

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

Marina Ferrarini, MD

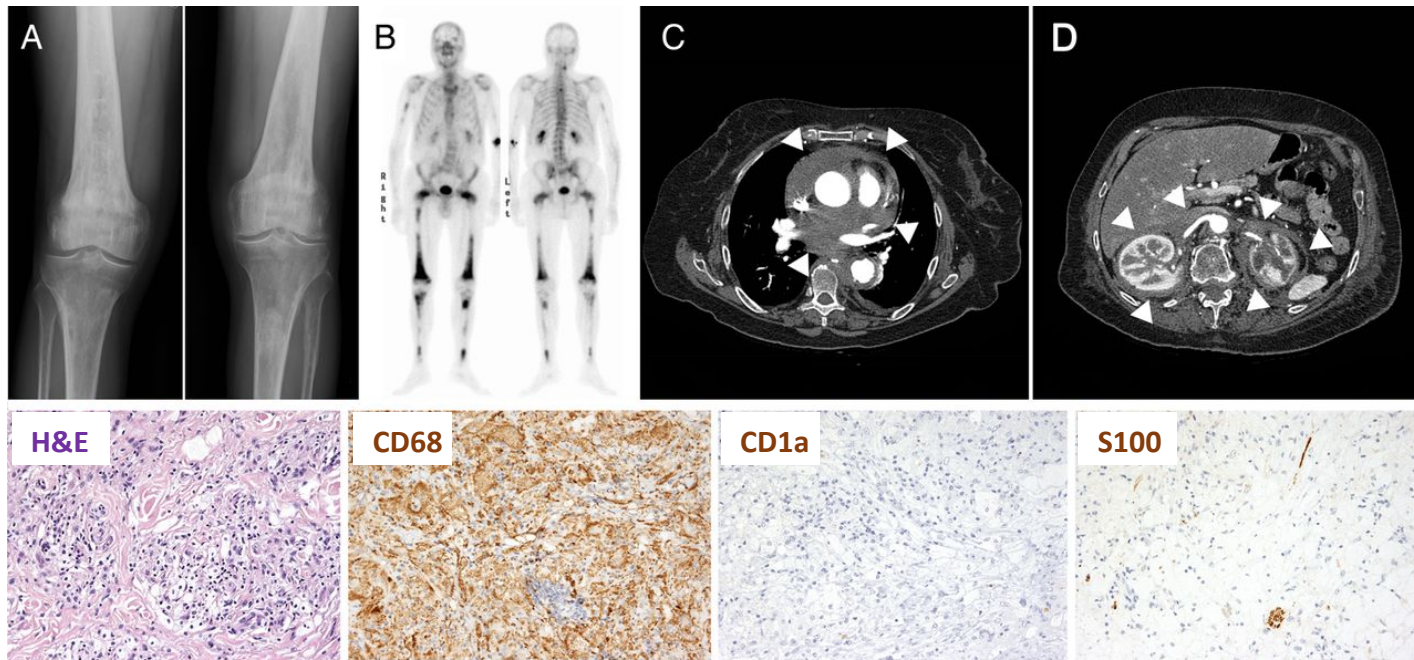
B-Cell Neoplasia Unit
Division of Experimental Oncology
IRCCS San Raffaele

7th Annual ECD Medical Symposium

Milan – July 11, 2019



Clinical, imaging and histological findings in patients with Erdheim–Chester disease

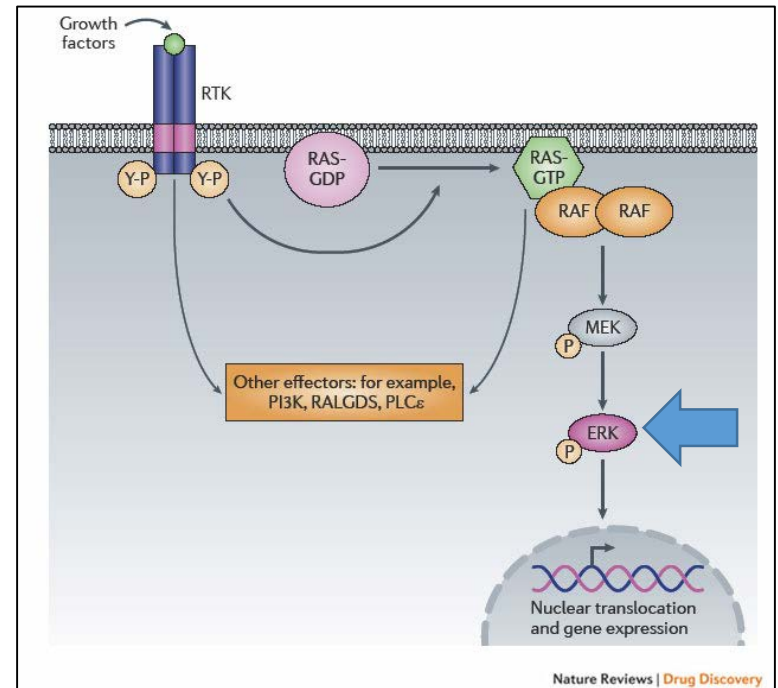
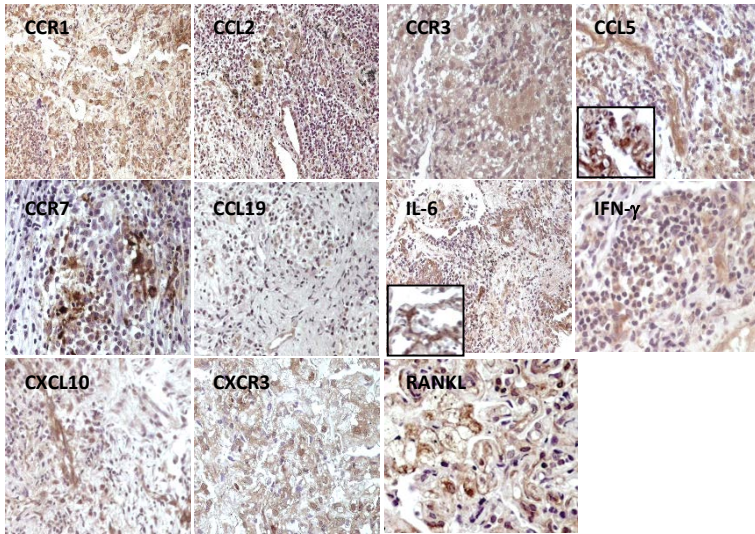


Clinical presentation

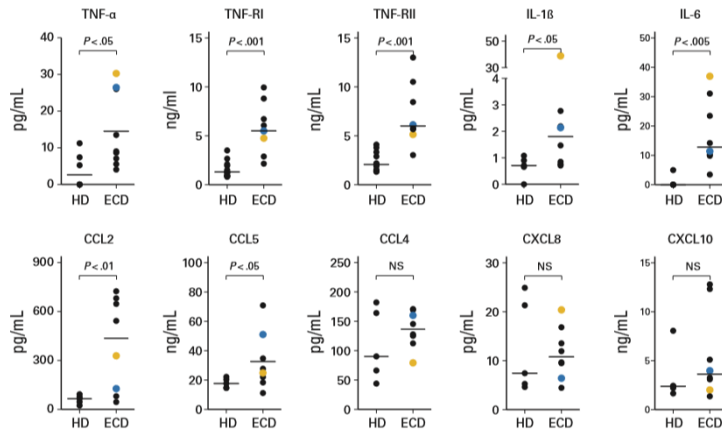
Bone pain (26%)	Pulmonary symptoms (12%) (dyspnea)
Neurological symptoms (23%) (<i>exophthalmos; gaze disturbances; gait ataxia</i>)	Cutaneous involvement (11%) (<i>xanthoma; xanthelasma</i>)
Diabetes Insipidus (22%)	Cardiovascular involvement (6%) (<i>pericardial effusion</i>)
Constitutional symptoms (20%)	Palpable mass (5%)
Retroperitoneal involvement (14%) (<i>renal failure; nephrovascular hypertension; hydronephrosis</i>)	Hypogonadism, panhypopituitarism (3%)

