3D culture of BRAF/KRAS-mutated Erdheim-Chester disease tissues unveils rewired histiocyte metabolism as a new therapeutic target

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Clinical presentation

Bone pain (26%)
Neurological symptoms (23%)
  (exophthalmos; gaze disturbances; gait ataxia)
Diabetes Insipidus (22%)
Constitutional symptoms (20%)
Retroperitoneal involvement (14%)
  (renal failure; nephrovascular hypertension; hydronephrosis)

Pulmonary symptoms (12%)
  (dyspnea)
Cutaneous involvement (11%)
  (xanthoma; xanthelasma)
Cardiovascular involvement (6%)
  (pericardial effusion)
Palpable mass (5%)
Hypogonadism, panhypopituitarism (3%)

Erdheim-Chester disease: an inflammatory myeloid neoplasm

Dagna et al, 2012 JCO
Arnaud et al, 2011 Blood

Stoppacciaro et al, 2006 Arthritis Rheum

Papo et al, 2019 Curr Oncol Rep
A unifying disease model for Erdheim-Chester disease

IL-6

CCL2

BRAFV600E

p-ERK

Stopacchiaro et al, 2006
Dagna et al, 2010
Arnaud et al, 2011
Dagna et al, 2012

Haroche et al, 2012
Blombery et al, 2012
Emile et al, 2014
Cangi et al, 2014

Cavalli et al, 2014
Cytokine inhibition in Erdheim-Chester disease

Rationale and efficacy of interleukin-1 targeting in Erdheim-Chester disease
Achille Aouba, Sophie Georgin-Lavialle, Christian Pegnoux, Nicolas Martin Silva, Amédaie Renand, Françoise Galateau-Salle, Sophie Le Toquin, Henri Bensadoun, Frédérique Larousserie, Stéphane Silvera, Nicole Provost, Sophie Candon, Raphaële Seror, Mathilde de Menthon, Olivier Hermine, Loïc Guillemin and Boris Bienvenu

BRIEF REPORT

Tocilizumab in patients with multisystem Erdheim–Chester disease
Alvise Berti\textsuperscript{a,b}, Giulio Cavalli\textsuperscript{a,b}, Barbara Guglielmi\textsuperscript{a}, Riccardo Biavasco\textsuperscript{b}, Corrado Campochiaro\textsuperscript{a,b}, Alessandro Tomelleri\textsuperscript{a,b}, Roberto Nicoletti\textsuperscript{a}, Andrea Panzacchi\textsuperscript{d}, Marina Ferrari\textsuperscript{a}, and Lorenzo Dagna\textsuperscript{a,b}
BRAF inhibition in Erdheim-Chester disease

Dramatic efficacy of vemurafenib in both multisystemic and refractory Erdheim-Chester disease and Langerhans cell histiocytosis harboring the BRAF V600E mutation

Julien Hercoffe,1,2* Fleur Cohen-Aubert,1,2* Jean-François Emile,3* Laurent Arnaud,1,2 Philippe Maksud,4 Frédéric Charlotte,4 Philippe Cluzol,4 Aurélie Drier,7 Baptiste Hamoir,1,2 Nataša Banamour,1 Sophie Rozard6 Jean Donadieu,1,2 and Zahir Amoura1,2

Vemurafenib in Multiple Nonmelanoma Cancers with BRAF V600 Mutations

David M. Hyman, M.D., Igor Puzanov, M.D., Vivek Subbiah, M.D., Jason E. Faris, M.D., Ian Chau, M.D., Jean-Yves Blay, M.D., Ph.D., Jürgen Wolf, M.D., Ph.D., Noopur S. Raje, M.D., Eli L. Diamond, M.D., Antoine Hollebecque, M.D., Radj Gervais, M.D., Maria Elena Elez-Fernandez, M.D., Antoine Italiano, M.D., Ph.D., Ralf-Dieter Hofheinz, M.D., Manuel Hidalgo, M.D., Ph.D., Emily Chan, M.D., Ph.D., Martin Schuler, M.D., Susan Frances Lasserre, M.Sc., Martina Makrutzki, M.D., Florin Sirzen, M.D., Ph.D., Maria Luisa Veronesi, M.D., Josep Taberner, M.D., Ph.D., and José Baselga, M.D., Ph.D.
vemurafenib treatment in ECD: limitations

• not all ECD patients carry a $BRAFV600E$ mutation

• vemurafenib treatment mostly results in **partial clinical responses** in ECD patients

• vemurafenib treatment is often associated with severe **side effects** and **recurrences** upon treatment discontinuation

**Targeted therapies in 54 patients with Erdheim-Chester disease, including follow-up after interruption (the LOVE study)**

Fleur Cohen Aubart, $^{1,2}$ Jean-François Emile, $^{3,4}$ Fabrice Carrat, $^{2,5,6}$ Frédéric Charlotte, $^{2,7}$ Neila Benamer, $^{8}$ Jean Donadieu, $^{9}$ Philippe Maksud, $^{10}$ Ahmed Idbaih, $^{11}$ Stéphane Barete, $^{12}$ Khé Hoang-Xuan, $^{11}$ Zahir Amoura, $^{1,2}$ and Julien Haroche $^{1,2}$
MEK inhibition in ECD: cobimetinib and trametinib

