Poster P392

Pharmacological Inhibition of SYK Confers Anti-proliferative and Novel Anti-tumor Immune Responses in AML

Luis A. Carvajal[1], Bridget Robinson[2], Yoko Kosaka[2], Thomas Jacob[2], Jinho Lee[2], Tressa Hood[1], Kristina Baker[2], Andy Kaempf[2], Suha Naffar-Abu Amara[1], Joanna Pucilowska[2], Evan Lind[2], Cristina Tognon[2], Jeffrey Tyner[2], Pavan Kumar[1], Tania Vu[2], Jorge DiMartino[1]

[1]Kronos Bio, Inc., San Mateo, CA, USA; [2]Oregon Health & Science University (OHSU), Portland, OR, USA

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, **Abstract P525**), 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 *FLT3*-mutated (*FLT3*m) AML (NCT05028751, **Abstract P524**).

Aims: To investigate the effects of ENTO and LANRA in T-cell responses in AML.

Methods: All patient-derived bone marrow (BM) and peripheral blood (PB) samples were obtained in accordance with Institutional Review Board (OHSU#004422) approval. Results from gene-expression profiling studies (RNA sequencing [RNA-seq]) in BM/PB mononuclear cells (MNCs) derived from 152 patients with AML, for which matching *NPM1* status and ex vivo sensitivity to ENTO was available, previously published by Tyner et al,[1] were reanalyzed using trimmed mean of M-values normalization and differential expression using edgeR. ENTO/LANRA sensitivity was assessed ex vivo after 72 hours in culture at 37 °C by MTS assay. T-cell functional assays were performed by culturing MNCs with anti-CD3 and treating with ENTO or LANRA, singly and in combination with anti-programmed death 1 (PD1). CD3+ T-cell proliferation as measured by Ki67 expression and phosphorylated SYK (pSYK) activation were assessed by quantitative single-cell immunofluorescence microscopy. Further, 6 formalin-fixed, paraffin-embedded BM biopsies from newly diagnosed AML patients were assessed for spatial expression of mRNA using a digital spatial-profiling method.

Results: In AML, mutations in *NPM1* with coexpression of *HOXA9/MEIS1* at baseline predicted antiproliferative activity to ENTO in ex vivo drug sensitivity studies. Accordingly, treatment of leukemic cells with either ENTO or LANRA inhibited SYK autophosphorylation in a dose-dependent manner. Pathway analysis of archival RNA-seq data from patients enrolled in the Leukemia and Lymphoma Society Beat AML Master Protocol (NCT03013998 [BAML-16-001-S6]) revealed gene expression signatures at baseline associated with the observed sensitivity to ENTO, including significant enrichment in the expression of genes associated with leukemogenesis, myeloid differentiation, and immune regulation. This was supported by T-cell functional studies, which demonstrated that ENTO and LANRA, singly as well as in combination with anti-PD1, could restore T-cell proliferation in primary AML patient samples that exhibited suppression. Last, Cancer Transcriptome Atlas analysis (GeoMx®) on archival BM biopsies obtained from these AML patients showed a strong correlation between immune checkpoint response and gene expression associated with immune pathway signaling.

SYK Mechanistic Model in AML

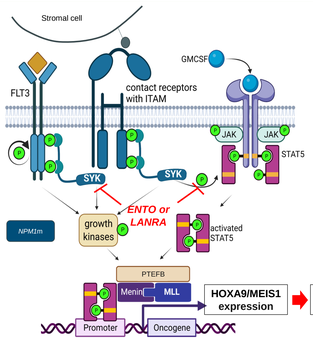

Figure 1.

- SYK integrates paracrine and contact signaling by reinforcing receptor tyrosine kinases (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 (eg, gilteritinib) can synergize with inhibition of a core node (eg, SYK) to potentiate antileukemic effects.
- LANRA shows strong antileukemic activity in combination with gilteritinib and other targeted agents in preclinical models **(Abstract P428)**.