Giulio Cavalli et al. *Ann Rheum Dis* 2013;72:1691-1695

Erdheim-Chester disease: an inflammatory myeloid neoplasm

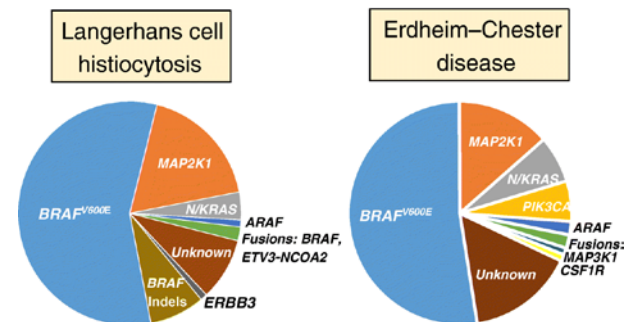


Stoppacciaro *et al*, 2006 *Arthritis Rheum*



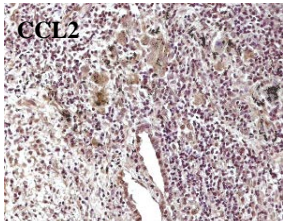
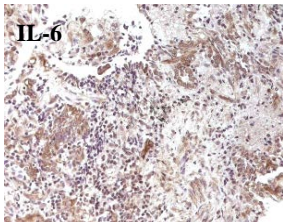
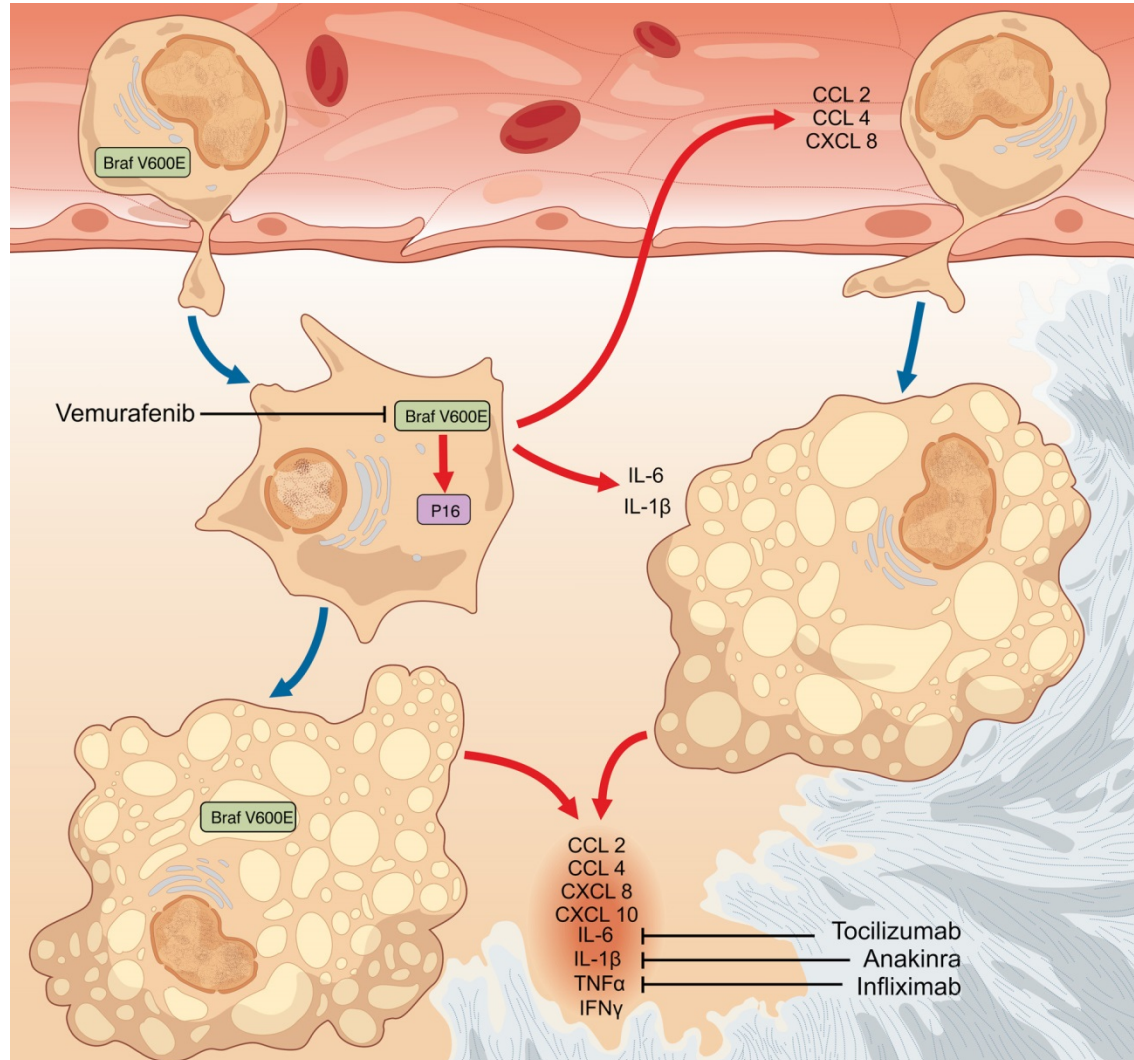
Dagna *et al*, 2012 *JCO*

Arnaud *et al*, 2011 *Blood*

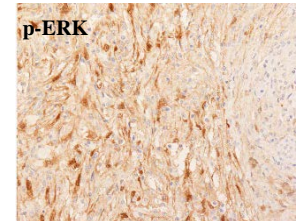


Papo *et al*, 2019 *Curr Oncol Rep*

A unifying disease model for Erdheim-Chester disease



Stoppacciaro *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

Cytokine inhibition in Erdheim-Chester disease

blood

Prepublished online Aug 19, 2010;
doi:10.1182/blood-2010-04-279240

Rationale and efficacy of interleukin-1 targeting in Erdheim-Chester disease

Achille Aouba, Sophie Geogin-Lavialle, Christian Pagnoux, Nicolas Martin Silva, Amédée Renand, Françoise Galateau-Salle, Sophie Le Toquin, Henri Bensadoun, Frederique Larousserie, Stéphane Silvera, Nicole Provost, Sophie Candon, Raphaële Seror, Mathilde de Menthon, Olivier Hermine, Loïc Guillevin and Boris Bienvenu

VOLUME 30 · NUMBER 28 · OCTOBER 1 2012

JOURNAL OF CLINICAL ONCOLOGY

Tumor Necrosis Factor α As a Master Regulator of Inflammation in Erdheim-Chester Disease: Rationale for the Treatment of Patients With Infliximab

Dagna L, Corti A, Langheim S, Guglielmi B, De Cobelli F, Doglioni C, Fragasso G, Sabbadini MG, Ferrarini M.

ONCOIMMUNOLOGY
2017, VOL. 6, NO. 6, e1318237 (6 pages)
<https://doi.org/10.1080/2162402X.2017.1318237>



Taylor & Francis
Taylor & Francis Group

BRIEF REPORT



Tocilizumab in patients with multisystem Erdheim–Chester disease

Alvise Berti^{a,b}, Giulio Cavalli^{a,b}, Barbara Guglielmi^a, Riccardo Biavasco^b, Corrado Campochiaro^{a,b}, Alessandro Tomelleri^{a,b}, Roberto Nicoletti^c, Andrea Panzacchi^d, Marina Ferrarini^e, and Lorenzo Dagna^{a,b}

BRAF inhibition in Erdheim-Chester disease

From www.bloodjournal.org by guest on October 21, 2014. For personal use only.

Plenary Paper

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

*Julien Haroche,^{1,2} *Fleur Cohen-Aubart,^{1,2} *Jean-François Emile,³ *Laurent Arnaud,^{1,2} Philippe Maksud,⁴ Frédéric Charlotte,⁵ Philippe Cluzel,⁶ Aurélie Drier,⁷ Baptiste Hervier,^{1,2} Neïla Benameur,⁸ Sophie Besnard,⁹ Jean Donadieu,¹⁰ and Zahir Amoura^{1,2}

VOLUME 33 · NUMBER 5 · FEBRUARY 10 2015

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Reproducible and Sustained Efficacy of Targeted Therapy With Vemurafenib in Patients With *BRAF*^{V600E}-Mutated Erdheim-Chester Disease

Julien Haroche, Fleur Cohen-Aubart, Jean-François Emile, Philippe Maksud, Aurélie Drier, Dan Tolédano, Stéphane Barete, Frédéric Charlotte, Philippe Cluzel, Jean Donadieu, Neïla Benameur, Philippe A. Grenier, Sophie Besnard, Jean-Paul Ory, François Lifermann, Ahmed Idbah, Brigitte Granel, Bruno Graffin, Baptiste Hervier, Laurent Arnaud, and Zahir Amoura

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

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 Veronese, M.D., Josep Tabernero, 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 Benameur,⁸ Jean Donadieu,⁹ Philippe Maksud,¹⁰ Ahmed Idbaih,¹¹ Stéphane Barete,¹² Khê Hoang-Xuan,¹¹ Zahir Amoura,^{1,2} and Julien Haroche^{1,2}

MEK inhibition in ECD: cobimetinib and trametinib

LETTER

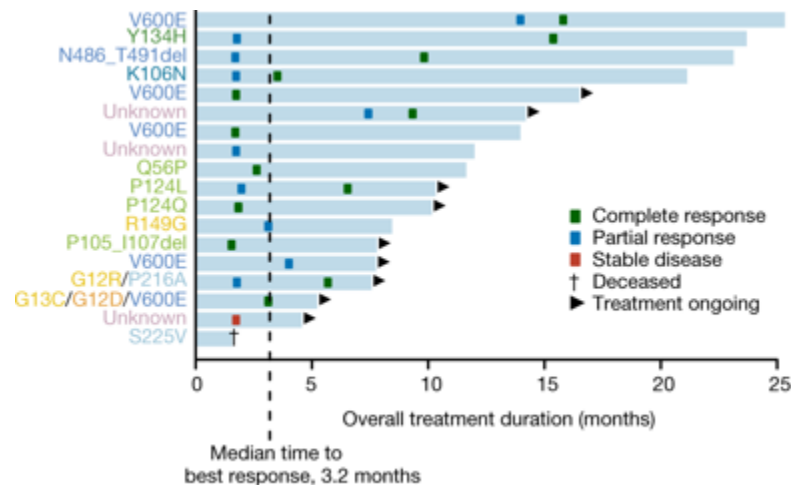
<https://doi.org/10.1038/s41586-019-1012-y>

