3-D culture of BRAF- and KRAS-mutated Erdheim-Chester disease tissues: impact of kinase inhibitors

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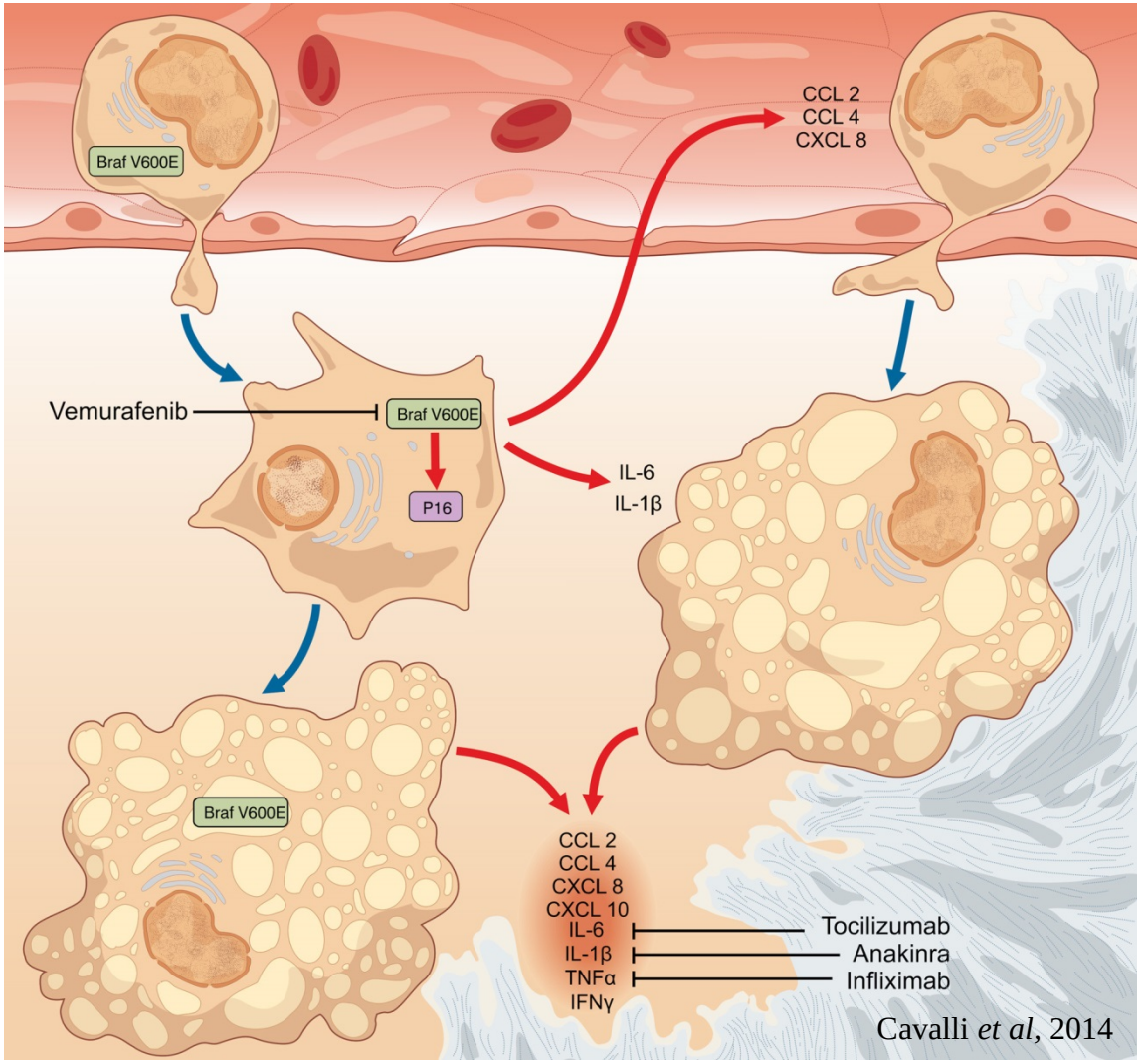
Division of Experimental Oncology
San Raffaele Scientific Institute, Milano

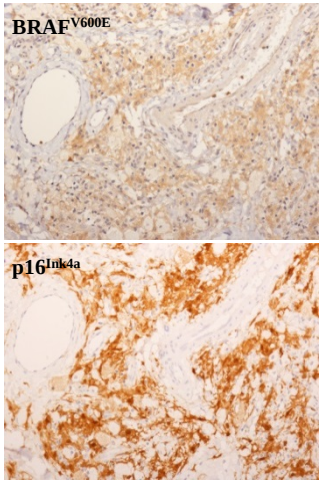
5th Annual ECD Medical Symposium
New York, NY
October 26, 2016

OSPEDALE SAN RAFFAELE

Università Vita-Salute San Raffaele

A unifying disease model for ECD





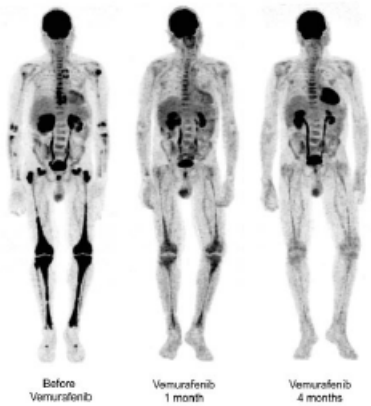
C. Doglioni

vemurafenib treatment in Erdheim-Chester disease

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 Idbaih, 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.