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

Sensitivity to ENTO Correlates Strongly with the Presence of *NPM1* and/or *FLT3* Mutation in AML Patient–derived Samples

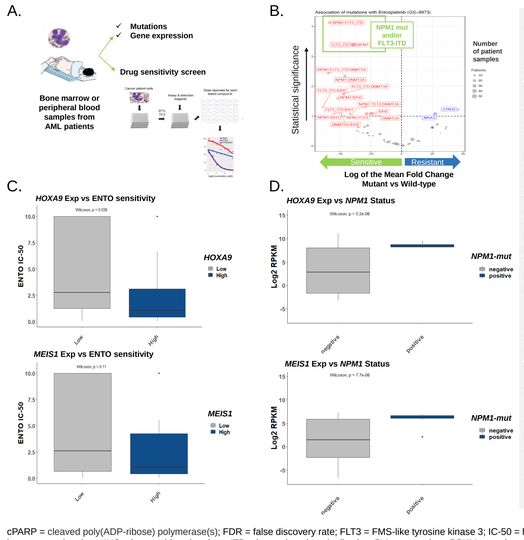

Figure 2.

A) Initial findings of the Beat AML Program included a cohort of 562 patients, from whom either BM or PB was collected. The specimens were assessed using whole exome sequencing for mutations, RNA-seq for gene expression, and ex vivo drug sensitivity analysis.[2] A subset of 152 samples were tested for sensitivity to ENTO (GS-9973) ex vivo.

B) ENTO sensitivity was determined by calculating the exposure-response area under the curve (AUC). The AUC average difference comparing various mutant vs wild-type genes implicated in leukemogenesis was determined using a 2-sided t-test from a linear model fit, plotted on the horizontal axis with FDR-corrected (using Benjamini-Hochberg[3]) q-value plotted on the vertical axis.[2] The mutations that showed the highest statistical significance associated with ENTO sensitivity were *NPM1* and/or *FLT3* internal tandem duplication (*FLT3*-ITD) mutations.

C) ENTO IC-50 was calculated for all samples and compared to *HOXA9* and *MEIS1* gene expression. Classifications of RPKM gene expression values were determined using the 75th percentile as "high" and 25th percentile as "low". High *HOXA9* expression was associated with increased sensitivity to ENTO with *MEIS1* trending in the same direction.

D) *HOXA9* and *MEIS1* $\log_2$ RPKM expression values were compared among samples that had mutations or were wild-type for *NPM1*. In both cases, *NPM1* mutants showed significantly higher expression for both genes.

cPARP = cleaved poly(ADP-ribose) polymerase(s); FDR = false discovery rate; FLT3 = FMS-like tyrosine kinase 3; IC-50 = half maximal inhibitory concentration; ICC = immunocytochemistry; IHC = immunohistochemistry; ITD = internal tandem duplication; PV, pervanadate; RPKM = reads per kilobase of transcript, per million mapped reads; RTK = receptor tyrosine kinase; UT = untreated.

Sensitivity to ENTO Shows an Association with Compromised Immune Response in AML Patient Samples at Baseline

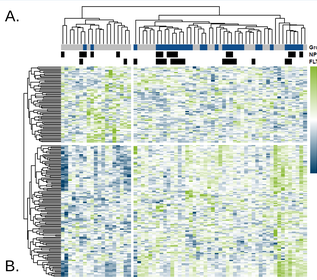

Figure 3.

A) A total of 66 samples that had baseline RNA-seq data were stratified into groups based on the ex vivo ENTO AUC results. Samples that had exposure-response AUCs in the lowest 20th percentile were classified as "sensitive" and those in the highest 20th percentile as "resistant". Differential gene expression was assessed comparing the 2 groups using edgeR.[4] Differentially expressed genes using Benjamini-Hochberg (BH)[3] false discovery rate (FDR) values of less than 0.05 were used for clustering analysis. The samples were additionally annotated with the mutation status for *NPM1* and *FLT3*-ITD. The resulting heat map shows gene expression profiles that are associated with ENTO sensitivity and *NPM1*m with or without comutation of *FLT3*.

B) Gene set enrichment analysis (GSEA)[5] was performed on the differentially expressed genes. The enrichment plots shown below are within the top 5 negatively enriched gene sets, ranked by normalized enrichment score (NES). These enriched pathways show that sensitivity to ENTO may be associated with downregulation of cell signaling and immune response pathways.