Efficacy of MEK inhibition in patients with histiocytic neoplasms

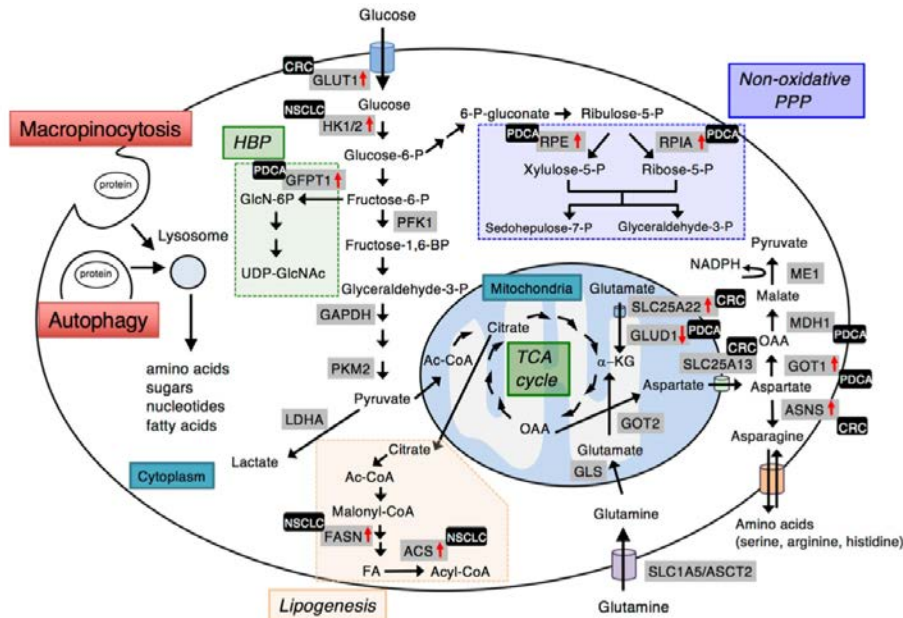
Eli L. Diamond^{1,2,12}, Benjamin H. Durham^{3,4,12}, Gary A. Ulaner^{2,5}, Esther Drill⁶, Justin Buthorn¹, Michelle Ki⁴, Lillian Bitner⁴, Hana Cho⁴, Robert J. Young^{2,5}, Jasmine H. Francis⁷, Raajit Rampal^{2,8}, Mario Lacouture^{2,9}, Lynn A. Brody⁵, Neval Ozkaya^{3,10}, Ahmet Dogan³, Neal Rosen^{2,8,11}, Alexia Iasonos^{2,6}, Omar Abdel-Wahab^{2,4,8*} & David M. Hyman^{2,8*}

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 Pathogenesis 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 Epidemiology and Biostatistics, Memorial Sloan Kettering Cancer Center, New York, NY, USA. ⁷Ophthalmic Oncology Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA. ⁸Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA. ⁹Dermatology Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA. ¹⁰Laboratory of Pathology, National Cancer Institute, Bethesda, MD, USA. ¹¹Molecular Pharmacology and Chemistry Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA. ¹²These authors contributed equally: Eli L. Diamond, Benjamin H. Durham.
*e-mail: abdelwao@mskcc.org; hymand@mskcc.org

NATURE | www.nature.com/nature



Approaching cancer metabolism in 3D culture



Targeting metabolic reprogramming in KRAS-driven cancers

Kenji Kawada¹ · Kosuke Toda¹ · Yoshiharu Sakai¹

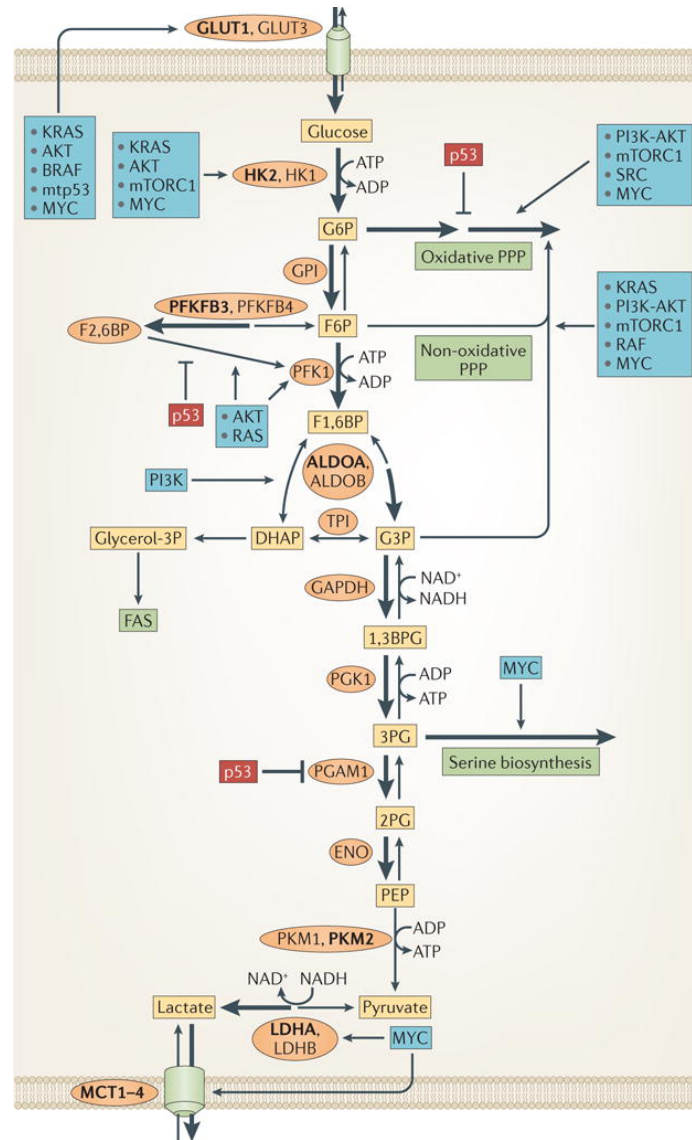
Aim: to identify

- tumor vulnerabilities
- pathogenic cues (oncometabolites, immunometabolites..)
- cross-talk with microenvironmental components



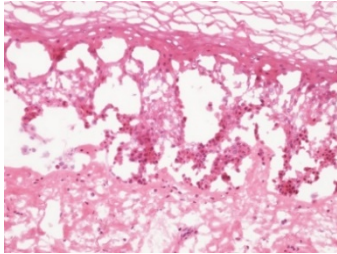
Improve treatment of cancer patients

Regulation of glucose metabolism by oncoproteins

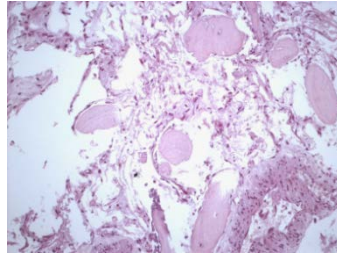


RCCS™ Bioreactor allows long-term culture of tissue explants

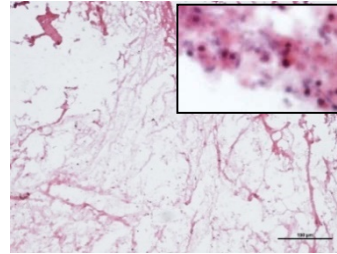
Skin



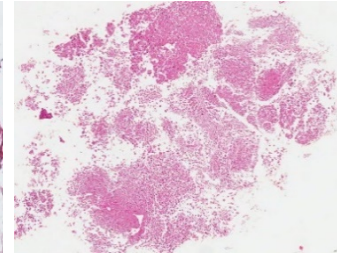
Bone Marrow



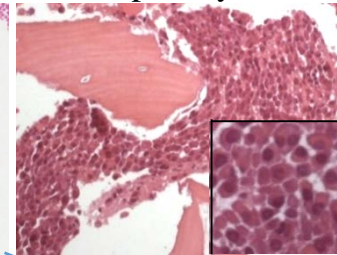
Renal Cell Carcinoma



Hepatocarcinoma

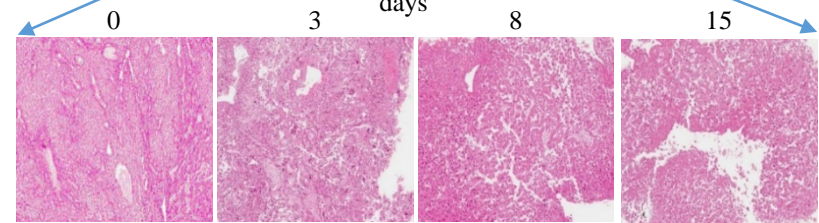


Multiple Myeloma



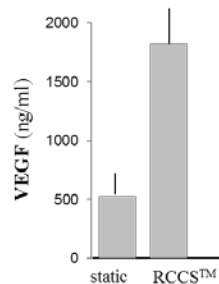
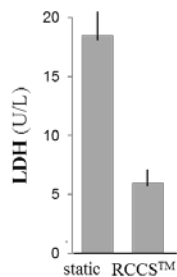
100X