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]

vemurafenib in Erdheim-Chester disease: unsolved issues

$BRAF^{V600E}$ is a targetable oncogene in ECD, however:

- not all ECD patients bear the $BRAF^{V600E}$ mutation or known targetable mutations
- vemurafenib treatment mostly results in partial clinical responses in ECD patients
- vemurafenib treatment is often associated with severe side effects and relapses upon treatment discontinuation

RCCS™ bioreactor preserves architecture of normal and cancer tissues

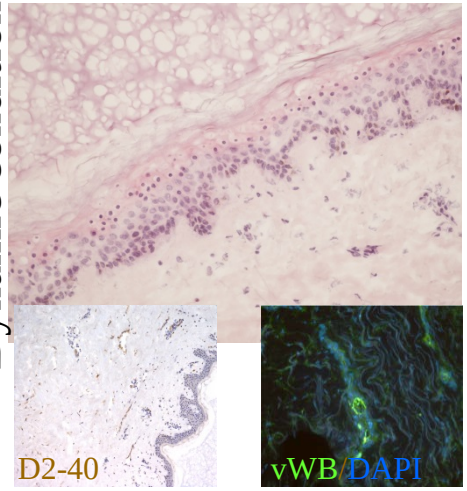
Skin

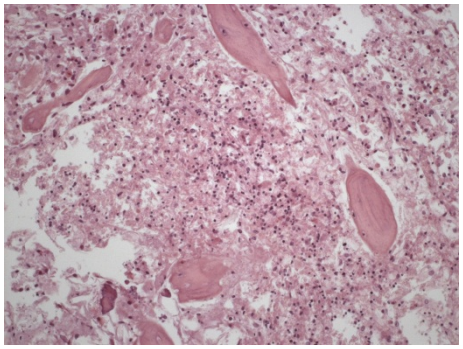
Bone Marrow

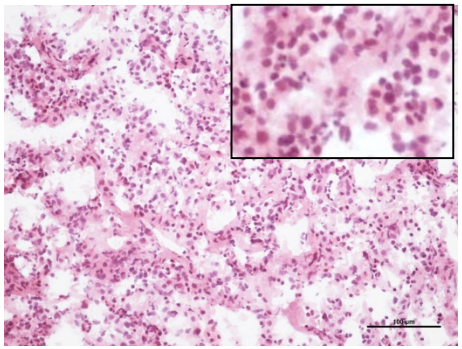
RCC

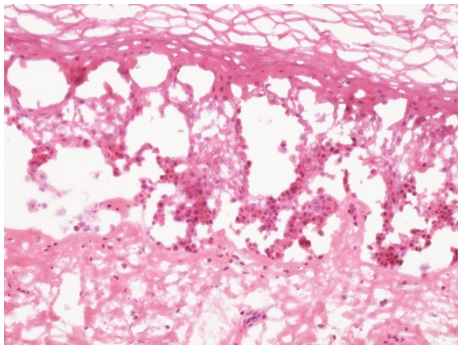
Dynamic condition

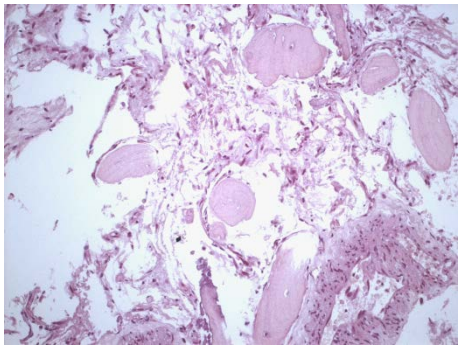
Static condition

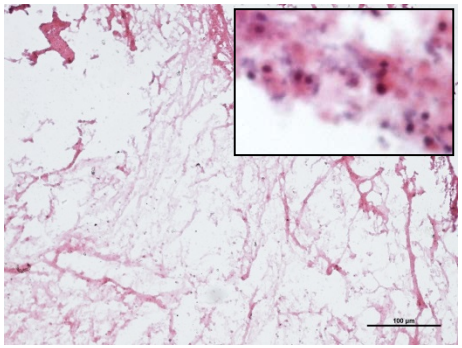




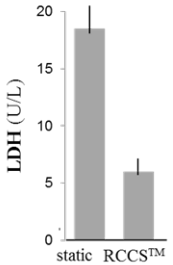


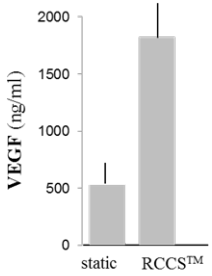






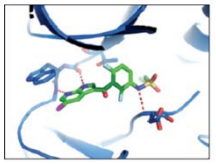
2 weeks

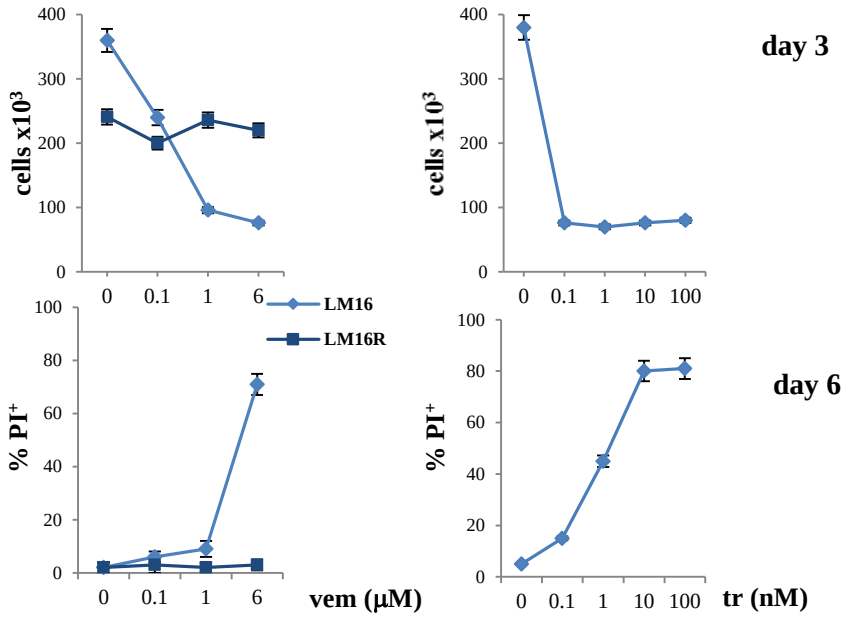


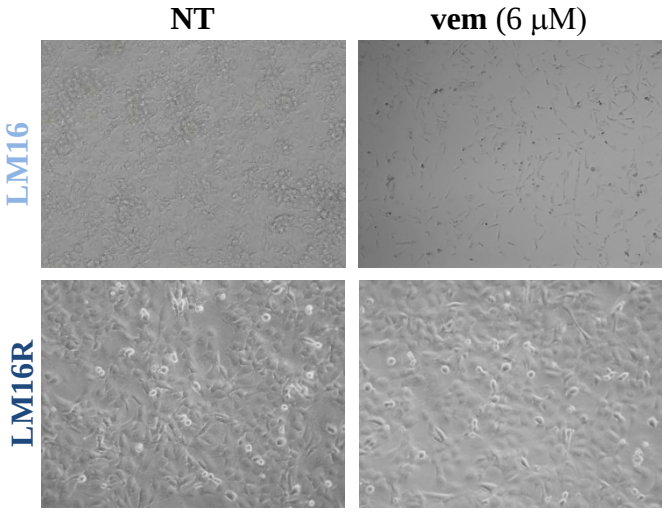


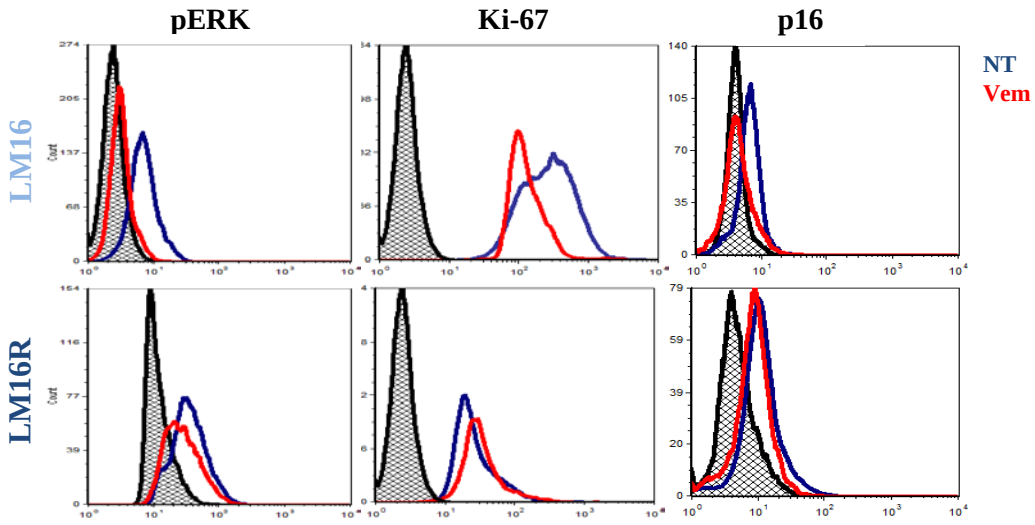
Ferrarini *et al*, 2013

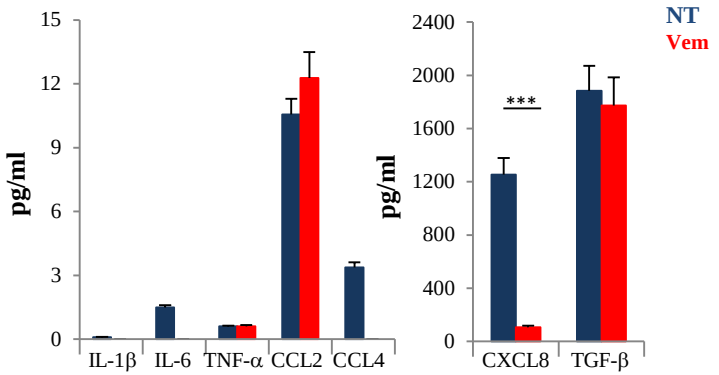
Effects of vemurafenib on BRAF-mutated melanoma cells