SYK Inhibition with ENTO or LANRA Can Restore T-cell Proliferation in AML Samples with Dysfunctional T-cell Proliferative Capacity

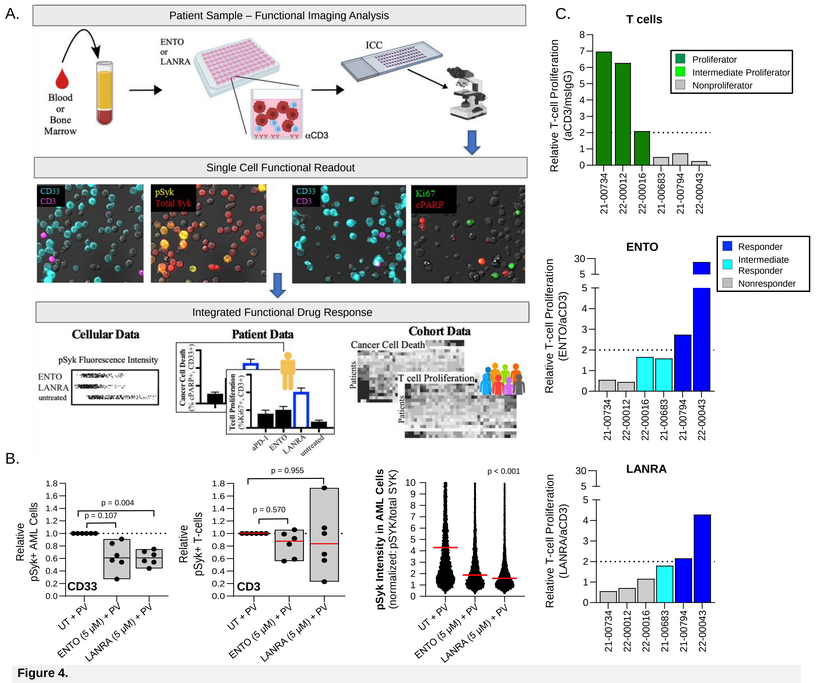

Figure 4.

A) Schematic of the workflow used to measure pSYK expression (4 h) and T-cell proliferation (72 h) after ex vivo treatment with ENTO or LANRA, using a single-cell, functional imaging assay. MNCs from 6 patients, newly diagnosed with AML, were treated ex vivo with ENTO or LANRA or an immune checkpoint inhibitor, α-PD1 (100,000 cells per condition). Cells were fixed, stained, and imaged for CD3, CD33, pSYK, total SYK, Ki67, and cPARP. Images were analyzed to measure fluorescence intensity of each marker, and populations were gated in FlowJo™ (BD Biosciences).

B) Treatment with ENTO or LANRA (4 h, ex vivo) decreased PV-induced pSYK levels 20%–70% in AML cells, relative to PV treatment alone, in the 6 AML samples tested (red line depicts median value). Intrasample differences in the percentage of pSYK+ cells between treatment groups were compared with Nemenyi test (AML cells: UT vs ENTO, p = 0.107; UT vs LANRA, p = 0.004; T cells: UT v ENTO, p = 0.570, LANRA, p = 0.955). Single-cell expression data from 1 AML specimen, 21-00794, is shown as an example (single-cell data correspond to white symbols in relative plots), and the normalized intensity of pSYK expression was also significantly reduced within individual AML cells after ENTO or LANRA treatment. Wilcoxon rank sum tests (with continuity corrections) were used to compare pSYK/total SYK intensity between treatment groups, with a Bonferroni correction applied; 3 pair-wise comparisons, p < 0.001.

C) Stimulation of AML specimens with αCD3 (72 h, ex vivo) demonstrates some patient samples contain T cells that do not adequately respond to this basic stimulus (nonproliferators, in gray), but can be rescued following treatment with αCD3 plus pSYK inhibitors, ENTO and LANRA (responders, in blue).

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AML Patients Without Inherent Proliferative Capacity Show Increased Proliferative Response to α-PD1 and ENTO and Can Be Stratified Based on Their Molecular Profiles

