Preclinical Activity of Selective SYK Inhibitors, Entospletinib and Lanraplenib, Alone or Combined With Targeted Agents in Ex Vivo AML Models With Diverse Mutational Backgrounds

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Abstract

Spleen tyrosine kinase (SYK) is a nonreceptor tyrosine kinase that mediates integrin and Fc receptor signaling in myeloid cells and has been implicated as an oncogenic driver in acute myeloid leukemia (AML). The oral SYK inhibitor entospletinib (ENTO) has demonstrated clinical activity in *HOXA9/MEIS1*-driven AML and is currently being investigated in a phase 3 trial, AGILITY (NCT05020665). Lanraplenib (LANRA) is a next-generation oral SYK inhibitor with potency, selectivity, and pharmacokinetic (PK) properties comparable to ENTO. Here we present data comparing the activity of ENTO and LANRA in ex vivo models of patient-derived AML cells, both as a single-agent and in combination with other AML therapies. ENTO and LANRA showed comparable effects on cell viability with no significant differences between the compounds when compared across 44 models representing different mutational backgrounds. Matrix combination assays were performed by combining ENTO or LANRA with either cytarabine (AraC; *NPM1* mut), gilteritinib (*FLT3* mut), or trametinib (*RAS* mut). Increased cell death in an additive manner was observed in all combinations tested, with results for ENTO and LANRA being similar, indicating the utility of both compounds in combinatorial treatment paradigms.

Spleen Tyrosine Kinase as an Oncogenic Driver in Acute Myeloid Leukemia[1,2]

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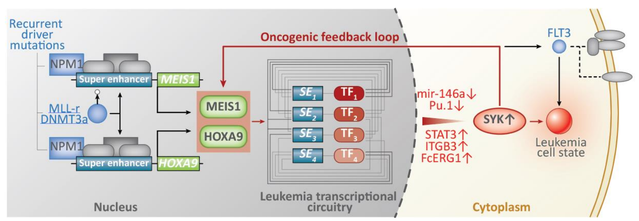

Adapted from Mohr S, et al. *Cancer Cell*. 2017;31(4):549-562.e11.

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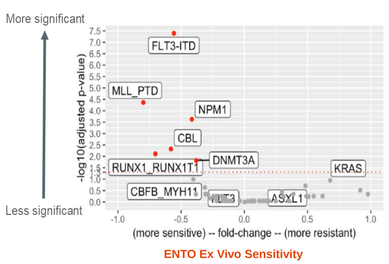

Figure 1: SYK is a critical node in *HOXA9/MEIS1* high AML. (A) *HOXA9/MEIS1* overexpression promotes leukemogenesis and is associated with high-risk AML. *MEIS1* increases SYK protein expression and activity, which then acts in a positive feedback loop by further promoting *MEIS1* expression thereby supporting leukemic cell survival and proliferation.[1,2] Inhibiting SYK disrupts the feedback loop and promotes apoptosis. **(B)** Ex vivo ENTO-treated AML samples were analyzed for mutations associated with sensitivity. Nucleophosmin-1 mutated (*NPM1c*) patients were among those sensitive to ENTO. (Figure 1B courtesy of Brian Drucker, MD of Oregon Health and Sciences University).

LANRA Pharmacokinetic Properties Compare Favorably With ENTO

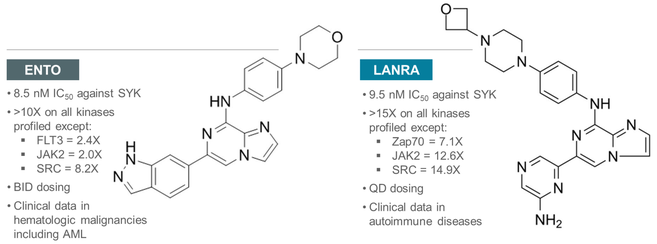

Figure 2: LANRA PK properties compare favorably with ENTO. LANRA is a next-generation oral SYK inhibitor with similar potency and selectivity as ENTO. LANRA has shown PK properties in human subjects that allow for once daily (QD) dosing as compared to twice daily (BID) dosing for ENTO. This poster compares the activity of LANRA to ENTO, both as a single agent and in combination with other AML therapies to support the clinical development of LANRA in AML.