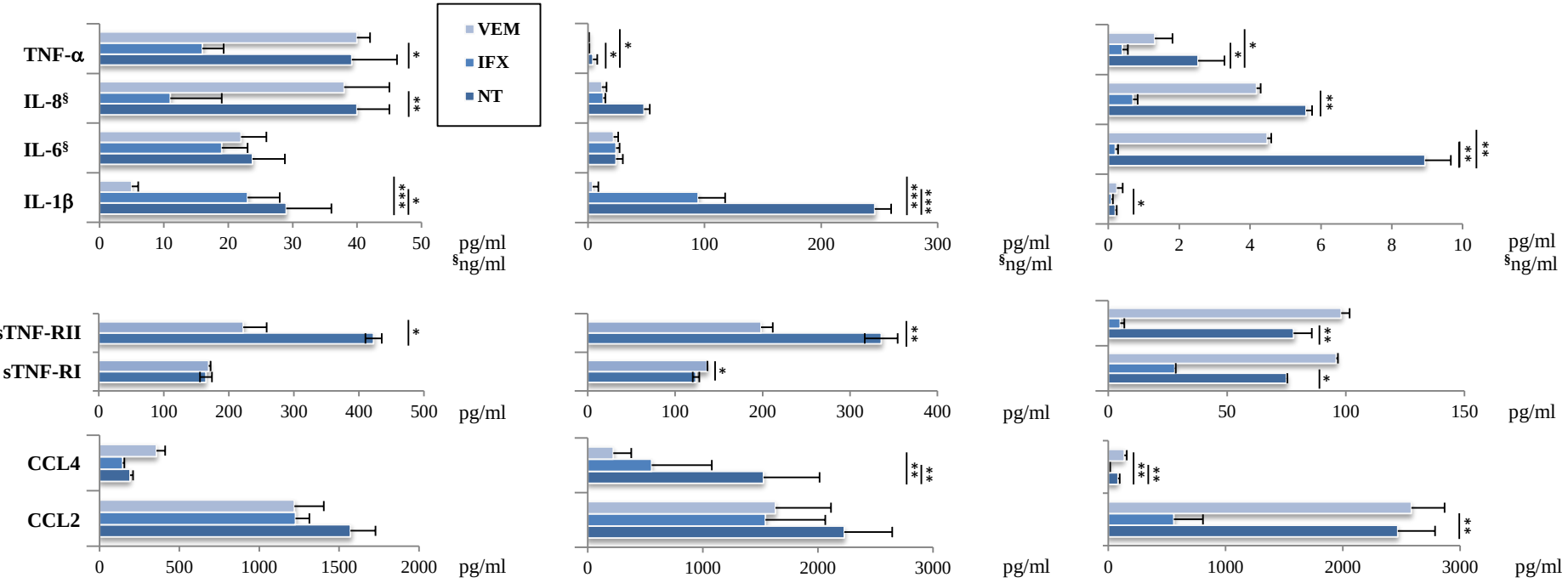


Cytokine- and BRAF-inhibitors affect cytokine release from ECD tissues

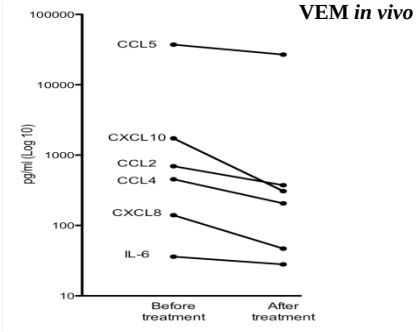
ECD 1
M/46-year-old
xanthelasma

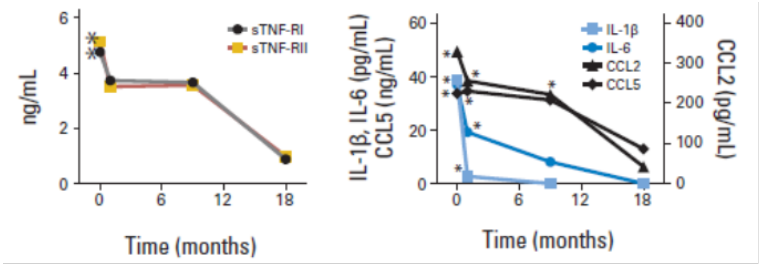
ECD 2
F/50-year-old
xanthelasma

ECD 3
F/69-year-old
pleural lesion



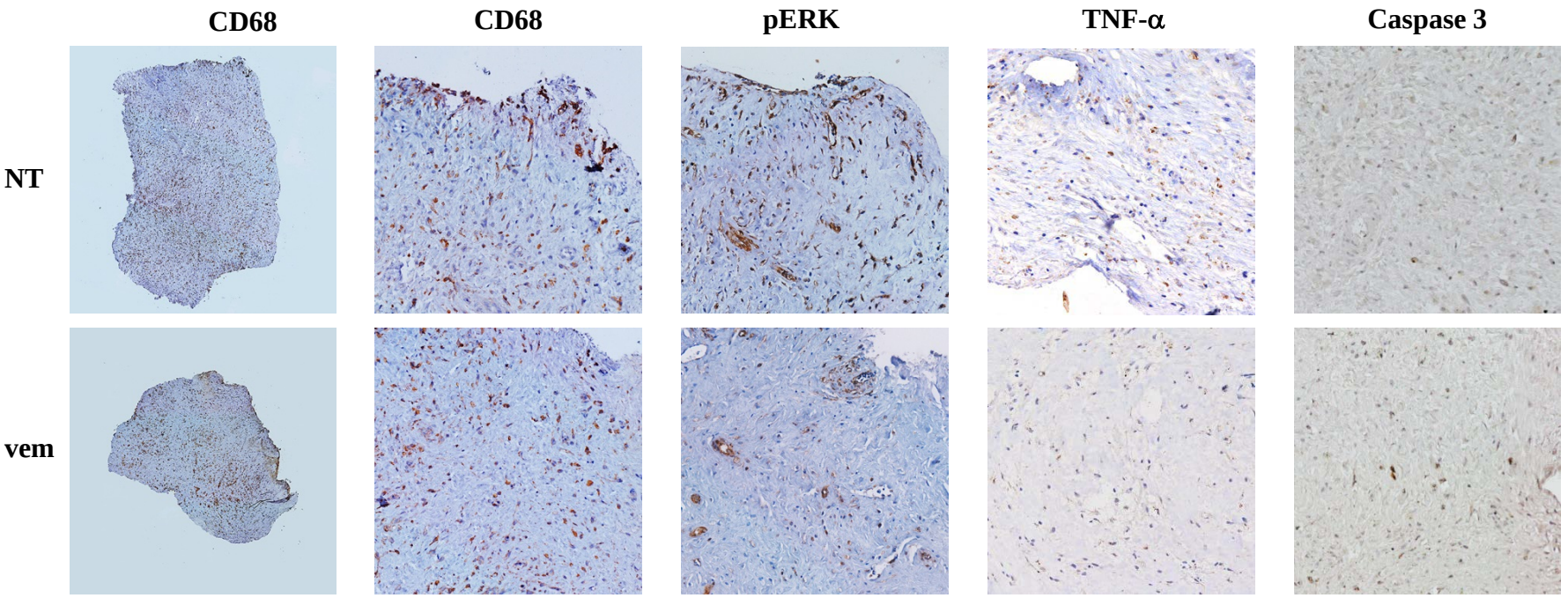
VEM *in vivo*



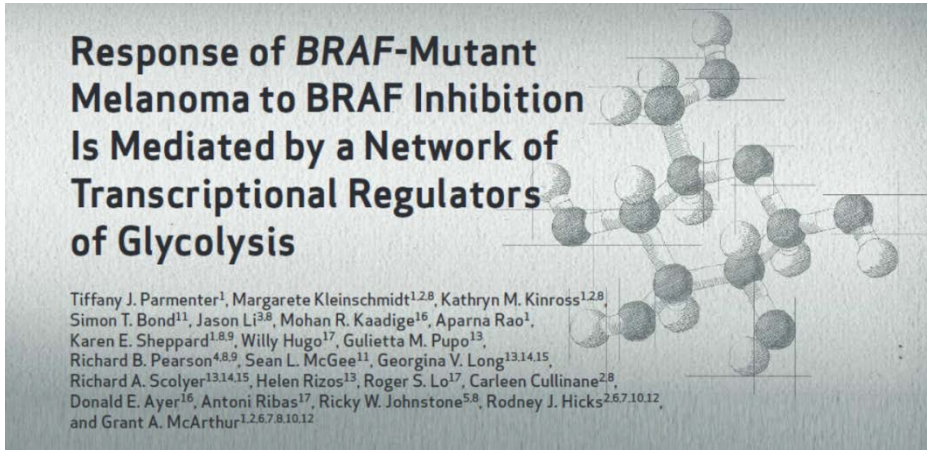


Dagna *et al*, 2012 *JCO*

Vemurafenib affects cytokine production but not histiocyte viability

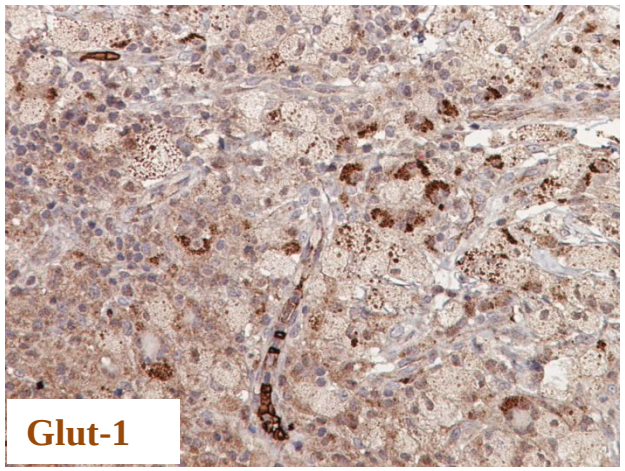


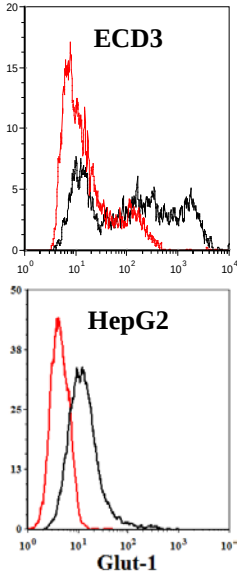
BRAF-mutation and metabolism in ECD histiocytes

