Novel Alterations in CSF1R, RET, and Other Diverse Kinases in the Histiocytoses with Biochemical and Structural Insights into Their Mechanisms of Activation

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Published Kinase Alterations in the Histiocytoses

Langerhans Cell Histiocytosis

- **BRAF V600E**
- **MAP2K1**
- **Fusions: BRAF, ETV3-NCOA2**
- **ERBB3**
- **ARAF**
- **BRAF Other**
- **K/NRAS**
- **BRAF Indels**

Unknown

- Lee, et al. JCI Insight 2017

Non-Langerhans Cell Histiocytosis

- **BRAF V600E**
- **MAP2K1**
- **Fusions: BRAF, ALK, NTRK1, ETV3-NCOA2**
- **MAPK1 (ERK2)**
- **MAP3K1 Amp. BRAF Other**
- **PIK3CA/D**
- **ARAF**
- **H/K/NRAS**

Unknown

- Kordes, et al. Leukemia 2015

- Lee, et al. JCI Insight 2017
- Chakraborty et al. Oncotarget 2017
- Techavichit, et al. Hum Pathol. 2017
- Garces, et al. Mod. Pathol. 2017
Questions

• What other novel alterations drive histiocytic neoplasms?
• Are there genetic differences across the diverse clinical and histologic subtypes of histiocytoses?
• What is/are the cell(s)-of-origin in the histiocytoses?
• What is the basis for familial histiocytoses?
Histiocytic Neoplasms Sequenced (n=270)

- Erdheim-Chester Disease (ECD) (37%; n=100)
- Langerhans Cell Histiocytosis (LCH) (34%; n=92)
- Juvenile Xanthogranuloma (JXG) (21%; n=55)
- Histiocytic Sarcoma (HS) (2%; n=6)
- Rosai-Dorfman Disease (RDD) (6%; n=17)

N = 270
Overall Histiocytoses Cohort (n=270)
Frequency of Kinase Alterations Identified (n = 270)
Erdheim-Chester Disease Cohort (N = 100)

Histiocytoses Subsets
- Langerhans Cell Histiocytosis (LCH)
- Erdheim-Chester Disease (ECD)
- Juvenile Xanthogranuloma (JXG)
- Rosai-Dorfman Disease (RDD)
- Histiocytic Sarcoma (HS)

Age
- Pediatric
- Adult

Sequencing Analyses
- Whole Exome Sequencing (WES) and/or Whole Transcriptome Sequencing (WT)
- Targeted DNA and/or RNA Sequencing

N = 100
Langerhans Cell Histiocytosis Cohort (N = 92)

Histioctyes Subsets
- Langerhans Cell Histiocytosis (LCH)
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Age
- Pediatric
- Adult

Sequencing Analyses
- Whole Exome Sequencing (WES) and/or
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- Targeted DNA and/or RNA Sequencing

N = 91
Juvenile Xanthogranuloma Cohort (N = 55)

Histiocytoses Subsets
- Langerhans Cell Histiocytosis (LCH)
- Rosai-Dorfman Disease (RDD)
- Erdheim-Chester Disease (ECD)
- Histiocytic Sarcoma (HS)
- Juvenile Xanthogranuloma (JXG)

Age
- Pediatric
- Adult

Sequencing Analyses
- Whole Exome Sequencing (WES) and/or Whole Transcriptome Sequencing (WT)
- Targeted DNA and/or RNA Sequencing

Jennifer Picarsic and Ronald Jaffe
Rosai-Dorfman Disease Cohort (N = 17)

Histiocytoses Subsets
- Langerhans Cell Histiocytosis (LCH)
- Rosai-Dorfman Disease (RDD)
- Erdheim-Chester Disease (ECD)
- Histiocytic Sarcoma (HS)
- Juvenile Xanthogranuloma (JXG)

Age
- Pediatric
- Adult

Sequencing Analyses
- Whole Exome Sequencing (WES) and/or Whole Transcriptome Sequencing (WT)
- Targeted DNA and/or RNA Sequencing
Histiocytic Sarcoma (HS) Cohort (N = 6)

Histiocytoses Subsets
- Langerhans Cell Histiocytosis (LCH)
- Rosai-Dorfman Disease (RDD)
- Erdheim-Chester Disease (ECD)
- Histiocytic Sarcoma (HS)
- Juvenile Xanthogranuloma (JXG)

Age
- Pediatric
- Adult

Sequencing Analyses
- Whole Exome Sequencing (WES) and/or Whole Transcriptome Sequencing (WT)
- Targeted DNA and/or RNA Sequencing
Correlation of Kinase Mutations with Histiocytosis Subtype

- Langerhans Cell Histiocytosis (LCH)
- Erdheim–Chester Disease (ECD)
- Juvenile Xanthogranuloma (JXG)
- Rosai–Dorfman Disease (RDD)
- Histiocytic Sarcoma (HS)

Frequency:
- 0
- 10
- 20
- 30
- 40

-log10 (p. values):
- 0
- 2
- 5
- 10
- 15
- 20
Recurrent CSF1R Mutations

CSF1R: The receptor for MCSF (Macrophage Colony Stimulating Factor) and IL-34

Controls production, differentiation, and function of macrophages

Expression is restricted to progenitor cells committed to the monocyte/macrophage lineage.

