Poster P428

SYK Inhibition Drives Deep Responses in a Biomarker Guided Subset of AML Alone and in Rational Combinations

Michael McKeown, Luis A. Carvajal, Melinda A. L. Day, Christina Noe, Pavan Kumar, Jorge DiMartino, Doug Saffran

Kronos Bio, Inc., San Mateo, CA, USA

KRONOS·BIO

Abstract

Background: Spleen tyrosine kinase (SYK) acts as a key integrator of signals from cell surface receptors containing an immunoreceptor tyrosine-based activation motif to boost cellular proliferation. In acute myeloid leukemia (AML), SYK serves as a relay to an oncogenic transcriptional regulatory network (TRN) linked to *NPM1*, *HOXA9*, and *MEIS1*.

The selective, orally bioavailable SYK inhibitor entospletinib (ENTO) has demonstrated clinical activity and tolerability in *HOXA9/MEIS1*-driven AML. ENTO is currently being investigated in a global phase 3 trial, AGILITY (NCT05020665), in combination with intensive induction/consolidation chemotherapy in patients with treatment-naive *NPM1*-mutated (*NPM1*m) AML. Lanraplenib (LANRA) is a next-generation SYK inhibitor with similar potency and selectivity to ENTO but with 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).

Aims: To assess the potential of biomarker-guided responses to ENTO and LANRA in mutationally defined subsets of AML and its activity in combination with other targeted agents in translationally relevant models.

Methods: Full kinome profiling was run at a fixed 1 µM concentration followed by dose response IC_{50} determination for top hits. AML and lymphoma cell lines were tested for antiproliferative effects using CellTiter-Glo® (CTG; Promega) at 5 days. AML patient–derived ex vivo models were tested in microtiter plate viability assays with CTG or in methylcellulose colony viability assays for the *NPM1*m/*FLT3*-ITD model used in combination studies.

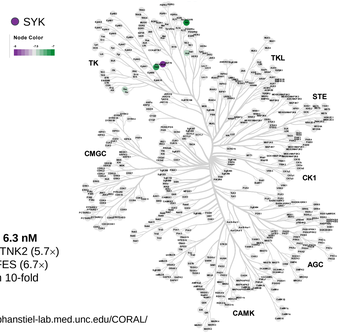
Results: Kinase selectivity profiling found >10-fold higher selectivity of ENTO and LANRA for SYK vs other kinases with improved potency and selectivity compared with the approved first-generation agent, fostamatinib. SYK inhibition with either agent showed robust antiproliferative activity in a panel of AML and lymphoma cell lines with varying mutational backgrounds. In addition, synergy was observed when ENTO or LANRA were combined with a menin inhibitor in *MLLr*, *FLT3*-ITD cell lines.

LANRA and ENTO showed strong antiproliferative activity in *NPM1*m AML patient samples validating *NPM1*m as a patient selection biomarker for ENTO and LANRA. In an analysis of a large public dataset of ex vivo patient samples, the presence of *NPM1*m and/or *FLT3*-ITD was significantly predictive of response to ENTO. In an ex vivo model of *NPM1*m/*FLT3*-ITD AML, consistent submicromolar response to LANRA was observed.

Combinations with AML standard-of-care and investigational agents, including azacitidine (AZA), showed at least additive effects. Strong synergy with the JAK inhibitor ruxolitinib was consistent mechanistically with a reporter model that demonstrated the ability of LANRA to block activation of STAT5 in response to proleukemic paracrine signaling. Finally, robust synergy across a range of LANRA concentrations was found when paired with venetoclax and gilteritinib. These results prompted further testing with an in vivo, patient-derived xenograft.