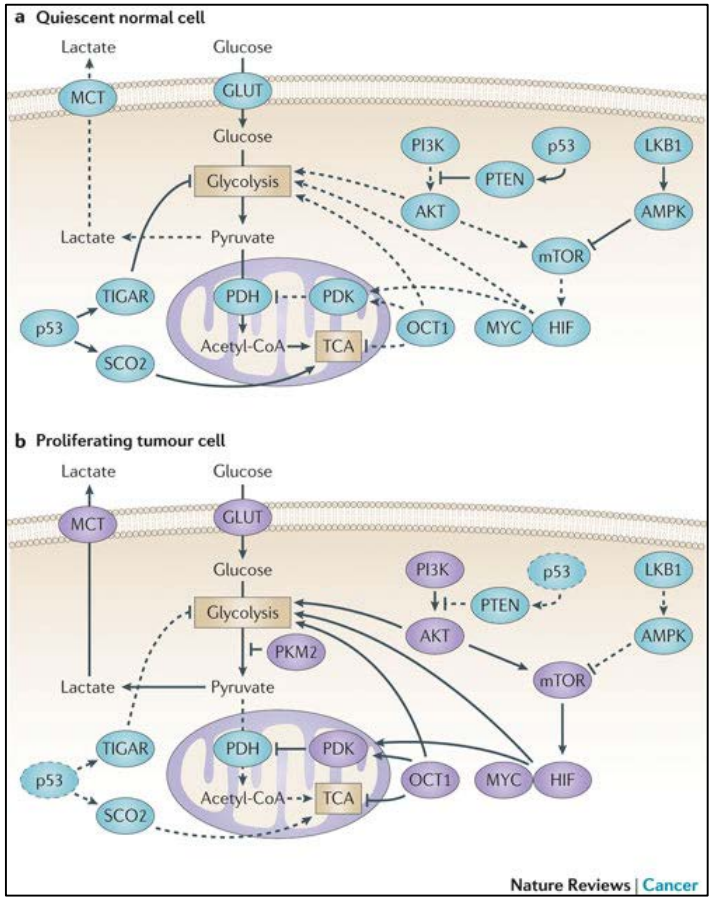


Response of *BRAF*-Mutant Melanoma to BRAF Inhibition Is Mediated by a Network of Transcriptional Regulators of Glycolysis

Tiffany J. Parmenter[1], Margarete Kleinschmidt[1,2,8], Kathryn M. Kinross[1,2,8], Simon T. Bond[11], Jason Li[3,8], Mohan R. Kaadige[16], Aparna Rao[1], Karen E. Sheppard[1,8,9], Willy Hugo[17], Gulietta M. Pupo[13], Richard B. Pearson[4,8,9], Sean L. McGee[11], Georgina V. Long[13,14,15], Richard A. Scolyer[13,14,15], Helen Rizos[13], Roger S. Lo[17], Carleen Cullinane[2,8], Donald E. Ayer[16], Antoni Ribas[17], Ricky W. Johnstone[5,8], Rodney J. Hicks[2,6,7,10,12], and Grant A. McArthur[1,2,6,7,8,10,12]

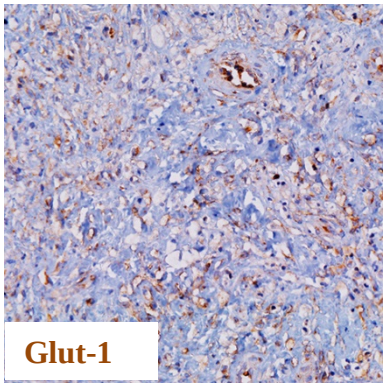




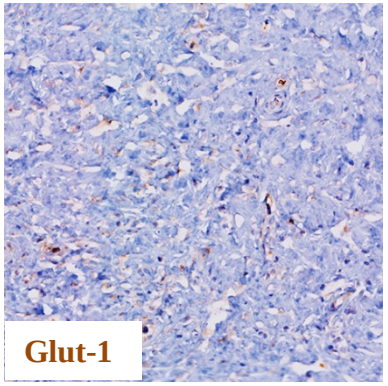


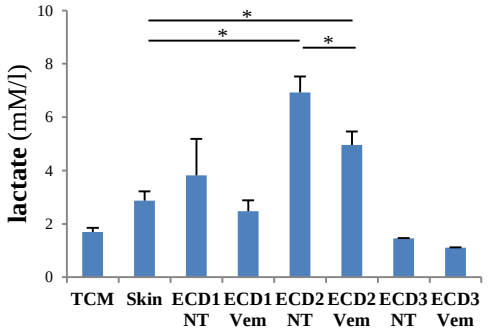
Effects of vemurafenib on BRAF-mutated ECD histiocytes: metabolism