LETTER

Efficacy of MEK inhibition in patients with histiocytic neoplasms

Eli L. Diamond1,2,22, Benjamin H. Durham3,4,12, Gary A. Ulaner2,5, Esther Drill6, Justin Buthorn1, Michelle Kj1, Lillian Bitner4, Hana Cho2, Robert J. Young2,6, Jasmine H. Francis2, Rajajith Rampal2,8, Mario Lacouture2,6, Lynn A. Brody2, Neval Ozkaya3,10, Ahmet Dogan3, Neal Rosen2,9,11, Alexia Iasonos2,6, Omar Abdel-Wahab2,4,8, & David M. Hyman7,8

1Department of Neurology, Memorial Sloan Kettering Cancer Center, New York, NY, USA. 2Weill Cornell Medical College, New York, NY, USA. 3Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, NY, USA. 4Human Oncology and Population Sciences Program, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA. 5Department of Radiology, Memorial Sloan Kettering Cancer Center, New York, NY, USA. 6Department of Medical Oncology, Memorial Sloan Kettering Cancer Center, New York, NY, USA. 7Laboratory of Pathology, National Cancer Institute, Bethesda, MD, USA. 8Laboratory of Pathology, Memorial Sloan Kettering Cancer Center, New York, NY, USA. 9Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA. 10Department of Radiation Oncology, Memorial Sloan Kettering Cancer Center, New York, NY, USA. 11Department of Genetics and Developmental Biology, Memorial Sloan Kettering Cancer Center, New York, NY, USA. 12These authors contributed equally. Eli L. Diamond, Benjamin H. Durham.

*E-mail: abdelwahab@mskcc.org; hyman@mskcc.org

Median time to best response, 3.2 months

Complete response
Partial response
 Stable disease
 Deceased
 Treatment ongoing

0 5 10 15 20 25
Overall treatment duration (months)
Approaching cancer metabolism in 3D culture

Targeting metabolic reprogramming in KRAS-driven cancers

Kenji Kawada¹ · Kosuke Toda¹ · Yoshiharu Sakai¹

**Aim:** to identify
- tumor vulnerabilities
- pathogenic cues (oncometabolites, immunometabolites..)
- cross-talk with microenvironmental components

Improve treatment of cancer patients
Regulation of glucose metabolism by oncoproteins

Hay, Nat Rev Cancer 2016
RCCSTM Bioreactor allows long-term culture of tissue explants

F Pedica.

Skin  Bone Marrow  Renal Cell Carcinoma  Hepatocarcinoma

Multiple Myeloma

Renal Cell Carcinoma

Ferrarini et al, PlosOne 2013
Belloni et al, Haematologica 2018
ECD tissues retain cytokine production in 3D culture

ECD 1
Xanthelasma, BRAF^{V600E}

ECD 2
Xanthelasma, BRAF^{V600E}

ECD 3
Pleural lesion, BRAF^{V600E}

IFX in vivo

VEM in vivo

3D culture of Erdheim-Chester disease tissues unveils histiocyte metabolism as a new therapeutic target
Antonello Villa, Daniela Belloni, Barbara Vergani, Simone Cenci, Giulio Cavalli, Riccardo Blavasco, Monica Rodolfo, Maria Giulia Cani, Claudio Degan, Lorenzo Dagna, Elisabetta Ferrero, Marina Ferrari

Ann Rheum Dis 2018;0:1–2. doi:10.1136/annrhumd-2018-214432
vemurafenib affects histiocyte metabolism but not viability

Villa et al, ARD 2018
trametinib inhibits cytokine release by KRAS-mutated histiocytes

**ECD 4**

BRAFwt, KRAS<sup>G12D</sup> xanthelasma

**trametinib**

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**Red Oil**

**manuscript in preparation**
trametinib counteracts metabolic reprogramming in histiocytes
Metabolomic analysis of ECD tissues cultured in bioreactor

Glycolysis

D-Glucose
2/3-Phospho-D-olivcerate
Phosphoenolpyruvate
D-Glucose 6-phosphate
Pyruvate
Lactate

TCA cycle

2-Oxoglutarate
Succinate
Citrate
Fumarate
Malate

GSH homeostasis

5-Oxoproline
Ascorbate
Dehydroascorbate

FA metabolism

L-Carnitine
acyl-C4 (butanoyl-l-carnitine)
acyl-C6 (hexanoyl-l-carnitine)
acyl-C5:1 (5:1/carnitine)
acyl-C10:1 (O-Decenoyl-L-carnitine)
acyl-C8 (L-octanoylcarnitine)
acyl-C10 (O-Deconoyl-L-carnitine)
acyl-C4:DC
acyl-C5:OH
acyl-C16 (L-Palmitoylcarbitine)
acyl-C18 (Octadecanoyl-L-carnitine)
acyl-C20:4