Disclosures

MALD reports current employment by Kronos Bio, Inc. previous employment by Cyteir Therapeutics, and equity in Kronos Bio, Inc. and Cyteir Therapeutics. **PS** has no financial relationships to disclose. **CAH** reports consulting fees from Oncopeptides, and research funding from Kronos Bio, Inc., Oncopeptides, Novartis, Orion Pharma, and Celgene/BMS. **AS** reports current employment by and equity in Kronos Bio, Inc. **NDO** reports current employment by and equity, stock, and options in Kronos Bio, Inc. **CYL** reports current employment by Kronos Bio, Inc. **PK**, **JD**, and **DCS** report current employment by and equity in Kronos Bio, Inc.

ENTO and LANRA Show Comparable Activity in *NPM1*- and *FLT3*-Mutated Peripheral Blood Derived Leukemic Blasts

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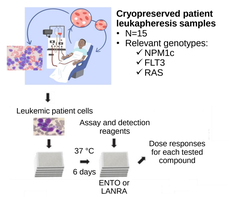

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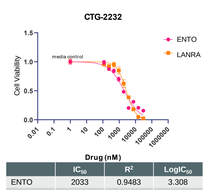

Drug (nM)	IC_{50}	R^2	$LogIC_{50}$
ENTO	2033	0.9483	3.308
LANRA	2706	0.9902	3.432

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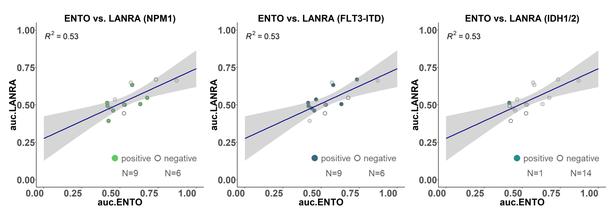

Figure 3: ENTO and LANRA display comparable antileukemic activity in *NPM1*- and/or *FLT3*-mutated AML blasts derived from peripheral blood. (A) Outline of the experiment. Blood was collected from patients, AML blasts isolated and cryopreserved. The cells were then thawed, placed in culture, and treated with varying concentrations ENTO or LANRA for 6 days. Cell viability was measured using CellTiter Glo. **(B)** Example cell viability curve for a *NPM1c* model, CTG-2232. Dose response curve with ENTO is in red and LANRA is in orange. **(C)** Comparison of ENTO and LANRA area under the curve (AUC) values across the 15 models showed a linear relationship indicating good correlation in response between the two inhibitors. (Work performed by Champions Oncology).

ENTO and LANRA Show Comparable Activity in *NPM1*- and *FLT3*-Mutated Bone Marrow Derived Leukemic Blasts

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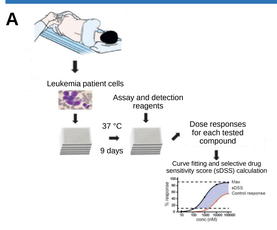

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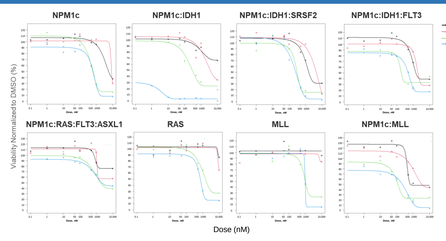

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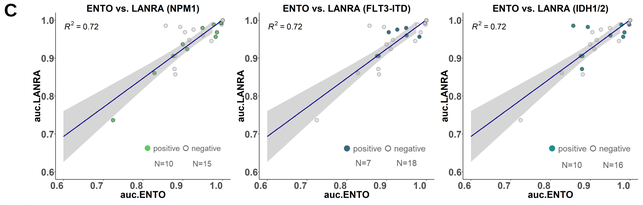

DMSO, dimethyl sulfoxide.

Figure 4: ENTO and LANRA display comparable antileukemic activity in *NPM1*- and/or *FLT3*-mutated AML blasts from bone marrow. (A) Cryopreserved AML cells from patient bone marrow samples were placed in culture and treated with increasing concentrations of ENTO, LANRA, fostamatinib or midostaurin for 9 days. Cell viability was measured with a flow cytometric assay using Annexin V and 7-aminoactinomycin D (7-AAD) staining. **(B)** Example cell viability curves in patient samples representing different mutational backgrounds. **(C)** Comparison of ENTO and LANRA AUC values across 29 models showed a linear relationship indicating good correlation in response between the two inhibitors.

References

1. Mohr S, et al. *Cancer Cell*. 2017;31(4):549-562.e11. 2. Tyner JW, et al. *Nature*. 2018;562(7728):526-531. 3. Puissant A, et al. *Cancer Cell*. 2014;25(2):226-242.