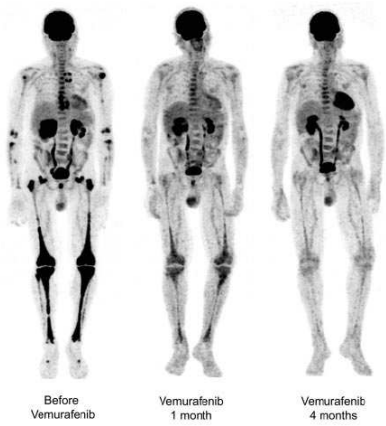
NT



vem





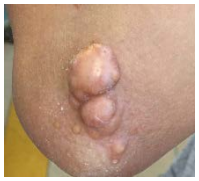


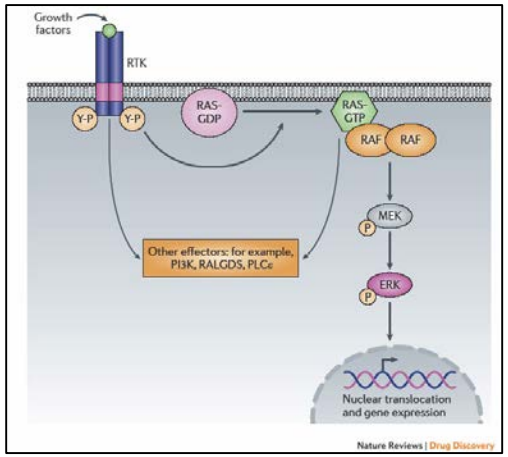
trametinib inhibits cyto-chemokine release by KRAS-mutated histiocytes *in vitro* and *ex-vivo*