Principles of Activation of Human CSF1R
Structural Mapping of CSF1R Activating Mutations and Proposed Impact of CSF1R Activation

- Enhance dimerization propensity in the absence of ligand
  - CSF1R<sup>P386L</sup>

- Enhance dimerization propensity in the absence of ligand
  - CSF1R<sup>W450_E456del</sup>

- Promotion of the receptor’s intrinsic kinase activity
  - Affect intracellular regions critical to enforcing the inactive state of the kinase domain in the absence of ligand
  - CSF1R<sup>Y546_K551del</sup>
CSF1R Mutations in the Histiocytoses are Activating

Ba/F3 Cell Cytokine-Independent Growth

Graph showing the growth of cells with different CSF1R mutations compared to controls.
CSF1R Mutations in Histiocytoses are Activating via Phospho-Flow Cytometry
CSF1R Deletion Mutations are Sensitive to CSF1R Inhibitors (Pexidartinib and BLZ 945)

![Graph showing IC50 values for different deletion mutations with Pexidartinib and BLZ945 inhibitors.](image-url)
Identical Twins with Histiocytosis

Monozygotic, dichorionic
Identical twin girls
Germline WES of the twins and their parents revealed no known pathological cancer predisposition syndromes.
Shared Somatic CSF1R and NF1 Mutations in Monozygotic Twins

Distinct mutations evolved within each child following CSF1R/NF1

CSF1R and NF1 mutations shared in the largest clones across the twins
JXG Mutational Signatures in Monozygotic Twins with the Highest Ranked Signature Being DNA Mismatch Repair

Twin 1

Twin 2

DNA MMR

Aging

Signature.1

Signature.6

Signature.15

unknown
Microsatellite Instability is Rare in the Histiocytoses – However, Both Twins Show Microsatellite Instability by Next-Generation Sequencing
CSF1R Mutants Expressed on Cell Surface
What cell type gives rise to histiocytoses?

- Fetal EMP
- Bone marrow derived hematopoietic stem cell?
- More committed monocyte/dendritic cell precursor?

Frederic Geissmann

What is the cell-of-origin of histiocytoses?

1. Shared CSF1R mutant yolk-sac precursor.
2. Hematogenous dissemination of shared precursor of histiocytosis in utero.
Spectrum of *CSF1R/CSF1* Mutant Diseases

Hereditary Diffuse Leukoencephalopathy with Spheroids: Germline LOF CSF1R mutations

*Rademakers, et al. Nat Gen 2012*

Tenosynovial Giant Cell Tumor: Ectopic overexpression of CSF1

*West, et al. PNAS 2006; Tap, et al. NEJM 2015*
Kinase Fusions in Histiocytoses

- RNF11-BRAF
- CLIP2-BRAF
- PACSIN2-BRAF
- BICD2-BRAF
- CSF2RA-BRAF
- SPPL2A-BRAF
- MS4A6A-BRAF
Kinase Fusions in Histiocytoses

- LMNA: NTRK1
- IRF2BP2: NTRK1
- SQSTM1: NTRK1
- TPM3: NTRK1
- KIF5B: ALK
- NCOA4: RET
**NCOA4-RET** Fusions in JXG/AXG are Activating
RET inhibitor Response in NCOA4-RET JXG/AXG

Pre-LOXO-292

Post-LOXO-292
RET inhibitor Response in NCOA4-RET JXG/AXG

![Diagram of NCOA4-RET sequence with ARA70 and Tyrosine Kinase domains.](image)

Pre-LOXO-292

Post-LOXO-292
MEK Inhibitor Response in \textit{BICD2-BRAF} Fusion LCH

Pre-Trametinib  
Post-Trametinib

Eli Diamond
ALK Inhibitor Response in *KIF5B-ALK* Fusion ECD

Pre-Treatment

Post-Treatment
Conclusions

- Diverse kinase mutations and fusions continue to drive systemic histiocytic neoplasms.

- Recurrent, activating CSF1R mutations in familial and sporadic histiocytoses, suggesting the cell-of-origin belongs to committed monocyte/macrophage progenitors. Highlights therapeutic potential for CSF1R inhibition in histiocytoses.

- First description of other kinase and receptor tyrosine kinase [MAPK7 (ERK5), MAPK3 (ERK1) ALK, KIT, MET, JAK3, and CSF3R] mutations and first RET fusions uncovered in the histiocytoses.

- BRAF<sup>V600E</sup> is prevalent in LCH and ECD but not in other histiocytoses subtypes. There is also an enrichment of NTRK1 fusions and CSF1R mutations in JXG and BRAF fusions and deletions in LCH compared to other histiocytoses in this cohort.

- Kinase alterations other than BRAF<sup>V600E</sup> have direct therapeutic implications.

Genetic Alterations

- **BRAF V600E**
- MAP2K1/2 Mutations
- Other RAF/MAPK Mutations
- Braf Fusions
- RET Fusions
- CSF1R Mutations
- ALK Fusions
- NTRK Fusions

Therapy

- Vemurafenib Debrafenib
- MEK Inhibition
- RET Inhibition
- CSF1R Inhibition
- ALK Inhibition
- NTRK1 Inhibition

- Interferon, Anakinra, other non-kinase drugs
THANK YOU

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