Matrix Combination Assays

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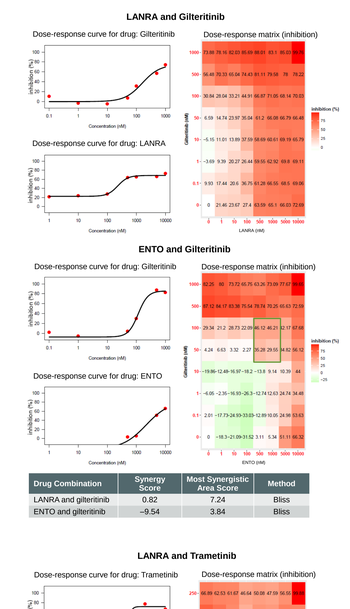

Drug Combination	Synergy Score	Most Synergistic Area Score	Method
LANRA and gilteritinib	0.82	7.24	Bliss
ENTO and gilteritinib	−9.54	3.84	Bliss

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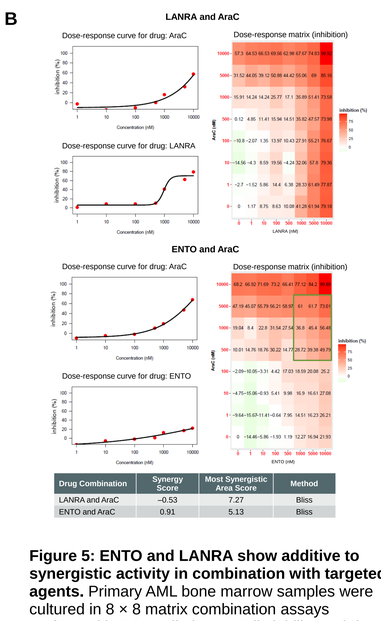

Drug Combination	Synergy Score	Most Synergistic Area Score	Method
LANRA and AraC	−0.53	7.27	Bliss
ENTO and AraC	0.91	5.13	Bliss

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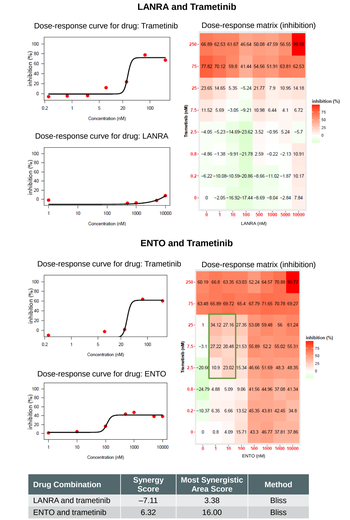

Drug Combination	Synergy Score	Most Synergistic Area Score	Method
LANRA and trametinib	−7.11	3.38	Bliss
ENTO and trametinib	6.32	16.00	Bliss

Figure 5: ENTO and LANRA show additive to synergistic activity in combination with targeted agents. Primary AML bone marrow samples were cultured in 8 × 8 matrix combination assays performed in 384 well plates. Cell viability and death were assessed after 3 days of incubation using CellTiter Glo. Data analysis was done by subtracting the background signal from all wells and then determining the percent viability of each treatment well by normalizing to the DMSO negative control well. Summary analysis of 2 models for each combination were combined, the outliers removed, and then the percent viability data analyzed using the SynergyFinder tool and the Bliss model of synergy. **(A)** Summary data of combinations of ENTO/LANRA with gilteritinib in 2 *FLT3* mutant models. Green box highlights area of synergy with ENTO and gilteritinib. **(B)** Summary data of combinations of ENTO/LANRA with cytarabine in 2 *NPM1c* models of AML. Green box highlights area of synergy with ENTO and cytarabine. **(C)** Summary data of combinations of ENTO/LANRA with trametinib in 2 *RAS* mutant AML models. Green box highlights area of synergy with ENTO and trametinib.

Conclusions

- LANRA and ENTO display comparable effects on viability among 44 AML patient-derived leukemic isolates.
- Only the *FLT3* mutational background showed differences between ENTO and LANRA, with slightly lower IC_{50} values in the presence of ENTO, most likely due to the inhibitory activity of ENTO against *FLT3*;[3] this is consistent with the hypothesis that SYK inhibition drives the majority of the activity.
- The results for LANRA and ENTO in the various combinations were similar, indicating the utility of both compounds in combinatorial treatment paradigms.

A phase 3 clinical trial, NCT05020665, with ENTO in combination with the 7 + 3 regimen in *NPM1*-mutated AML patients is currently enrolling.

A phase 1/2 clinical trial, NCT05028751, with LANRA in combination with gilteritinib in *FLT3*-mutated AML patients is currently enrolling.

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