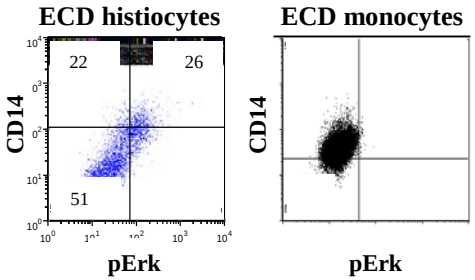
Patient 4

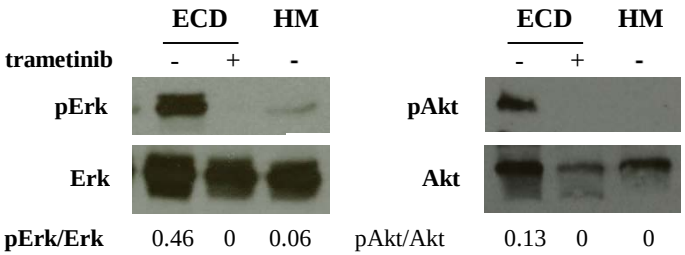
BRAFwt, KRAS^{G12V}

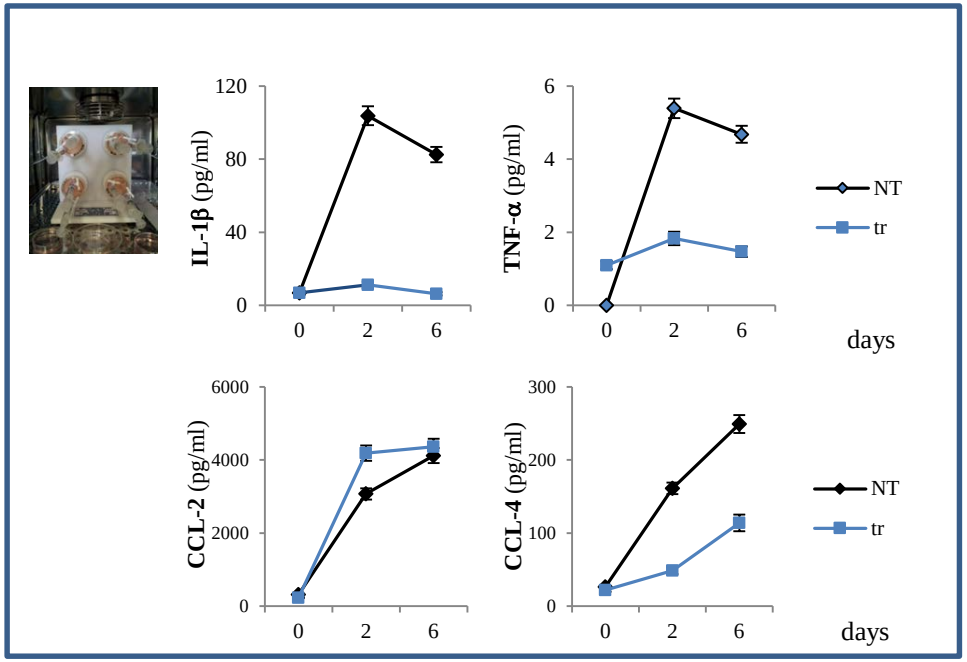
xanthelasma

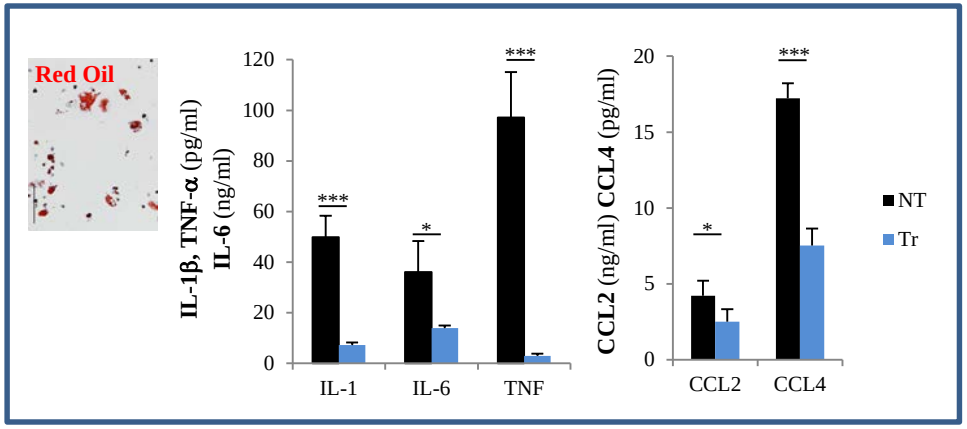






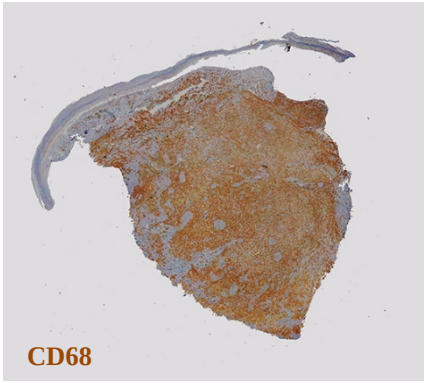


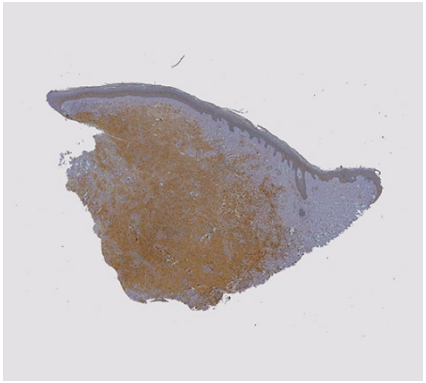


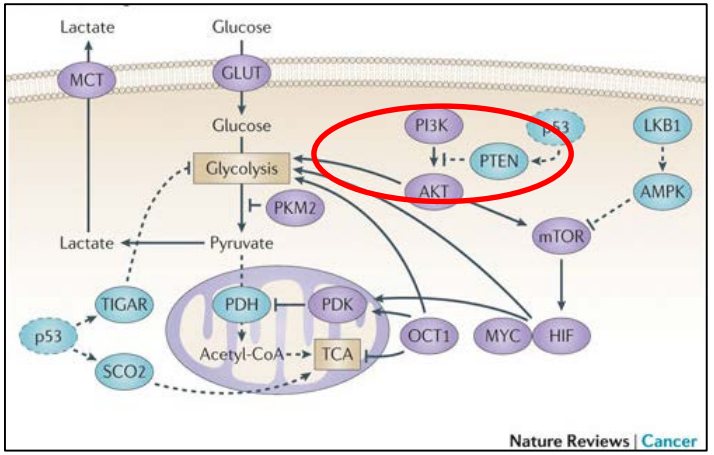


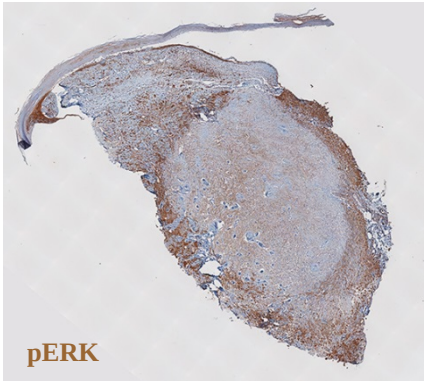
NT

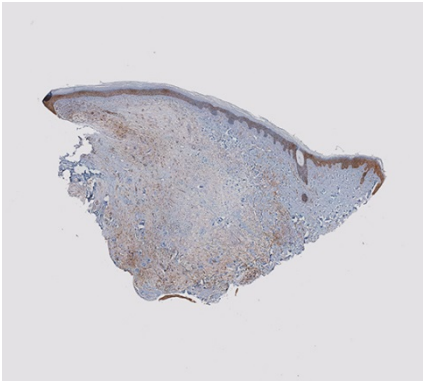
trametinib

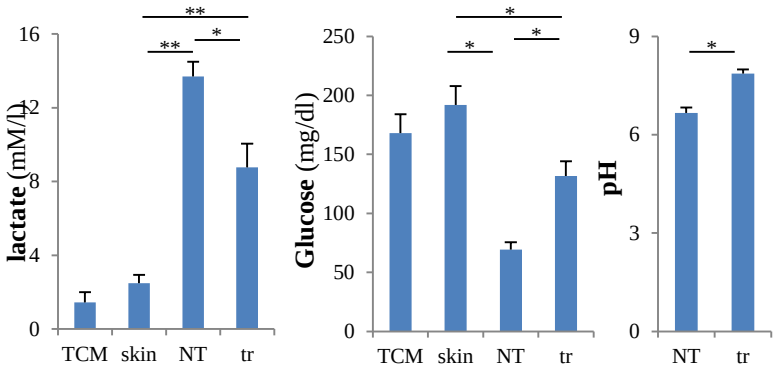


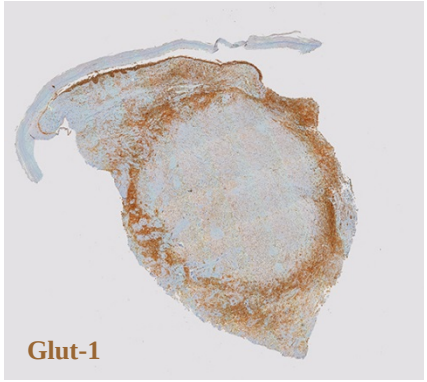


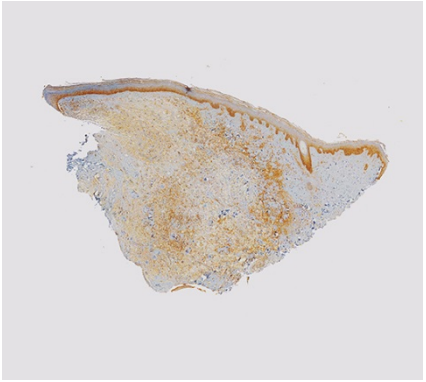


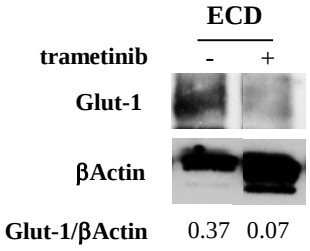












Conclusions

- 3-D dynamic culture in bioreactor of ECD tissues is suitable for drug testing
- vemurafenib and trametinib affect cytokine and chemokine production but not viability of ECD histiocytes in short-term culture
- kinase inhibitors counteract the up-regulated aerobic glycolysis in BRAF-mutated and KRAS-mutated ECD histiocytes
- The technology can be further exploited as a novel tool to investigate ECD pathophysiology and also to identify mechanisms of action of BRAF/MEK inhibitors on ECD histiocytes for future combination therapies