ENTO and LANRA Show High Selectivity for SYK Compared to First-Generation Inhibitors

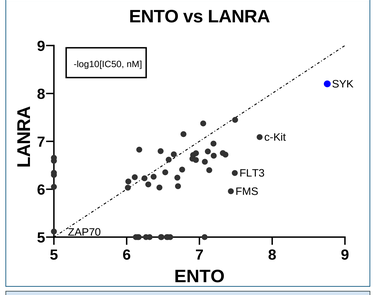
LANRA is highly selective and potent with only 5 kinases having IC_{50} <100 nM



SYK: 6.3 nM
Only TNK2 (5.7×) and FES (6.7×) within 10-fold

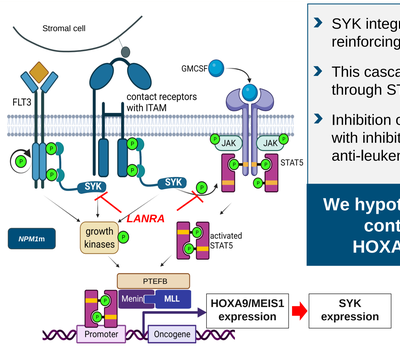
http://phanstiel-lab.med.unc.edu/CORAL/

ENTO and LANRA are potent and selective SYK inhibitors



Kinome panel evaluated at Reaction Biology Corporation with single point and dose response on top binders.

SYK Mechanistic Model in AML

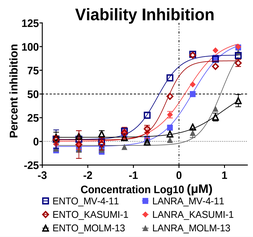


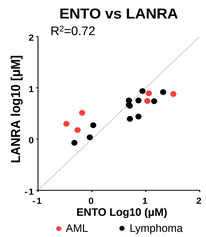
- SYK integrates paracrine and contact signaling by reinforcing RTKs like FLT3.
- This cascades downstream to the HOXA9/MEIS1 TRN through STAT and kinase transduction.
- Inhibition of upstream RTKs (eg, gilteritinib) can synergize with inhibition of a core node (eg, SYK) to potentiate anti-leukemic effects.

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

Also see **Poster P392** for expanded biomarker and patient model studies.

ENTO and LANRA Show Strong Activity and Correlation in AML and Lymphoid Cell Lines

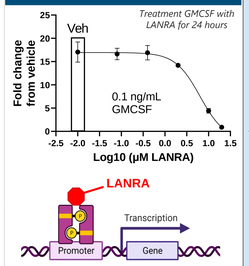




- ENTO and LANRA show comparable and well-correlated effects in a panel of AML cell-line models assessed by viability with CTG.
- A subset including AML and lymphomas showed particularly low µM to nM sensitivity to both agents.

LANRA Significantly Attenuates Pro-leukemic STAT5 Pathway Signaling

LANRA shows dose-dependent reduction in STAT5 activation



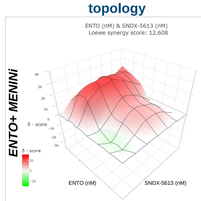
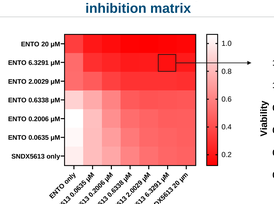
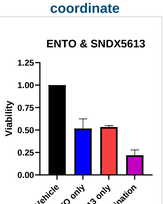
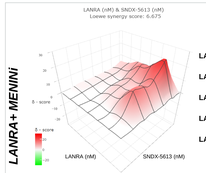
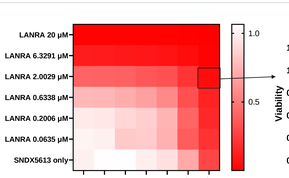
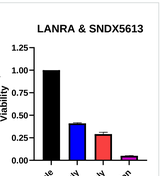
- A U937 AML reporter with STAT5-driven luciferase is responsive to GMCSF.
- LANRA shows dose-dependent suppression of activation linking SYK to JAK/STAT signaling.

SYK Inhibition Shows Synergy with Menin Inhibition in *MLLr*/*FLT3*-ITD Models

MOLM13 MLLr/FLT3-ITD model with ENTO

Culture cell lines for 5 days with SYKi + MENINi and assess viability by CTG

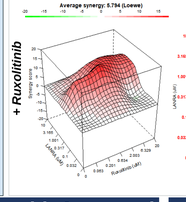
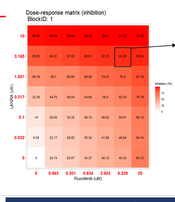
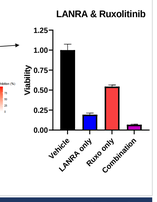
MV411 MLLr/FLT3-ITD model with LANRA

