targeted therapies in AML.
- In preclinical studies, LANRA-mediated SYK inhibition shows compelling antiproliferative activity in combination with the BCL-2 inhibitor, venetoclax, and the menin inhibitor, SNDX-5613, in genetically defined subsets of AML.
- In an *FLT3*-ITD PDX model, LANRA in combination with gilteritinib is well tolerated, shows strong antileukemic effects, and extends OS.

References

1. Hahn CK, et al. *Cancer Cell.* 2009;16(4):281-294.
2. Puissant A, et al. *Cancer Cell*. 2014;25(2):226-242.
3. Mohr S, et al. *Cancer Cell*. 2017;31(4):549-562.
4. Walker AR, et al. *Clin Cancer Res*. 2020;26(22):5852-5859.
5. Tyner JW, et al. *Nature*. 2018;562(7728):526-531.

Presented at the ASH 64th Annual Meeting and Exposition; December 10-13, 2022; New Orleans, LA.