200X
days



F. Pedica.

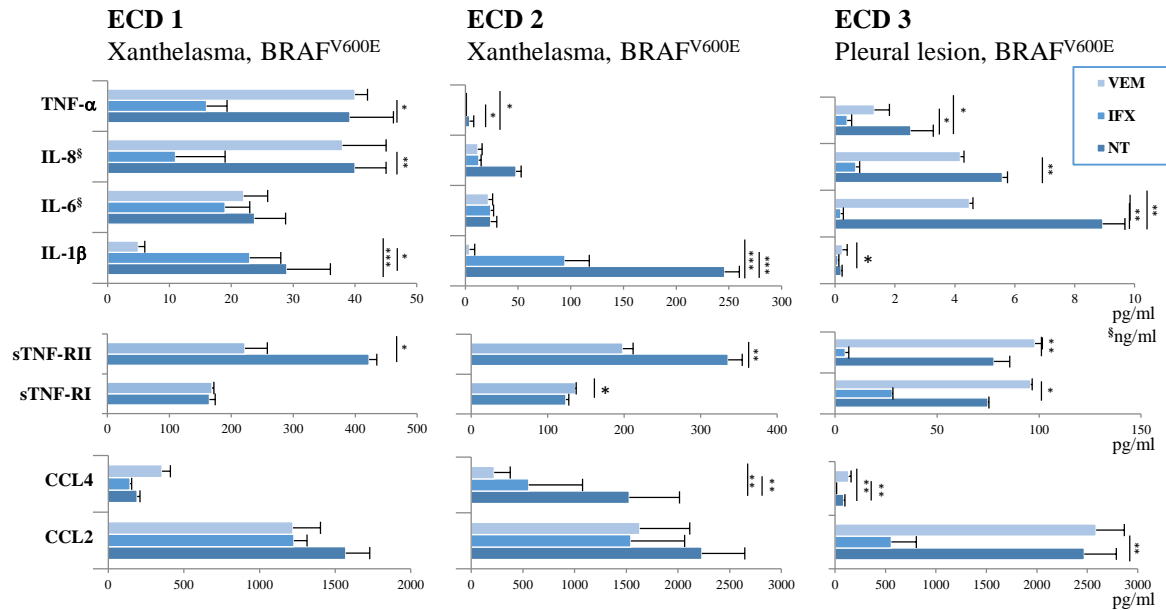
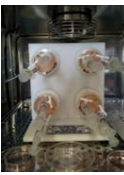
Renal Cell Carcinoma



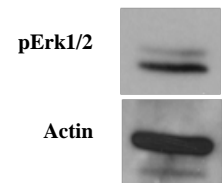
Ferrarini *et al*, PlosOne 2013

Belloni *et al*, Haematologica 2018

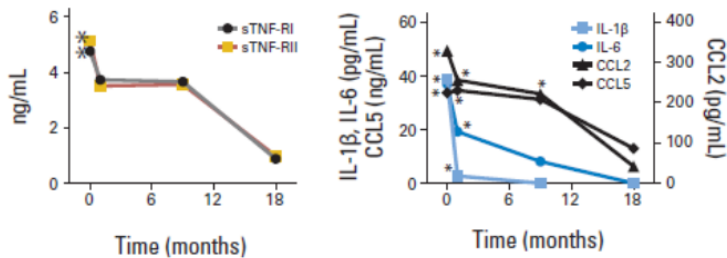
ECD tissues retain cytokine production in 3D culture



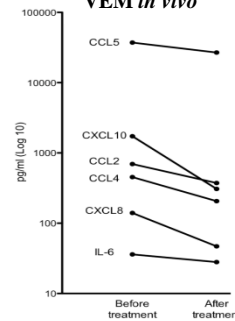
ECD 2 skin biopsy



IFX *in vivo*



VEM *in vivo*

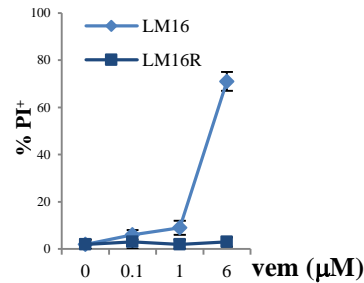
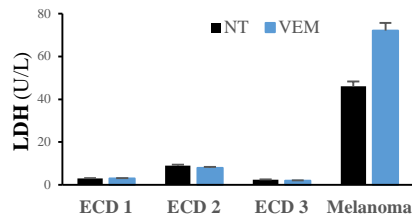
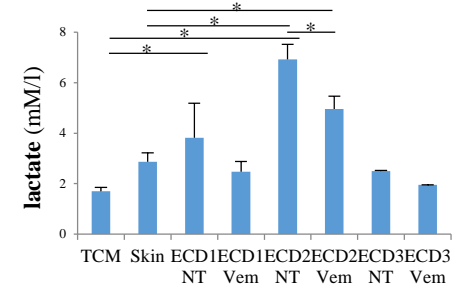
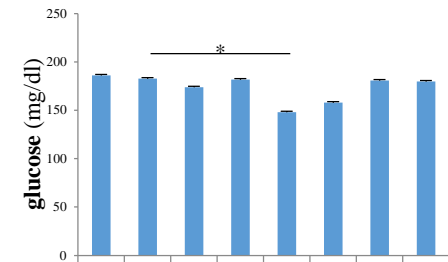
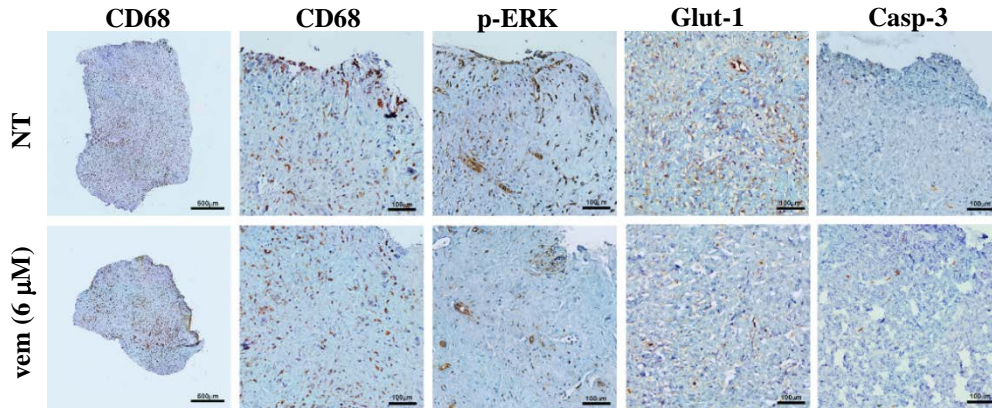
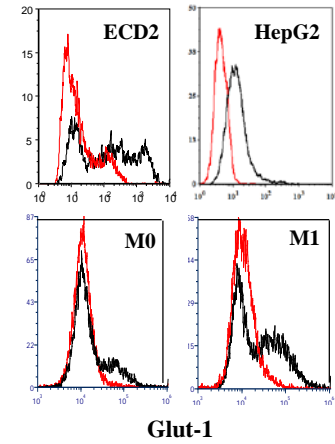
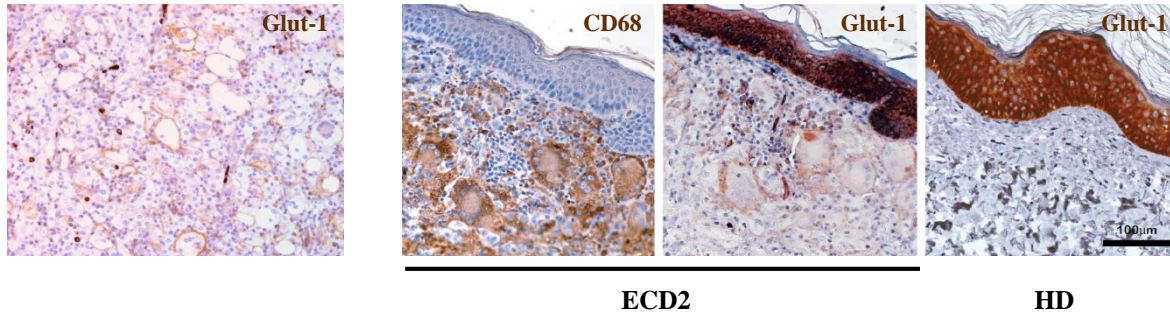
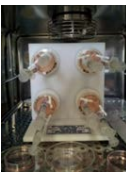


3D culture of Erdheim-Chester disease tissues unveils histiocyte metabolism as a new therapeutic target

Antonello Villa,¹ Daniela Belloni,² Barbara Vergani,¹ Simone Cenci,³ Giulio Cavalli,^{4,5} Riccardo Biavasco,^{5,6} Monica Rodolfo,⁷ Maria Giulia Cangi,⁸ Claudio Doglioni,^{5,8} Lorenzo Dagna,^{4,5} Elisabetta Ferrero,² Marina Ferrarini²

