2259

The Novel Profiling Relative Inhibition Simultaneously in Mixtures (PRISM) Platform Identifies Synergistic Activity of Lanraplenib and Ruxolitinib in Hematological Malignancies

Akanksha Pandey[1], Luis A. Carvajal[1], Andrew S. Boghossian[2], Matthew G. Rees[2], Melissa M. Ronan[2], Jennifer A. Roth[2], **Tressa R. Hood[1]**, Linlin Guo[1], Tamara D. Hopkins[1], Nikolaus Obholzer, Michael R. McKeown[1], Jorge DiMartino[1], Charles Y. Lin[1]

[1]Kronos Bio, Inc., Cambridge, MA, USA; [2]Broad Institute of MIT and Harvard, Cambridge MA, USA

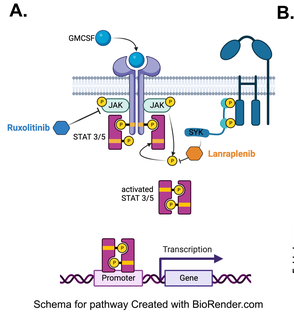
Abstract

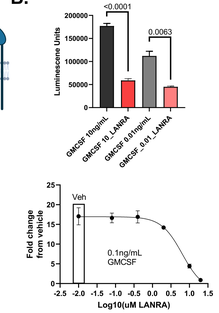
Background: Investigation of drug combinations across different contexts can provide useful insights on the anti-cancer mechanism and can ultimately lead to new treatments. However, conventional drug combination screening methods are limited by throughput. Efforts to systematically identify the most effective active combinations and the optimal molecular contexts in a high throughput screening (HTS) format could greatly accelerate the development of combination treatments. Spleen Tyrosine Kinase, SYK, is a non-receptor tyrosine kinase known to regulate intracellular signaling, including FLT3, AKT/mTOR and STAT5 pathways, via its immunoreceptor tyrosine-based activation motif (ITAM). Deregulated SYK signaling has been reported to play a central role in pathogenesis of allergic and autoimmune diseases as well as hematological malignancies. Lanraplenib (LANRA) is a next-generation SYK inhibitor currently being evaluated in combination with gilteritinib, a FLT3 inhibitor, in patients with relapsed or refractory (R/R) *FLT3*–mutated acute myeloid leukemia (AML) (NCT05028751). Given its critical role in intracellular signaling and interaction with receptor tyrosine kinases (RTKs), we hypothesized that lanraplenib could synergize with ruxolitinib, a JAK inhibitor. To address this hypothesis, we performed a high throughput drug combination screen using the Broad Institute's Profiling Relative Inhibition Simultaneously in Mixtures (PRISM) platform, which enables rapid screening of thousands of compounds in a 930-cell line panel across 45 different lineages.

Methods:
In this study, we performed a combination screen using the PRISM platform with ruxolitinib as the test compound, with a 10 µM top dose (7 dose concentrations with 3-fold dilutions), in combination with lanraplenib at two anchor doses, 2 µM and10 µM. PRISM is a pooled, multiplexed cell viability assay that provides 7-pointdose response curves, IC, AUC values, and relative abundance of unique cell line barcodes. To understand the drug synergy landscape across different lineages, we developed a bioinformatics pipeline which uses PRISM viability data to calculate synergy scores across all the cell lines and drug combinations. Secondary validation studies of the combinations used CellTiter-Glo (CTG) viability measurements. Phospho-SYK expression was evaluated in archival formalin-fixed paraffin-embedded (FFPE) bone marrow biopsies from patients with myeloid proliferative neoplasm (MPN) by immunohistochemistry (IHC). RNA-seq was performed to evaluate differential changes in gene expression in response to lanraplenib. Gene set enrichment analysis (GSEA) was performed to evaluate perturbation in leukemogenic signaling pathways.