Itaconate

NT   tr   N1   tr  TCM
RCCS  histio

 manuscipt in preparation
activation of the glycolysis pathway
accumulation of TCA metabolites
accumulation of citrate, suggestive of activation of the cholesterol synthesis pathway
activation of tryptophan metabolism, most likely by activation of IDO1
high induction of itaconate
overall, the metabolic profile resembles that of activated monocytes and macrophages
all these noted metabolic changes were counteracted by culture of tissue samples with trametinib
Energy metabolism in ECD histiocytes and activated macrophages

Amino acids
- L-alanine
- L-glutamine
- L-asparagine

Glycolis
- D-Glucose
- 2/3-Phospho-D-glycerate
- Phosphoenolpyruvate
- D-Glucose 6-phosphate
- Pyruvate
- Lactate

TCA cycle
- 2-Oxoglutarate
- Succinate
- Citrate
- 2-Oxoglutaramate
- Fumarate
- Malate

GSH homeostasis
- 5-Oxoproline
- Ascorbate
- Dehydroascorbate

FA metabolism
- L-Carnitine
- acyl-C4 (butanoyl-l-carnitine)
- acyl-C6 (hexanoyl-l-carnitine)
- acyl-C5:1 (Tetrayl-carnitine)
- acyl-C10:1 (O-Decanoyl-l-carnitine)
- acyl-C8 (L-octanoyl-carnitine)
- acyl-C10 (O-Decanoyl-l-carnitine)
- acyl-C4-DC
- acyl-C5-OH
- acyl-C16 (L-Palmitoyl-carnitine)
- acyl-C18 (Octadecanoyl-l-carnitine)
- acyl-C20:4

Itaconate
- NT tr
- N1 tr
- TCM

Minhas et al, 2018 Nat Immunol
G. Dell’Antonio

ECD: a disease of “foamy” histiocytes

Uber lipoidgranulomatose
Chester W, 1930

Clinical and Population Studies

Hypoalphalipoproteinemia and $BRAF^{V600E}$ Mutation Are Major Predictors of Aortic Infiltration in the Erdheim-Chester Disease

Fleur Cohen-Aubart, Maryse Guerin, Lucie Poupel, Philippe Cluzel, Flora Saint-Charles, Frédéric Charlotte, Youssif Arsaﬁ, Jean-François Émile, Eric Frisadal, Carine Le Goff,
Jean Donadieu, Zahir Amoura, Philippe Lesnik, Julien Haroche, Wilfried Le Goff

$BRAF^{V600E}$

↓ CXCL1

↓ CX3CR1

↑ CC7

↑ IL1RA

↑ CCL2

Low HDL-C

Low CEC

Blood CD14+

AORTIC INFILTRATION

G. Dell’Antonio

VEMURAFENIB
Itaconate: an emerging determinant of inflammation in activated macrophages

Itaconate: the poster child of metabolic reprogramming in macrophage function

Luke A. J. O’Neill1,2* and Maxim N. Artyomov1,2*

Nature Reviews | Immunology

Itaconic acid mediates crosstalk between macrophage metabolism and peritoneal tumors

Jonathan M. Weiss1, Luke E. Davies1, Megan Karwan1, Lilia Ileva1, Michelle K. Orzal1, Robert Y.S. Cheng1, Lisa A. Ridmer1, Christina M. Amunstanga1, David A. Wink1 and Daniel W. McVicar1

1Cancer and Inflammation Program, Center for Cancer Research, National Cancer Institute (NCI), Frederick, Maryland, USA; 2Cardiff University, Division of Infection and Immunology, Cardiff, United Kingdom; 3Yale Comprehensive Cancer Center, Yale University, New Haven, Connecticut, USA; 4Laboratory for Metabolic Immunology, Center for Cancer Research, National Cancer Institute (NCI), Frederick, Maryland, USA; 5Women’s Malignancies Branch, Center for Cancer Research (CCR), NCI, Frederick, Maryland, USA.
Overview of mechanisms of T cell exhaustion

Expression of Programmed Cell Death 1 Ligands (PD-L1 and PD-L2) in Histiocytic and Dendritic Cell Disorders

Wherry & Kurachi, Nat Rev Immunol. 2015
Targeting ECD metabolism in 3D culture: 2-DG

Muñoz-Pinedo C et al, Cell Death and Disease 2012
Targeting ECD metabolism in 3D culture: metformin

Steinberg GR & Carling D, Nat Rev Drug Discovery 2019

- Reduction in lipid storage
  - increased fatty acid oxidation
  - inhibition of fatty acid and cholesterol synthesis

- Regulation of carbohydrate metabolism
  - increased GLUT1-dependent glucose uptake
  - cell-type-specific increased glycolysis
Conclusions

• Dynamic 3D culture in bioreactor is suitable for pathogenic studies and for drug testing in ECD

• The technology allowed us to define outcomes down-stream oncogenic mutations, and specifically to identify rewired metabolism as a peculiar feature of ECD histiocytes.

• Our model can be further exploited to design new therapeutic strategies for ECD and conceivably for other forms of histiocytosis.
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ECD patients and families
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