Ann Rheum Dis 2018;0:1–2. doi:10.1136/annrheumdis-2018-214432

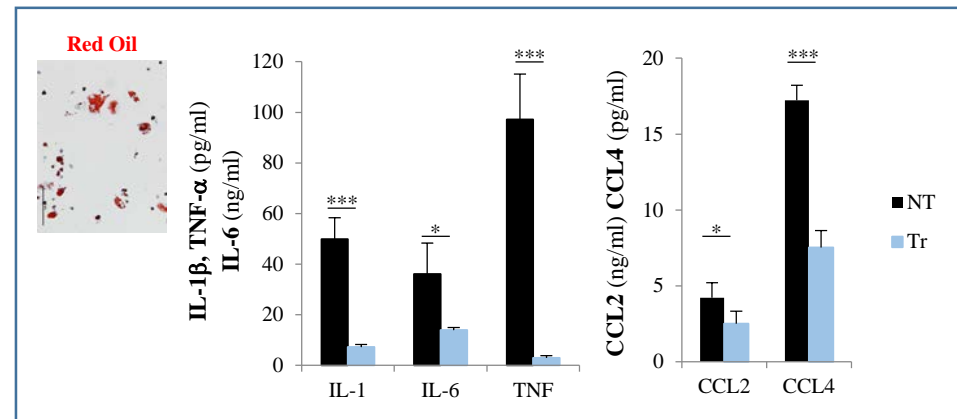
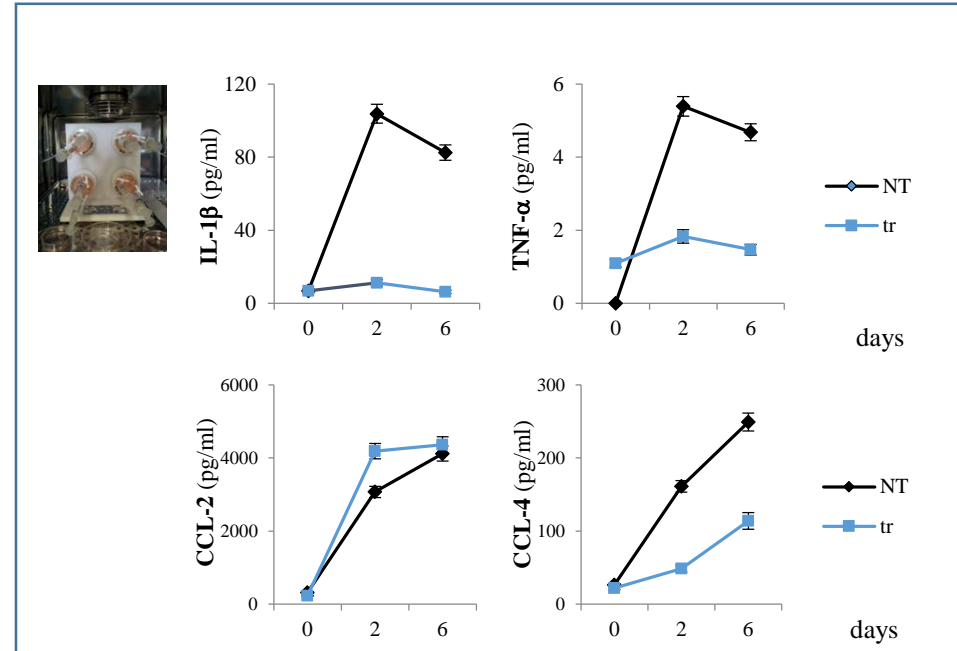
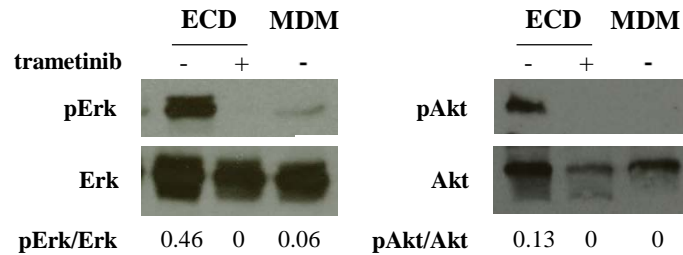
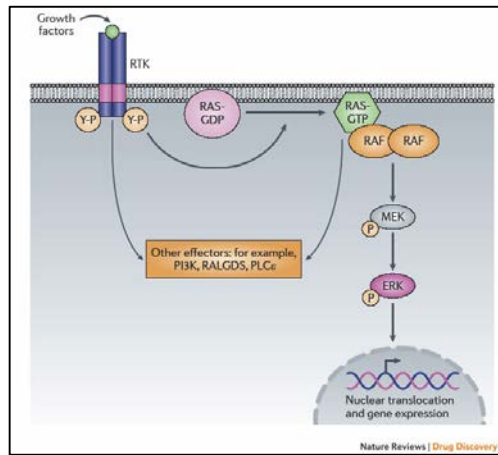
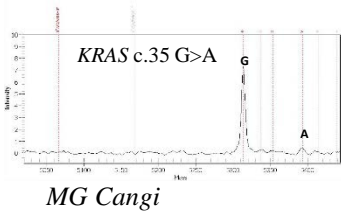
vemurafenib affects histiocyte metabolism but not viability



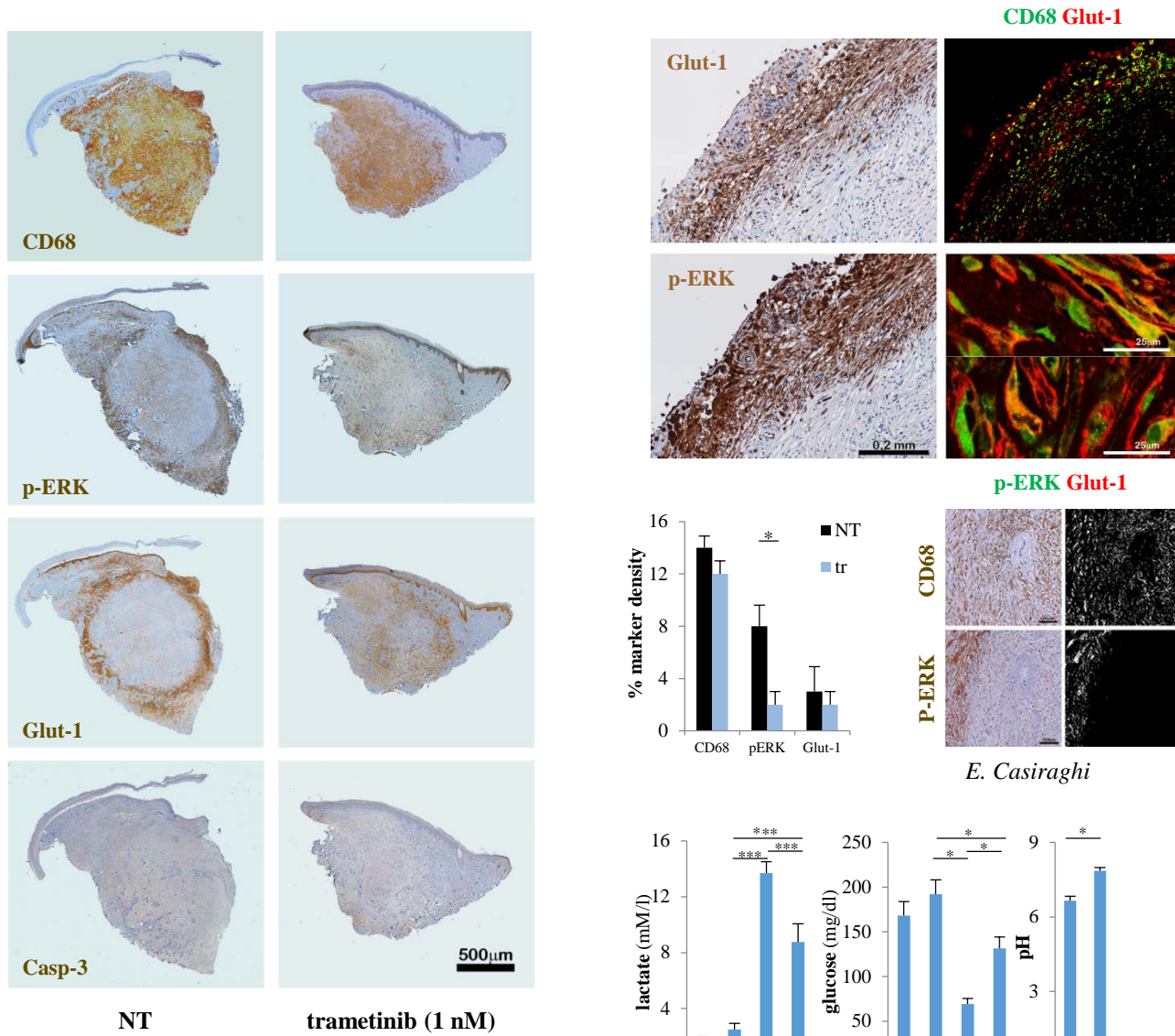
trametinib inhibits cytokine release by KRAS-mutated histiocytes