Synergistic effect topology	Normalized viability inhibition matrix	Effect from indicated coordinate
		
Broad and deep shift toward increased sensitivity for both drugs in combination		
		
Broad and deep shift toward increased sensitivity for both drugs in combination		

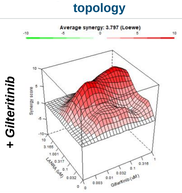
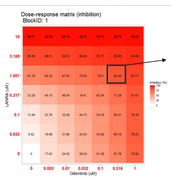
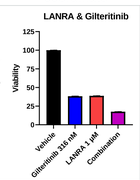
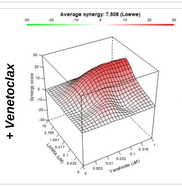
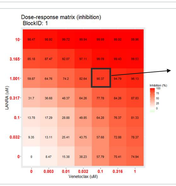
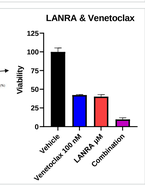
- ENTO and LANRA were found to be synergistic with both models in combination with SNDX5613 across a broad range of concentrations.
- This supports potential future in vivo studies testing the potential of this combination.

LANRA Shows Synergy with JAK Inhibition in Ex Vivo AML Patient Samples

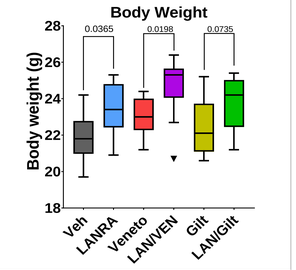
Right: Consistent with the effects of LANRA on the JAK/STAT pathway found in a reporter model, LANRA showed strong synergy in combination with ruxolitinib. Although not commonly used in AML, this could support a rational combination in MDS.
Below: Two of the strongest combinations for LANRA with immediate applicability to clinical translation were FLT3 inhibition by gilteritinib and BCL2 inhibition by venetoclax. In both cases, synergy was observed across a broad range of concentrations, which is important given varying exposure over time for 2 drugs with in vivo studies. This ex vivo was then followed up with a 21-day treatment window study.

Synergistic effect topology	Viability inhibition matrix	Effect from indicated coordinate
		

LANRA Shows Synergistic Activity with Approved Agents in Combination in an AML Patient Sample

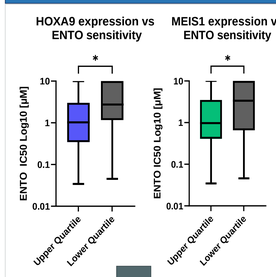
Synergistic effect topology	Viability inhibition matrix	Effect from indicated coordinate
		
		

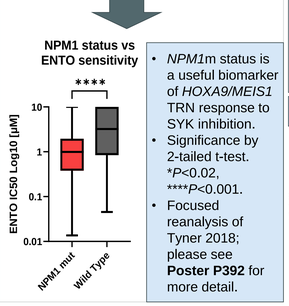
Combination Shows Improved Body Weight and Supports LANRA as a Combination Partner



Disseminated AML PDX model. Although vehicle mice showed significant body-weight loss from peak, combinations showed improvement over vehicle and single agents in maintaining mouse body weights (2-tailed t-test with Welch's correction).

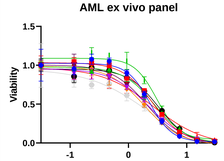
*NPM1*m is a Biomarker for SYK Inhibitor Response in AML Patient Samples





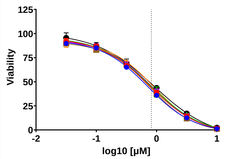
- *NPM1*m status is a useful biomarker of *HOXA9/MEIS1* TRN response to SYK inhibition.
- Significance by 2-tailed t-test. *P<0.02, ****P<0.001.
- Focused reanalysis of Tyner 2018; please see **Poster P392** for more detail.

Panel of AML ex vivo models

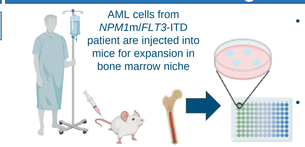


Top: LANRA dose response across a panel of *NPM1*m models shows robust and consistent activity.
Bottom: An *NPM1*m/*FLT3*-ITD model grown in semi-solid colony-forming conditions shows potent and consistent response across 7 independent assays runs each in triplicate.

