Insights into the Cell-of-Origin of the Histiocytoses Using Patient-Derived Xenograft Models

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### What Cells Give Rise to Histiocytoses?

**Historical**
- LCH arises from Langerhans cells (LCs)
  - Shared antigenic markers
  - Birbeck granules

**2010**
- LCH cell transcriptional profile more similar to monocyte-derived precursors than LCs

**2012**
- Epidermal LCs arise from fetal liver monocytes rather than bone marrow hematopoietic precursors

**2014**
- BRAF V600E detected in bone marrow precursors of high-risk BRAF V600E-mutated LCH patients

**2015**
- LCH and non-LCH neoplastic cells have distinct transcriptional profiles

**References**
Ontogeny of Macrophages and the Potential Cell(s)-of-Origin of Systemic Histiocytic Neoplasms

Mesoderm

Intra-embryonic hemogenic endothelium

Hematopoietic Stem Cells

Self renewal

MDPs

Epidermal LCs

Tissue-resident macrophages

Kupffer cells
Microglia
Langerhans cells

Self renewal
Cell-of-Origin Studies of Histiocytosis

- Berres et al. suggested LCH is a clonal disorder arising from HSPCs that acquire a somatic mutation in an oncogenic pathway linked to the histiocytoses

- *BRAF* V600E mutation has not been detected in the CD34+ compartment of all *BRAF* V600E-mutated LCH patients

- Expression of *Braf* V600E in the murine dendritic cell precursors does not recapitulate all phenotypic consequences of human LCH.
  - Opens the possibility of alternate cells or origin for the systemic histiocytoses
    - Tissue-resident macrophages arising from yolk sac-derived EMPs

- Comparable studies have not been published in ECD and other non-LCH neoplasms, and whether or not HSPCs from histiocytosis patients have functional self-renewal potential is unknown.

Experimental Questions

• Can hematopoietic stem and progenitor cells from systemic histiocytoses patients functionally give rise to these disorders?

• Can we generate patient-derived xenograft models of histiocytic neoplasms for further functional analyses or *in vivo* treatment?
### Diagnostic and Mutational Data for the Eight Xenografted Histiocytosis Patient Samples

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Histiocytic Neoplasm</th>
<th>Histiocytic Mutation</th>
<th>WHO-Classified Myeloid Neoplasm</th>
<th>Myeloid Neoplasm Mutation(s)</th>
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<tbody>
<tr>
<td>1</td>
<td>ECD</td>
<td>BRAF V600E</td>
<td>MPN/MDS</td>
<td>NRAS G13D ASXL1 Q733X</td>
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<td>2</td>
<td>LCH</td>
<td>BRAF V600E</td>
<td>MPN/MDS</td>
<td>TET2 X1268_Splice KRAS A146P</td>
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<td>ECD</td>
<td>BRAF V600E</td>
<td>MPN</td>
<td>JAK2 V617F NRAS G12S TET2 L757fs*56 U2AF1Q157P</td>
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<td>BRAF V600E</td>
<td>MDS/AML</td>
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<td>8</td>
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</table>
Evaluation of Histiocytoses and Histiocytoses Plus Other Myeloid Neoplasm Co-occurrence via Patient-Derived Xenograft (PDX) Models

Harvest BM
Enrichment of CD34+ HSCs Via Immunomagnetic Selection

Transplant NSGS (NSG-SGM3) Mice (NOD-scid gamma IL3-GM-SF)

Monitor PB Monthly for Engraftment until Sacrifice

Patient 1
ECD: BRAF V600E
CMML: ASXL1 p.Q733

Patient 2
LCH: BRAF V600E

Patient 3
ECD: BRAF V600E
CMML: TET2

Patient 4
MPN: JAK2 p.V617F
ECD: BRAF V600E

Patient 5
CMML: ASXL1

Patient 6
TET2

Patient 7
ECD: KRAS G12S

Patient 8
ECD: BRAF V600E

Sublethal Irradiation
ECD Patient 7 Heart Biopsy with Non-Langerhans Cell Histiocytosis \textit{KRAS} p.G12S

**Patient 7 Heart Biopsy**

![H&E Image]

![Human CD68 Image]
CBC Results for NSGS PDX Model from ECD Patient 7

White Blood Cells

Red Blood Cells

Platelets

Hemoglobin

Hematocrit

MCV

<table>
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<tr>
<th>Months</th>
<th>WBC (K/μL)</th>
<th>RBC (M/μL)</th>
<th>Platelets (K/μL)</th>
<th>Hgb (g/dL)</th>
<th>Hct (%)</th>
<th>MCV (fL)</th>
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</table>

Months
Evidence of Engraftment of Human ECD Patient 7 hCD45+ Cells in NSGS PDX Model

Month 1

0.35%

Month 2

0.45%

Month 3

0.73%
ECD Patient 7 PDX Model Tissue FACS Analysis

Bone Marrow
- hCD45: 8.84%
- mCD45.1

Spleen
- hCD45: 4.52%
- hCD14

Liver
- hCD45: 19.8%
- hCD14

Lung
- hCD45: 9.17%
- hCD14

Kidney
- hCD45: 0.66%
- hCD14

Bone Marrow
- hCD33: 26.9%
- hCD3: 18.7%

Spleen
- hCD33: 11.6%
- hCD3: 14.7%

Liver
- hCD33: 31.7%
- hCD3: 52.6%

Lung
- hCD33: 15.0%
- hCD3: 53.8%

Kidney
- hCD33: 33.3%
- hCD3: 45.8%
PDX Model Demonstrates Infiltration of Hematopoietic Tissues with Human CD45+ Foamy Histiocytes

PDX Bone Marrow

PDX Spleen

H&E

Human CD45
PDX Model Demonstrates Infiltration of Hematopoietic Tissues with Human CD68+ and CD1a- Foamy Histiocytes

PDX Bone Marrow

PDX Spleen

Human CD68

Human CD1a
PDX Model Demonstrates Infiltration of Hematopoietic Tissues with Human CD163+ Foamy Histiocytes
PDX Model Demonstrates Infiltration of Other Tissues with Human CD45+ and CD163+ Foamy Histiocytes
PDX Model Demonstrates Patient 7
*KRAS c.34G>A; p.G12S*

PDX Bone Marrow

PDX Spleen
Evaluation of Histiocytoses and Histiocytoses Plus Other Myeloid Neoplasm Co-occurrence via Patient-Derived Xenograft (PDX) Models

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CD34+ HSCs

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Patient 5

Patient 6

Patient 7

ECD: KRAS G12S

Patient 8

ECD: BRAF V600E
Additional Evidence of Engraftment of Patient 3 and Patient 8 hCD45+ Cells in NSGS PDX Models

Patient 3 PDX BMA

Patient 8 PDX BMA
Conclusions and Future Directions

• Functional evidence that the CD34+ compartment can initiate histiocytosis

• Current studies involve adult histiocytic disorders only, but pediatric histiocytoses may have a different cell of origin

• Further work is needed to determine the frequency of successful engraftment of CD34+ cells in histiocytoses
  • Based on current experience the engraftment rate is 1/8

• Further PDX murine model characterization underway
  • Continuing to monitor surviving ECD (2), ECD/LCH (1), LCH (1) PDX models for engraftment
  • Serial transplantation ongoing using hCD34+ cells from engrafted NSGS PDX murine models
  • Monitoring NSGS mice recently transplanted with normal human CD34+ cord blood

• Interrogation of different purified cell subsets from systemic histiocytoses patients is needed to refine the cell(s)-of-origin of the systemic histiocytoses
Thank you

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