ECD 4

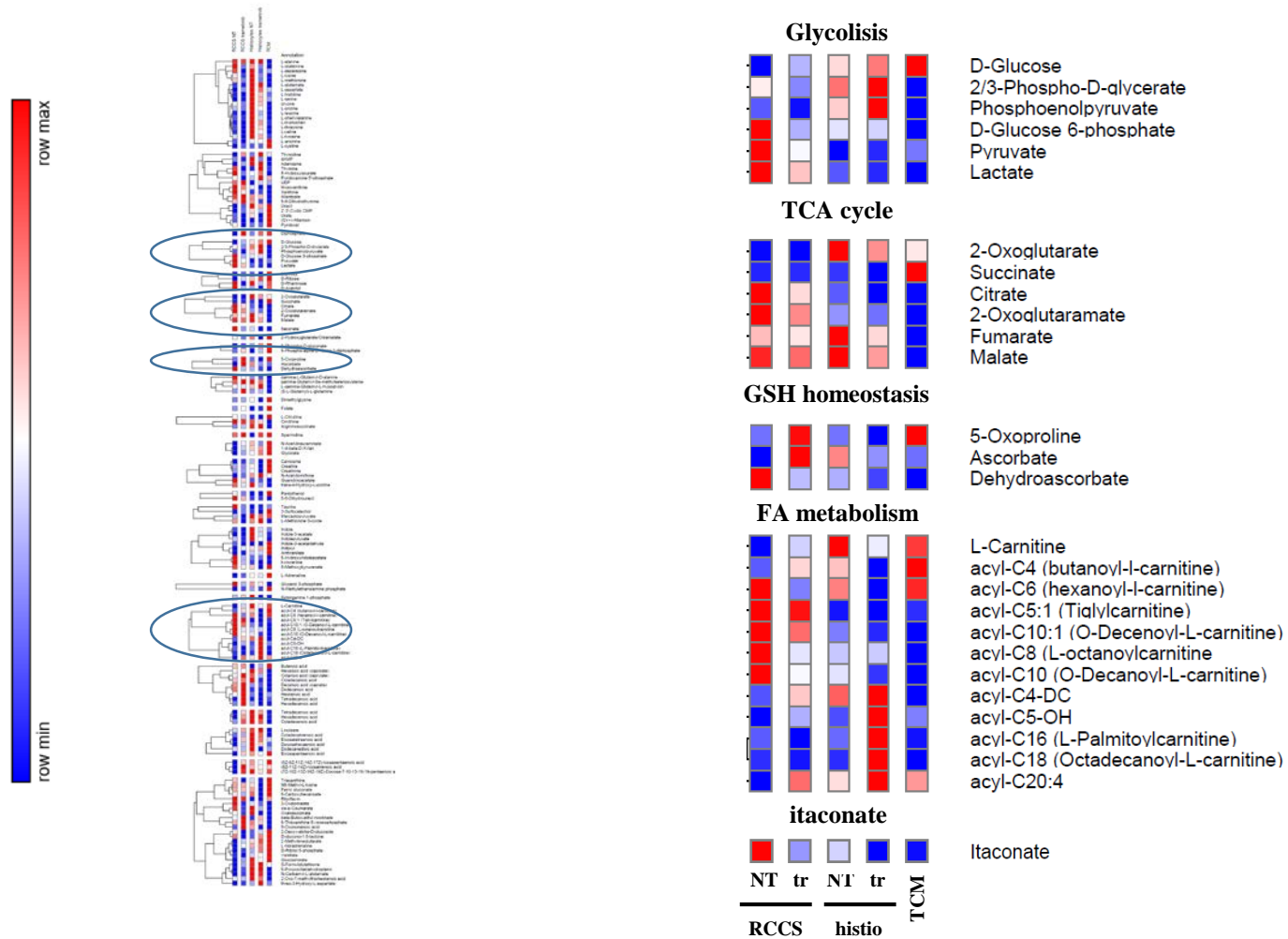
BRAF^{wt}, KRAS^{G12D}
xanthelasma



trametinib counteracts metabolic reprogramming in histiocytes



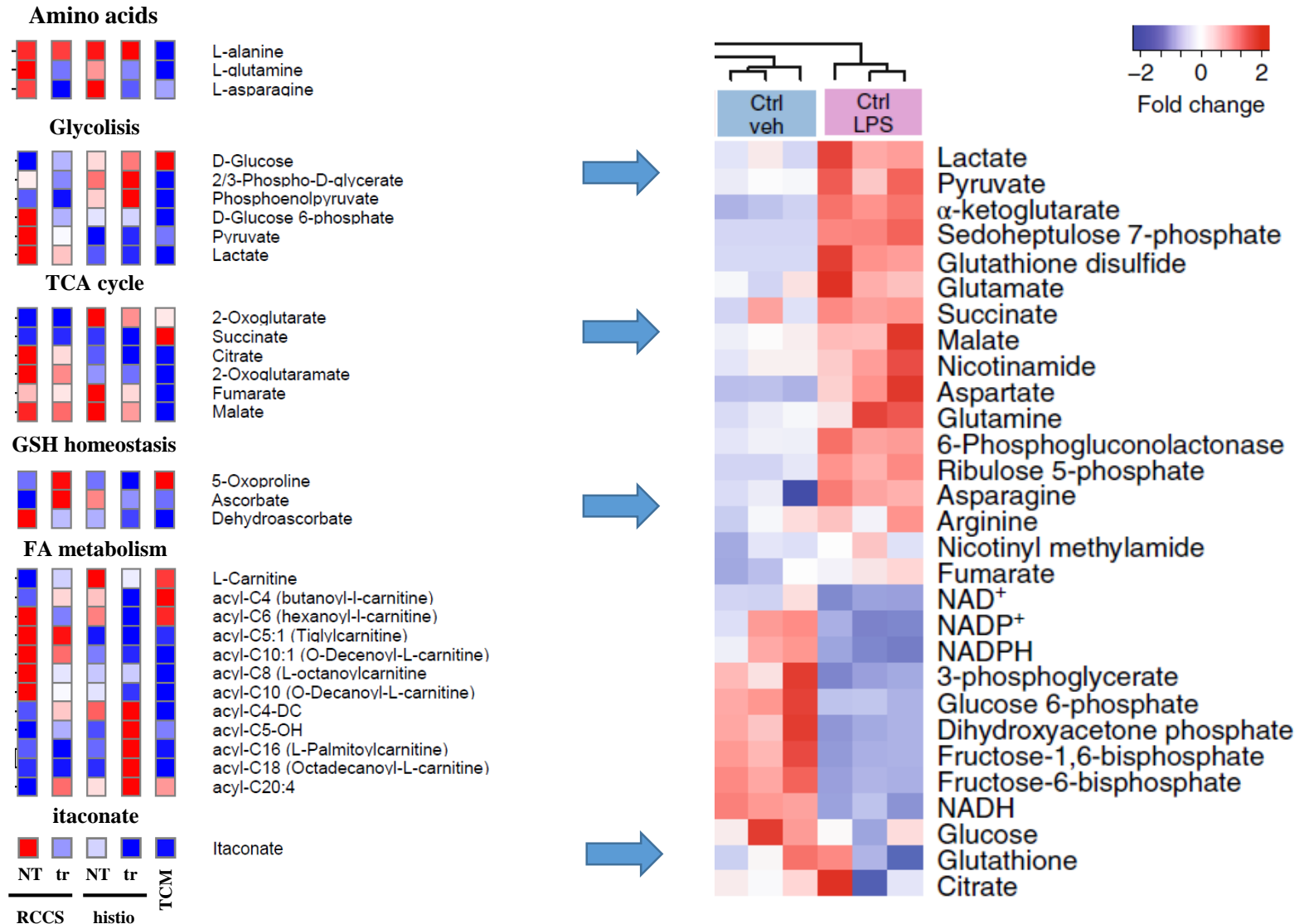
Metabolomic analysis of ECD tissues cultured in bioreactor



Metabolomics analysis of ECD tissues

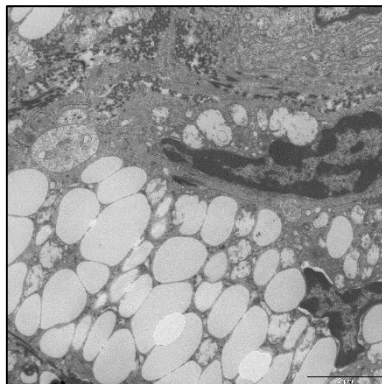
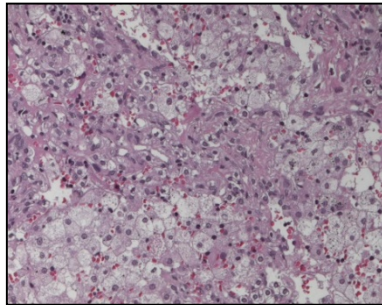
- activation of the glycolysis pathway
- accumulation of TCA metabolites
- accumulation of citrate, suggestive of activation of the cholesterol synthesis pathway
- activation of tryptophan metabolism, most likely by activation of IDO1
- high induction of itaconate
- overall, the metabolic profile resembles 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