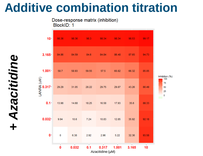
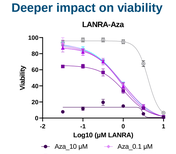
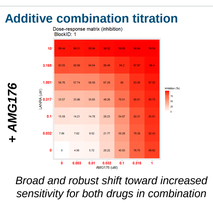
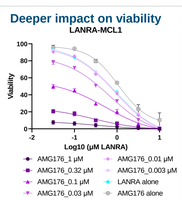
LANRA AM7577 *NPM1*m/*FLT3*-ITD model used for follow-up combo studies



LANRA Showed Additional Additive Benefit with SOC and Investigational Agents

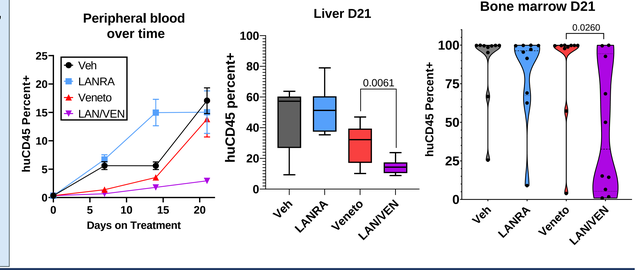


- Culture cells ex vivo for 5 days with LANRA + combo agent and assess viability.
- Semi-solid media for niche-like cell interactions.

Additive combination titration	Deeper impact on viability
	
Azacitidine has limited antiproliferative effect on its own but was additive to LANRA	
	
Broad and robust shift toward increased sensitivity for both drugs in combination	

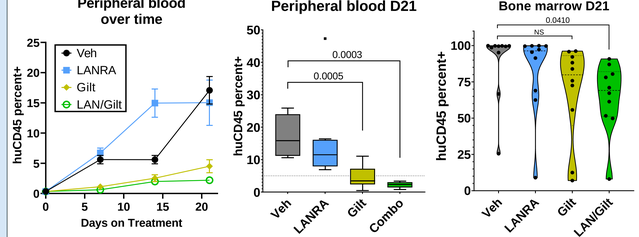
LANRA and Venetoclax Show Strong Combination Benefit in AML PDX Model

- LANRA (50 mpk, BID) and venetoclax (50 mpk, QD) showed improved antileukemic effects in combination.
- Significant reductions were noted in peripheral blood and were especially strong in tissues where some mice were essentially cleared of disease.
- Data may suggest a rational doublet approach in the clinic.
- A follow-up PDX study for survival is ongoing.



Combination of LANRA with Gilteritinib Showed Improved Tumor Burden Reduction

- LANRA (50 mpk, BID) and gilteritinib (3 mpk, QD) showed enhanced effect in combination.
- While this model is strongly sensitive to *FLT3* inhibition in peripheral burden, the combination showed significant benefit in the bone marrow.
- Data support current clinical trial effort with gilteritinib combination.
- A follow-up PDX study for survival is ongoing.



Conclusions

- SYK is a promising therapeutic target for subsets of AML patients defined by specific mutations. The highly predictive response for SYK inhibition with concurrent *NPM1*m/*FLT3*-ITD mutations and synergy with gilteritinib observed in patient-derived models is supportive of its clinical evaluation in combination with a *FLT3* inhibitor.
- Our work further supports the rationale for an ongoing phase 1b/2 study of LANRA in combination with gilteritinib in patients with R/R *FLT3*m AML (NCT05028751).

BID = twice daily; GMCSF = granulocyte-macrophage colony-stimulating factor; IC50 = inhibitory concentration; JAK = Janus kinase; RTK = receptor tyrosine kinase; SYKi = SYK inhibitior; MDS = myelodysplastic syndromes; SOC = standard of care; MCL1 = myeloid cell leukemia-1; MENINi = menin inhibitor; PDX = patient-derived xenograph; QD = once daily; Veh = vehicle.

Presented at the EHA 2022 Congress; June 9-12, 2022; Vienna, Austria.

Select images created with BioRender.com.