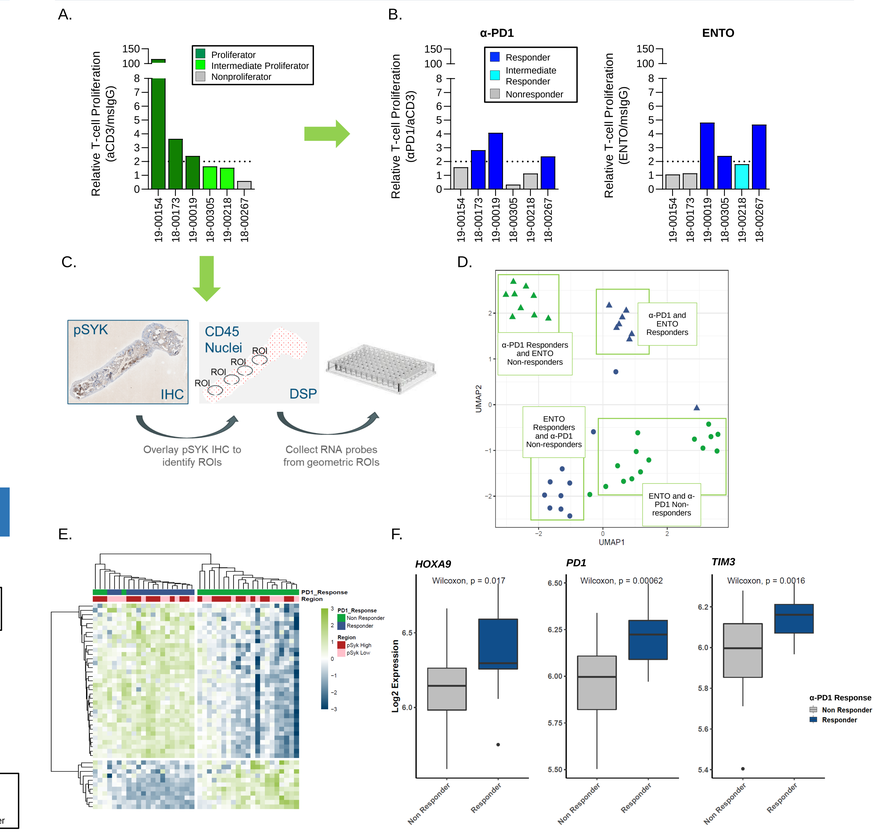

Figure 5.

A, B) Six AML specimens were stimulated with aCD3 (72 h, ex vivo) and analyzed via functional imaging analysis, as in Figure 4, again demonstrating that some AML patients contain T cells that do not proliferate in response to basic stimuli (nonproliferators in gray), but can be rescued following immune checkpoint inhibition, with α-PD1, or pSYK inhibition, with ENTO (responders in blue).

C) Schematic of digital spatial profiler (DSP) workflow. An image of a tissue section stained for pSYK (chromogenic) is overlayed over sequential tissue section prepared for DSP analysis. RNA probes were collected from regions of interests (ROIs) with high and low pSYK expression.

D) Uniform Manifold Approximation and Projection (UMAP)[6] dimension reduction technique was performed on the 42 ROIs that passed quality control. UMAP plot shows clear clustering and separation of the α-PD1 responders and ENTO responders vs nonresponders.

E) Differential gene expression was done comparing α-PD1 responders and nonresponders using a linear mixed model (LMM). LMM accounts for the subsampling per tissue that are not independent observations. The resulting differentially expressed genes using p-values of less than 0.01 were used for clustering analysis. The data show clear differences in gene expression, which can be used to stratify patients more likely to respond to α-PD1.

F) A total of 42 ROIs were analyzed as independent observations. Normalized expression of *HOXA9*, *PD1*, and *TIM3* is shown as $\log_2$ Q3 (3rd quartile of all selected targets) with Wilcoxon statistics. The data show an association between response to α-PD1 and immune tolerance markers, PD1, and TIM3, as well as the leukemic driver *HOXA9*.

Conclusions

- Inhibition of SYK is a promising therapeutic approach in *NPM1*- and *FLT3*-mutated AML.
- Our studies illustrate that ENTO and LANRA may restore T-cell proliferation in a subset of AML patients with dysfunctional T-cell responses, suggesting a novel mechanism of action. These results are consistent with prior studies demonstrating that functional T-cell impairment in a subset of AML patients may be restored with immune checkpoint inhibitors.[7]
- Additional studies are required to fully understand the mechanism of SYK-mediated antitumor immune responses in AML and to identify biomarkers for patient selection.

References

1. Tyner JW, et al. *Nature*. 2018;562(7728):526-531. 2. Mohr S, et al. *Cancer Cell*. 2017;31(4):549-562. 3. Benjamini Y, et al. *J Royal Statistical Soc Ser B*. 1995;57(1):289-300. 4. Robinson MD, et al. *Bioinformatics*. 2010:26(1):139-140. 5. Subramanian A, et al. *Proc Natl Acad Sci U S A*. 2005;102(43):15545-15550. 6. McInnes L, et al. *arXiv*. 2020:1802.03426. 7. Lamble AJ, et al. *Proc Natl Acad Sci U S A*. 2020;117(25):14331-14341.