ECD: a disease of “foamy” histiocytes

Über lipoidgranulomatose
Chester W, 1930

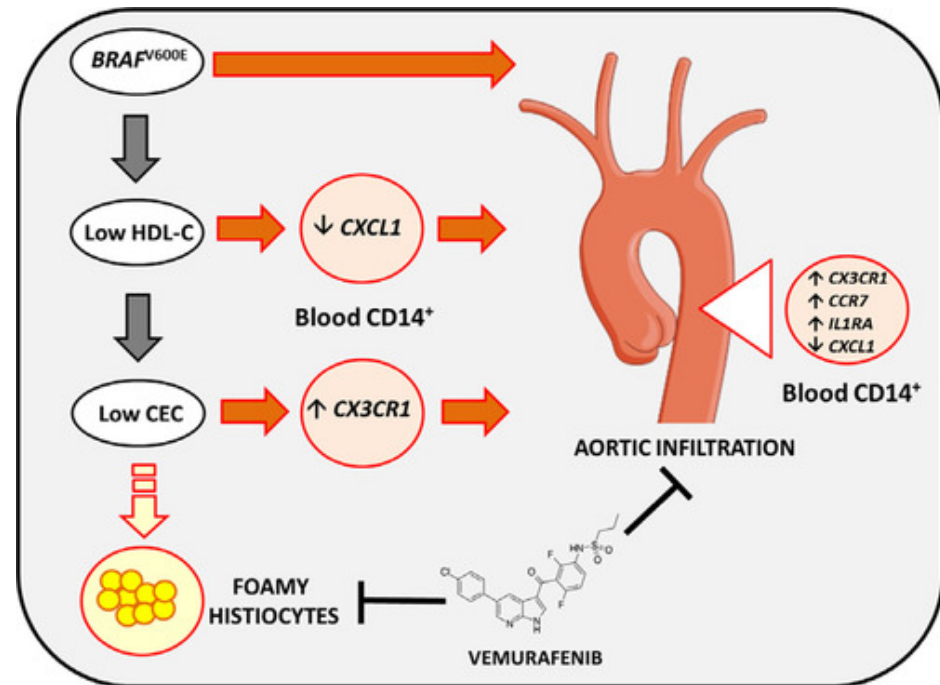


G. Dell'Antonio

Clinical and Population Studies

Hypoalbuminemia 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, Youssef Arsafi, Jean-François Emile, Eric Frisdal, Carine Le Goff, Jean Donadieu, Zahir Amoura, Philippe Lesnik, Julien Haroche, Wilfried Le Goff



REVIEW

Itaconate: an emerging determinant of inflammation in activated macrophages

IMMUNOMETABOLISM

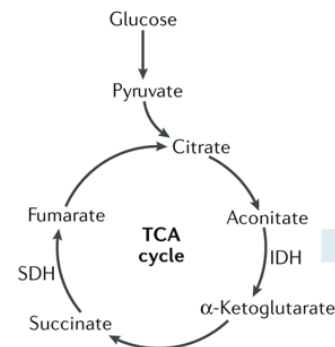
REVIEWS

Itaconate: the poster child of metabolic reprogramming in macrophage function

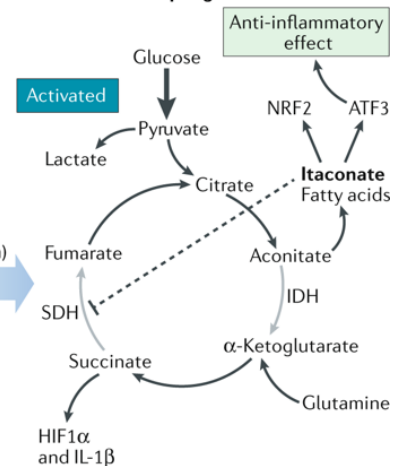
Luke A. J. O'Neill^{1*} and Maxim N. Artyomov^{2*}

NATURE REVIEWS | IMMUNOLOGY

Resting macrophage metabolism



Activated macrophage metabolism



RESEARCH ARTICLE

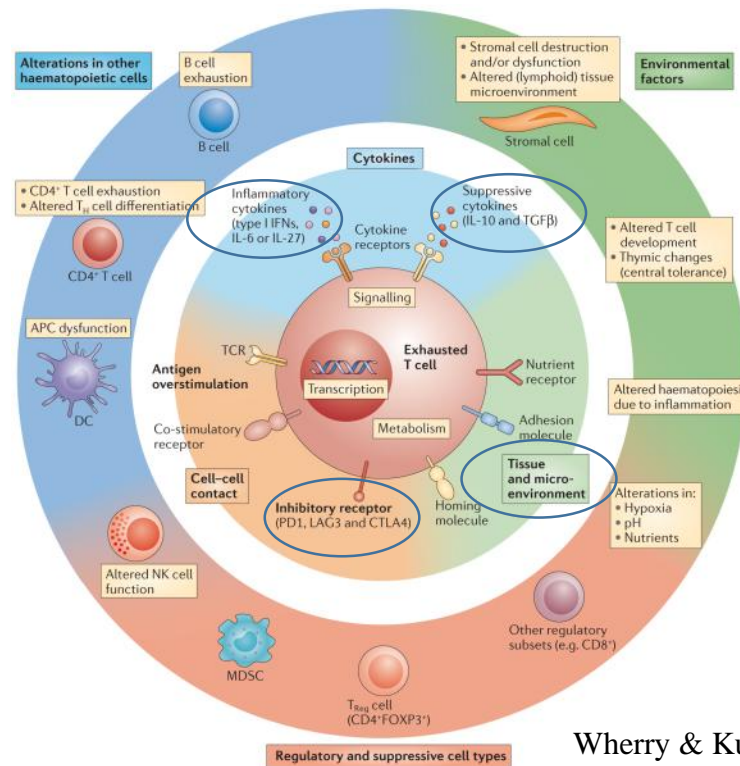
The Journal of Clinical Investigation

Itaconic acid mediates crosstalk between macrophage metabolism and peritoneal tumors

Jonathan M. Weiss,¹ Luke C. Davies,² Megan Karwan,³ Liifa Ileva,³ Michelle K. Ozaki,⁴ Robert Y.S. Cheng,¹ Lisa A. Ridnour,¹ Christina M. Annunziata,⁴ David A. Wink,¹ and Daniel W. McVicar¹

¹Cancer and Inflammation Program, Center for Cancer Research, National Cancer Institute (NCI) at Frederick, Frederick, Maryland, USA. ²Cardiff University, Division of Infection and Immunity, Cardiff, United Kingdom. ³Frederick National Laboratory for Cancer Research, Leidos Biomedical Research Inc., Frederick, Maryland, USA. ⁴Women's Malignancies Branch, Center for Cancer Research (CCR), NCI, Bethesda, Maryland, USA.

Overview of mechanisms of T cell exhaustion



Wherry & Kurachi, Nat Rev Immunol. 2015

ORIGINAL ARTICLE

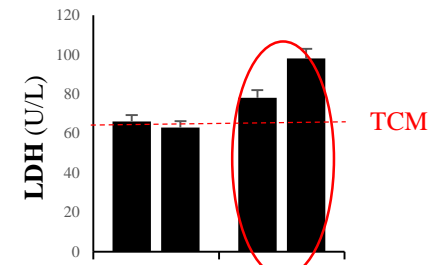
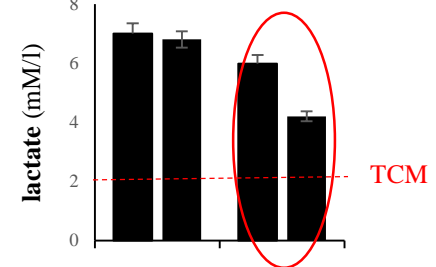
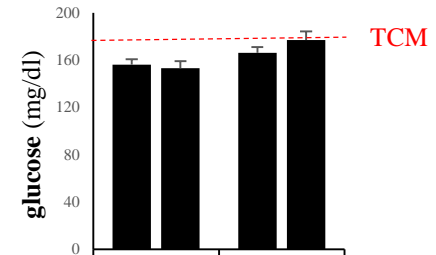
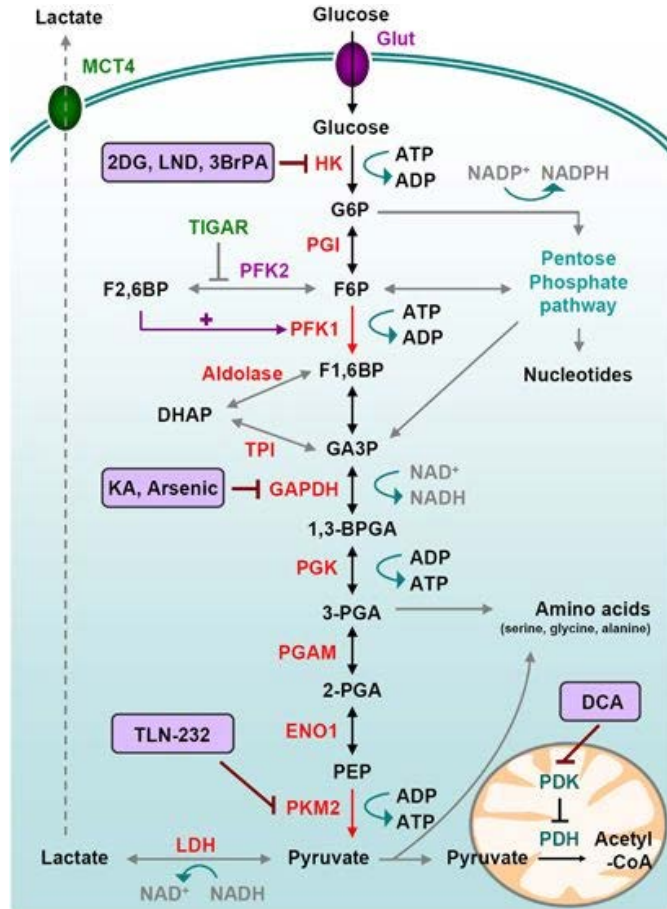
Expression of Programmed Cell Death 1 Ligands (PD-L1 and PD-L2) in Histiocytic and Dendritic Cell Disorders