Results:
In the PRISM cell line panel, ruxolitinib in combination with lanraplenib demonstrated synergistic activity in hematological malignancy cell lines. Among AML cell lines in the panel, OCIAML5, OCIM1, HL60, HEL, EOL1, MONOMAC6, NB4, U937, PL21,and TF1 showed the highest synergy scores. The most sensitive cell lines to the combination showed up-regulation of JAK-STAT and inflammatory signaling pathways in a gene set enrichment analysis (GSEA) prior to treatment. Consistent with this, phosphorylated SYK was associated with inflammatory megakaryocytes and fibrosis in primary samples from patients with MPN. Lanraplenib down-regulated JAK-STAT signaling in a reporter cell line and repressed gene expression associated with dysregulated inflammatory pathways in AML cells. Additionally, the combination of lanraplenib and ruxolitinib showed synergistic antiproliferative activity across a broad range of concentrations in FLT3-ITD primary AML cells and a panel of hematological malignancy cell lines, confirming the PRISM results.

SYK and JAK/STAT signaling cooperate to drive oncogenic signaling



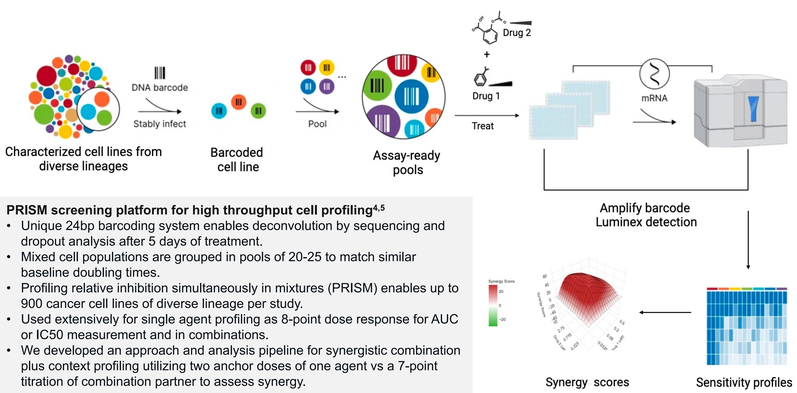


(A) SYK is a key co-factor for receptor tyrosine kinases, integrins and fc-gamma receptors.[1,2,3]
- SYK binds to ITAM containing cell membrane proteins, triggering a phosphorylation cascade and oncogenic signaling.
- SYK cooperates with JAK to boost intracellular signaling by directly phosphorylating STATs and may amplify the constitutively active oncogenic mutant variants.

(B) Lanraplenib robustly blocks downstream STAT5 activation and shows dose dependent reduction in STAT5 activation.
- A U937 AML reporter with STAT5 driven luciferase is responsive to GMCSF 10ng/mL. Lanraplenib shows dose dependent suppression of activation linking SYK to JAK/STAT signaling.

Hypothesis:
Concomitant pharmacological inhibition of both JAK and SYK synergistically suppresses oncogenic signaling in hematological malignancies.

PRISM enables the generation of high throughput anti-proliferative profiling

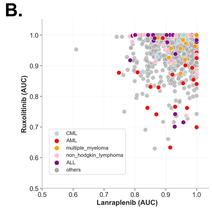


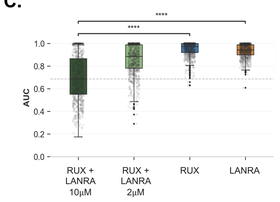
PRISM screening platform for high throughput cell profiling[4,5]
- Unique 24bp barcoding system enables deconvolution by sequencing and dropout analysis after 5 days of treatment.
- Mixed cell populations are grouped in pools of 20-25 to match similar baseline doubling times.
- Profiling relative inhibition simultaneously in mixtures (PRISM) enables up to 900 cancer cell lines of diverse lineage per study.
- Used extensively for single agent profiling as 8-point dose response for AUC or IC50 measurement and in combinations.
- We developed an approach and analysis pipeline for synergistic combination plus context profiling utilizing two anchor doses of one agent vs a 7-point titration of combination partner to assess synergy.

PRISM combination screen identifies synergy with ruxolitinib and lanraplenib combination

A.

