Niche-based screening identifies small-molecule inhibitors of leukemia stem cells

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Definitions and Abbreviations

- **LSC**: Leukemia Stem Cells
- **LSCe**: Leukemia Stem Cell (enriched)
  - isolated from the bone marrow of leukemic animals by fluorescence-activated cell sorting
- **OP9**: Stromal Cells
  - From mouse bone marrow that has hematopoietic supportive capacity.
- **HPSCs**: Normal Hematopoietic stem and progenitor cells
- **CAFC**: Cobblestone Area-Forming Cells
Background: AML

- Acute Myeloid Leukemia (AML) is a fast forming cancer of the blood [1]
- Each year AML affects about 20,000 patients, and has a 5 year survival rate of about 26% [2]
Background: The bone marrow niche

- Heterogenous with a chemoprotective role
- Within AML is a population of cells with the capacity for self-renewal, disease initiation and disease propagation termed LSCs
- This microenvironment is therefore a prime target for AML
  - LCS are similar to normal hematopoietic stem and progenitor cells (HSPCs)
  - Can’t model leukemia cells with high-throughput small molecule screening
Primary Findings (Results)

● Screened 14,718 compounds in a leukemia-stroma co-culture system for Colony Forming Area Cells (CFACs).
● Lovastatin showed potential to inhibit leukemia and was unique.
  ○ Works via inhibiting HMG-CoA reductase
● BRD7116 also showed inhibition potential.
Figure 1: LSCe cells through co-culture with stromal cells to form CAFCs
Supplementary Figure 1c: Using c-Kit as a marker
Supplementary Figure 1d: Transplanting LSCE
Supplementary Figure 1e: CellProfiler software
Small molecule co-culture screen to find chemical compounds

Screened 14,718 small molecules in duplicate

~ 1920 bioactive molecules
~ 1600 natural products
~ 2800 compounds (generated via diversity oriented synthesis)
Co-culture screen data

- Values normalized to means of neutral and positive controls
Filtering Steps

- Initially: 14,718 small molecules
- 415 compounds inhibiting leukemic CAFCs in coculture
- 240 non-toxic (RETEST)
  - 8 point dose response on LSCe
  - Coculture
Filtering Steps CONT.

- In presence of one of the stromal types:
  - 196 compounds exhibiting dose response

- In presence of both types:
  - 139 compounds displayed activity

- At low concentrations (<20uM) 36 compounds exhibited stromal toxicity

- 155 prioritized compounds that inhibited LSCe cells relative to HSPCs
Small molecule screening cont.

- Assessed the selectivity of the 155 prioritized hits
- Preferential activity against LSCs- 100-fold selectivity against leukemia cells compared to normal HSPCs
  - Celastrol and parthenolide

<table>
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<tr>
<th>Name</th>
<th>Structure</th>
<th>LSCe assay EC$_{50}$ (nM)</th>
<th>HSPC assay EC$_{50}$ (nM)</th>
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<td><img src="image" alt="Celastrol Structure" /></td>
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<td>1,300</td>
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</table>
Small molecule screening cont.

- Tested 155 compounds on six human AML cell lines
  - NOMO-1 and THP-1 (MLL-AF9 oncogene)
- 8 concentrations in the absence of co-culturing
  - Subset 1: displayed potent activity in AML cell line
  - Subset 2: >10-fold higher potency on primary LSCe cells in co-culture compared to their mean potency across AML cell line.
<table>
<thead>
<tr>
<th>BRD7116§</th>
<th>lovastatin⁺</th>
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<tbody>
<tr>
<td>[Chemical Structure of BRD7116]</td>
<td>[Chemical Structure of Lovastatin]</td>
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</table>
BRD7116

- Bis-aryl sulfone
- Stroma-mediated anti-LSCe activity
- EC50 of 200 nM for co-culture LSCe cells
- Induced transcriptional changes consistent with myeloid differentiation
Supplementary Figure 3a

Stromal Pretreatment Screen

Plate OP9 stroma \(\rightarrow\) 1 Day \(\rightarrow\) Add Compound (155 tested total) \(\rightarrow\) 3 Days \(\rightarrow\) Remove Compound, Plate LSCe cells \(\rightarrow\) 6 Days \(\rightarrow\) Cobblestone area quantification
Supplementary Figure 3b

**troglitazone**

**Co-culture**

EC$_{50}$ = 0.42µM

**Stromal Pretreatment**

EC$_{50}$ = 1.6µM
Supplementary Figure 3d, 3e

**d**

**Cell Lines**

% viability (normalized) vs. Troglitazone concentration (μM)

- OCI-AML3
- U937
- NB4
- SKM-1
- NOMO-1
- THP-1

**e**

**Cell Lines**

% viability (normalized) vs. BRD7116 concentration (μM)

- OCI-AML3
- U937
- NB4
- SKM-1
- NOMO-1
- THP-1
Supplementary Figure 3f

The figure shows a graph with the x-axis representing BRD7116 concentration (μM) and the y-axis representing the percentage of replicates positive for cobblestone areas. The graph compares different patient samples, with each line representing a different sample, including AML 1 to AML 6 and Normal.
Figure 2: BRD7116 targets LSCs - Dose response curve (a) and effect of pre treatment of stroma (b)
Figure 2: BRD7116 has cell autonomous (c) and non autonomous effects (d)
Lovastatin inhibits leukemia cells and lowers CAFC activity in mouse and human cells

- Lovastatin is an FDA approved drug used for hypercholesterolemia.
- It inhibits CAFCs with an EC$_{50} < 200$ nM without harming mouse AML cell lines or HSPCs at concentrations as high as 20 µM.
- The inhibitory effects of lovastatin were cell autonomous, meaning lovastatin did not score in the previous stromal pretreatment assay.
- LSCs generated using an alternative oncogene, MOZ-TIF2, were sensitive to lovastatin in co-culture.
Figure 3: Lovastatin inhibits leukemia cells in mice (a) and lowers CAFC activity over time in human cells (b)

Leukemia selective activity of Lovastatin compared to HSPCs in co-culture with BMSCs

Effects of lovastatin on CAFC activity in normal and leukemic patient samples
Figure 3: Lovastatin inhibits leukemia cells (a) and lowers CAFC activity over time in human cells (b)

Lovastatin selectively inhibits LSCE while sparing HSPCs
Figure 4: Mevalonate rescues anti leukemia effect
Figure 4: Genetic pathway of mevalonate shows that HMGCR inhibition selectively targets LSCs
Figure 4: Post transplant limiting dilution analysis (c) and Ex vivo three component treatment (d) to shows cells developing leukemia.
What is the purpose of the untreated WT helper splenocytes?
Long term chimerism analysis further supported that lovastatin did not impair normal HSPC function.
Key takeaways

- Potential for success with high throughput, low cost drug screens
- Recreation of bone marrow niche
- Identification of new targets for AML
- Power of physiologically relevant, complex biology to small molecule screening for AML
**Next Steps**

- Follow up studies with naturally arising, primary human leukemia cells
- Extension to other leukemia models
- Scale up to larger compounds
- Understanding the mechanisms of BRD7116
- Exploration of the mevalonate pathways  
  - Effector molecules specifically
  - Systemic assessment of mevalonate dependent effects
References