Jie Xu, MD, PhD,* Heather H. Sun, BA,* Christopher D.M. Fletcher, MD,* Jason L. Hornick, MD, PhD,* Elizabeth A. Morgan, MD,* Gordon J. Freeman, PhD,† F. Stephen Hodi, MD,†‡
Geraldine S. Pinkus, MD,* and Scott J. Rodig, MD, PhD*‡

Targeting ECD metabolism in 3D culture: 2-DG

ECD 5

Xanthelasma, BRAF^{V600E}

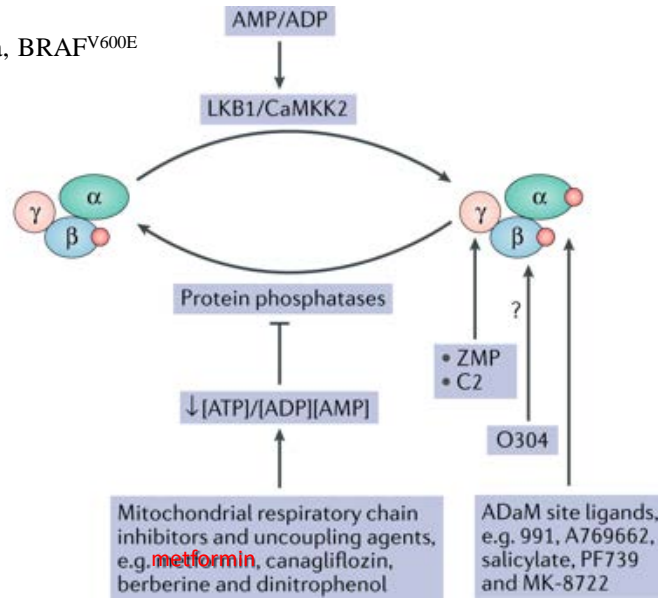


vem 6μM	-	-	+	+
2-DG 1mM	-	+	-	+

Targeting ECD metabolism in 3D culture: metformin

ECD 5

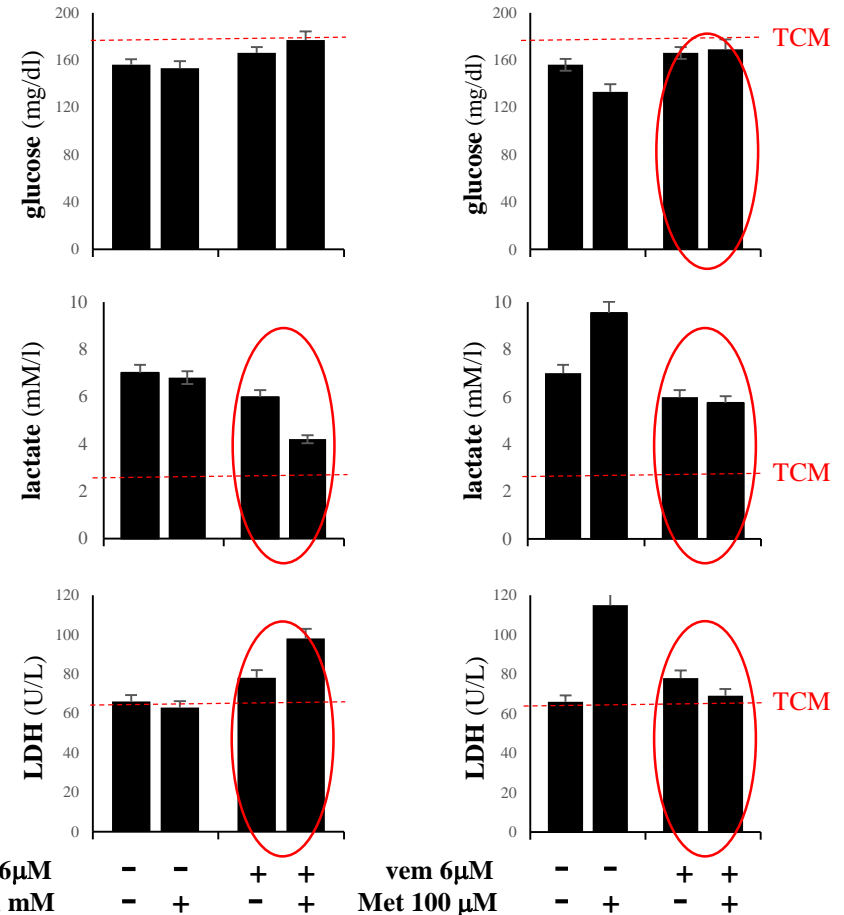
Xanthelasma, BRAF^{V600E}



Steinberg GR & Carling D, Nat Rev Drug Discovery 2019

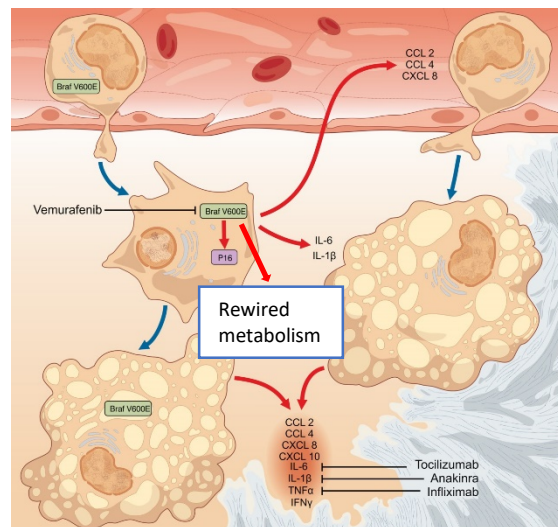


- ✓ Reduction in lipid storage
 - increased fatty acid oxidation
 - inhibition of fatty acid and cholesterol synthesis
- ✓ Regulation of carbohydrate metabolism
 - increased GLUT1-dependent glucose uptake
 - cell-type-specific increased glycolysis



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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Daniela Belloni
Silvia Heltai
Elisabetta Ferrero
Marina Ferrarini
B-cell Neoplasia Unit
San Raffaele Scientific Institute, Milano

Giulio Cavalli
Alvise Berti
Corrado Campochiaro
Giacomo De Luca
Lorenzo Dagna
Unit of Medicine and Clinical Immunology
San Raffaele Scientific Institute, Milano

Barbara Vergani
Antonello Villa
Consorzio MIA
University of Milano-Bicocca

Elisabetta Casiraghi
Department of Computer Science
University of Milano.

Giulia Cangi
Claudio Doglioni
Pathology Unit
San Raffaele Scientific Institute, Milano

Travis Nemkov
Angelo D'Alessandro
Department of Biochemistry and Molecular
Genetics, University of Colorado, Denver

Riccardo Biavasco
Eugenio Montini
SR-Tiget
San Raffaele Scientific Institute, Milano



Kathy Brewer
ECD Global Alliance,
DeRidder, LA

ECD patients and families

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Daniela Belloni
Silvia Heltai
Elisabetta Ferrero
Marina Ferrarini
B-cell Neoplasia Unit
San Raffaele Scientific Institute, Milano

Giulia Cangi
Claudio Doglioni
Pathology Unit
San Raffaele Scientific Institute, Milano

Giulio Cavalli
Alvise Berti
Corrado Campochiaro
Giacomo De Luca
Lorenzo Dagna
Unit of Medicine and Clinical Immunology
San Raffaele Scientific Institute, Milano

Travis Nemkov
Angelo D'Alessandro
Department of Biochemistry and Molecular
Genetics, University of Colorado, Denver

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Antonello Villa
Consorzio MIA
University of Milano-Bicocca

Elisabetta Casiraghi
Department of Computer Science
University of Milano.

Riccardo Biavasco
Eugenio Montini
SR-Tiget
San Raffaele Scientific Institute, Milano



Kathy Brewer
ECD Global Alliance,
DeRidder, LA