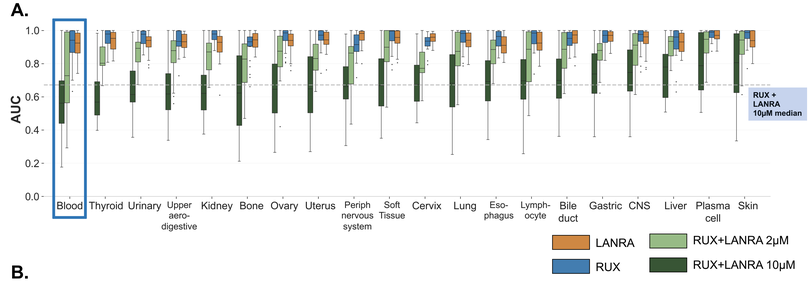
PRISM screen	Number of cell lines	Number of Lineages
lanraplenib (Single agent)	870	29
ruxolitinib (Single agent)	873	29
ruxolitinib + lanraplenib [2µM] (Combination)	881	28
ruxolitinib + lanraplenib [10µM] (Combination)	878	28

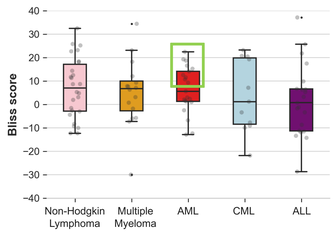




Single agent profiling identified heme models as sensitive
(A) >870 models screened and passed QC for 8-point dose-response curves at 3x dilution from 10µM for each compound.
(B) Among the combos tested, ruxolitinib and lanraplenib showed enriched activity in convergent lineages.
(C) Across all models and lineages, the combination showed increased anti-proliferative effect at both lanraplenib dose levels. Two anchor doses of lanraplenib at 2µM and 10µM were profiled against a 7-point titration of ruxolitinib.

PRISM combination effect is most strongly observed in hematological malignancies

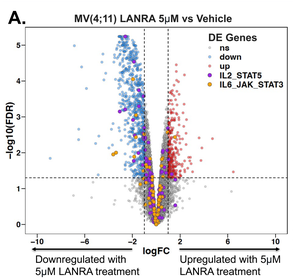


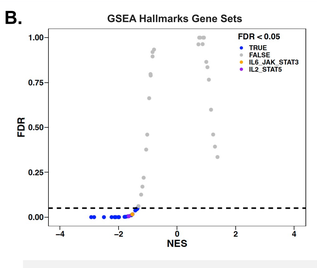


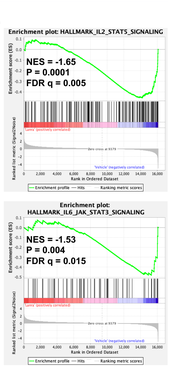
(A) Combination effect most strongly observed in blood lineage cancer models
- Rank ordering of lineages by the median of the ruxolitinib + lanraplenib 10µM dataset.
- Blood cancers (blue box) showed a strong potentiation at both 2µM and 10µM anchor doses of lanraplenib.

(B) Bliss synergy score by lineage for heme oncology models
- Synergy was calculated for each cell line using a 2 x 7 matrix of dose concentrations.
- >10 is synergistic and less than -10 is antagonistic.
- AML shows an enrichment with 9/20 models having strong synergy (green box).

JAK/STAT signaling pathways are downregulated in response to lanraplenib





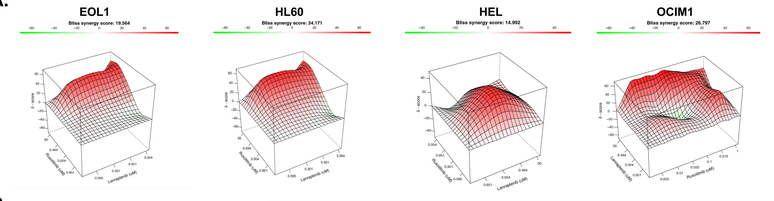


(A) Differential expression shows downregulated JAK/STAT signaling genes when treated with lanraplenib
- RNA-seq on MV(4;11) cells treated with 5µM lanraplenib and vehicle (DMSO) for 48hrs.
- Differential expression using EdgeR[6] with a log fold-change (logFC) threshold of -1 or 1 and false discovery rate (FDR) of 0.05.

(B) GSEA hallmark analysis identifying significant enrichment of JAK/STAT pathways
- Gene set enrichment analysis (GSEA)[7] results of treatment with 5µM lanraplenib.
- Normalized enrichment score (NES) for all hallmark pathways and using FDR cutoff of 0.05.

(C) Enrichment plots for JAK/STAT pathways
- Profiles of the running enrichment score (ES) and positions of gene set members on the rank-ordered list.

Ruxolitinib synergizes with lanraplenib in targeted AML cell line panel

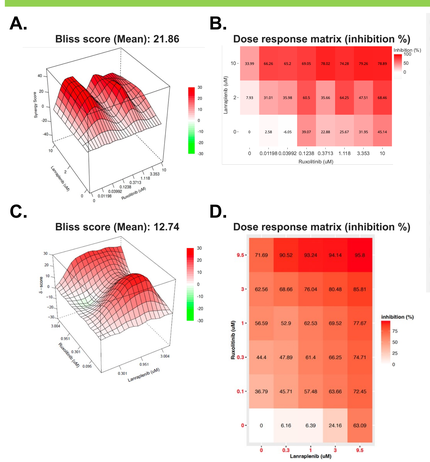


B.

Cell line	PRISM screen (Bliss score)	Retesting (Bliss score)
OCIAML5	21.86	12.74
OCIM1	19.22	26.80
HL60	18.68	24.17
HEL	16.52	14.99
EOL1	15.20	19.56

Discrete retesting cell line panel reproduces the PRISM combination screen results
(A) Five AML cell lines were chosen for discrete, orthogonal retesting using a 5-day in vitro assay with CellTiter-Glo (CTG) readout. Graphs display topography plots for bliss synergy demonstrating a broad range of robustly synergistic activity. OCI-AML5 data is shown below with comparison to screen.
(B) All five showed strong synergy on repeat consistent with the original screening data. This validates the multiplex high throughput approach as a potential synergy screen.

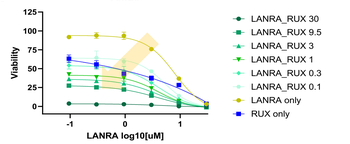
Orthogonal retesting validates screening data and approach



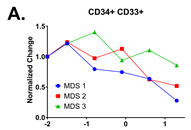
OCI-AML5 exemplar data from PRISM screen compared to discrete retesting

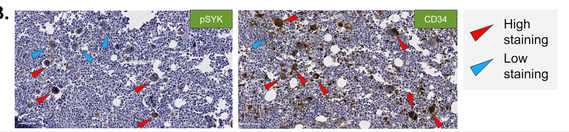
From screen
(A) Bliss synergy score topography map illustrates a broad range of doses all showing robust synergistic interaction.
(B) Dose response matrix shows the 7 x 2 matrix with strong anti-proliferative activity observed for combination.

From retest
(C) Bliss synergy topography plot recapitulated the broad range of synergistic doses
(D) Dose response matrix depicts strong viability reduction of the combo as assessed by CellTiter-Glo (CTG).
(E) Dose response curves from matrix data. Yellow shows lanraplenib alone and blue for ruxolitinib. Shades of green illustrate the increasing left and down shift of increasing anti-proliferative effect.



Myeloid dysplastic syndrome (MDS) patient samples are responsive to lanraplenib





(A) MDS bone marrow patient samples were treated with lanraplenib *ex vivo* and analyzed by flow cytometry. The blast population, defined by CD34+/CD33+ showed a marked reduction in response to SYK inhibition. **(B)** Representative image of an MDS patient sample presenting with Refractory Anaemia with Excess Blasts (RAEB), hypercellularity, dysmegakaryocytopoiesis, dyserythropoiesis, and dysgranulopoiesis. Slides were stained with a phosphorylation specific SYK (pSYK) or CD34 antibody. Strong pSYK staining was observed in megakaryocytes which are implicated in MDS/MF pathogenesis. These irregular MK cells also displayed a high rate of CD34+ which is consistent with their aberrant state in MDS/MF.

Conclusions

These studies demonstrate the utility of PRISM as a platform to rapidly identify rational combination agents. Importantly, lanraplenib is effective in combination with ruxolitinib in AML and other hematological malignancy preclinical models. This finding is consistent with the observation that SYK can regulate STAT signaling and cooperate with other RTKs like FLT3. Given the central role of SYK in regulation of oncogenic and inflammatory signaling, SYK inhibition with lanraplenib in combination with ruxolitinib may be a promising strategy for patients with myeloid malignancies. Lanraplenib is currently being studied in a different RTK combo with gilteritinib in NCT05028751.

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