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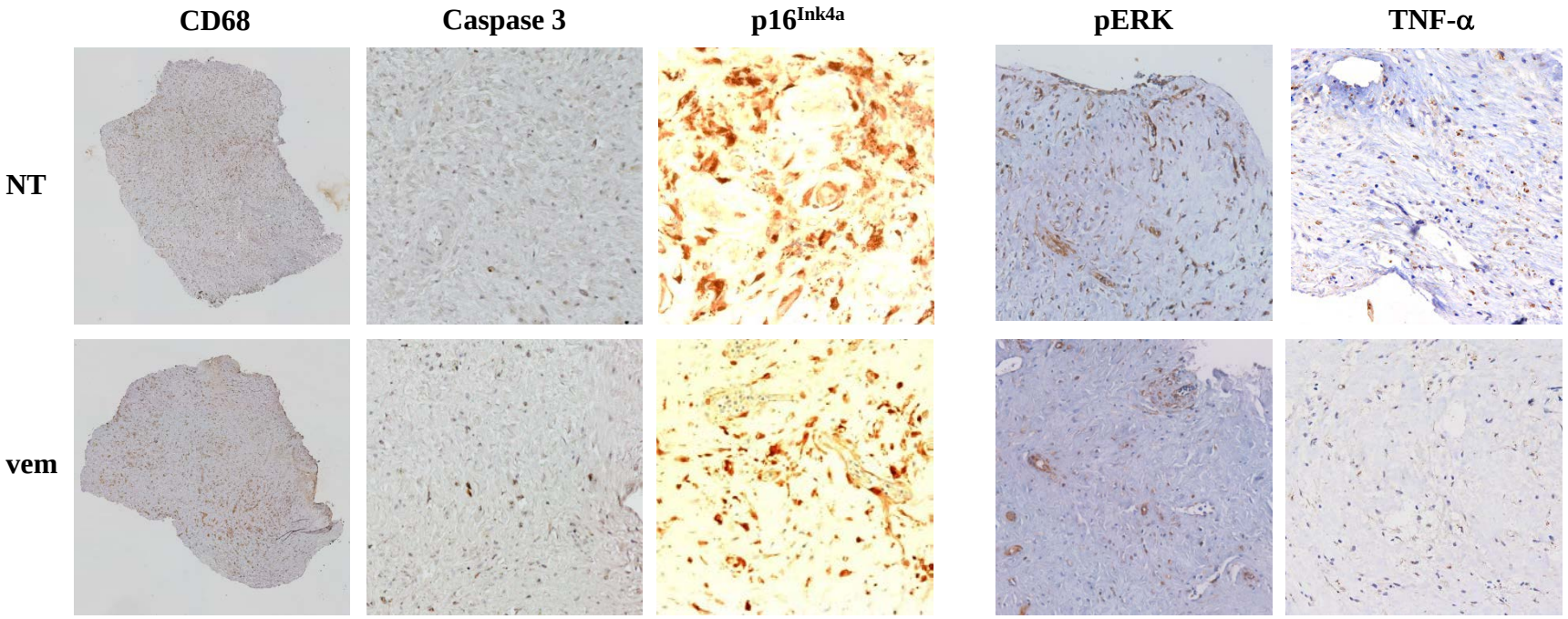
Eugenio Montini
SR-Tiget
San Raffaele Scientific Institute, Milano

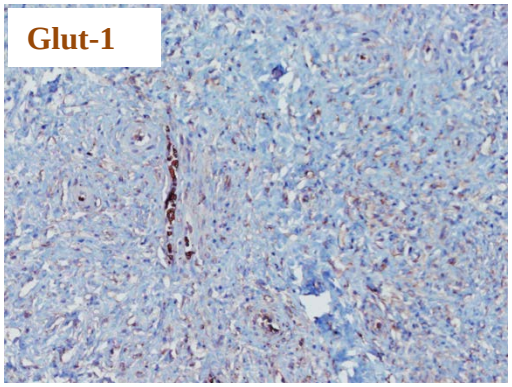
Kathy Brewer
ECD Global Alliance,
DeRidder, LA

Vemurafenib affects cytokine production but not histiocyte viability

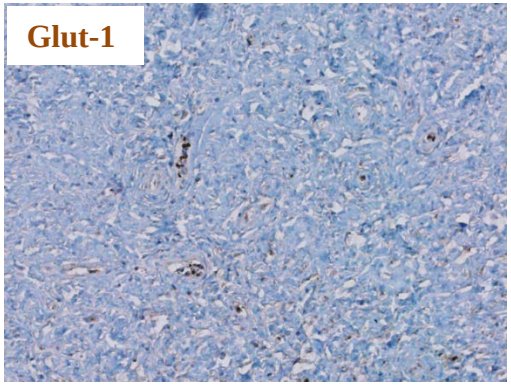


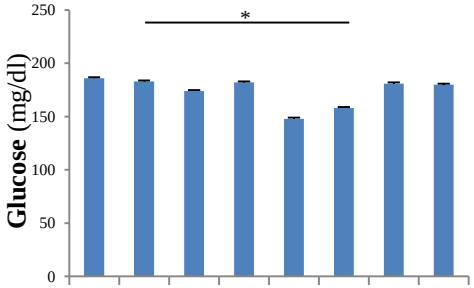
Effects of vemurafenib on BRAF-mutated ECD histiocytes: metabolism

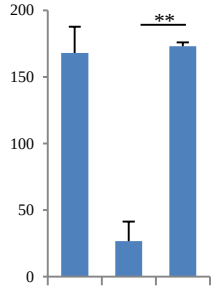
NT

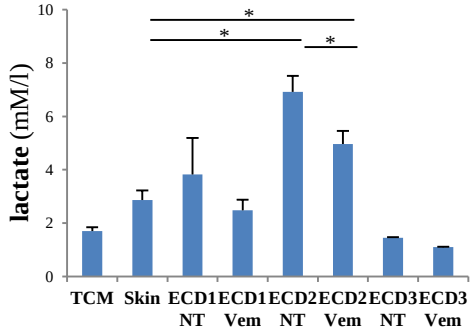


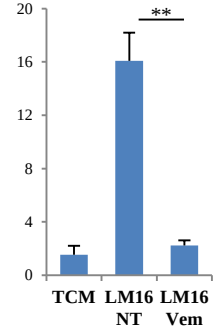
vem

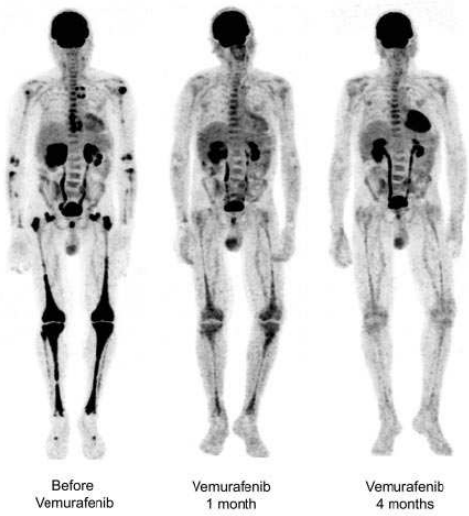


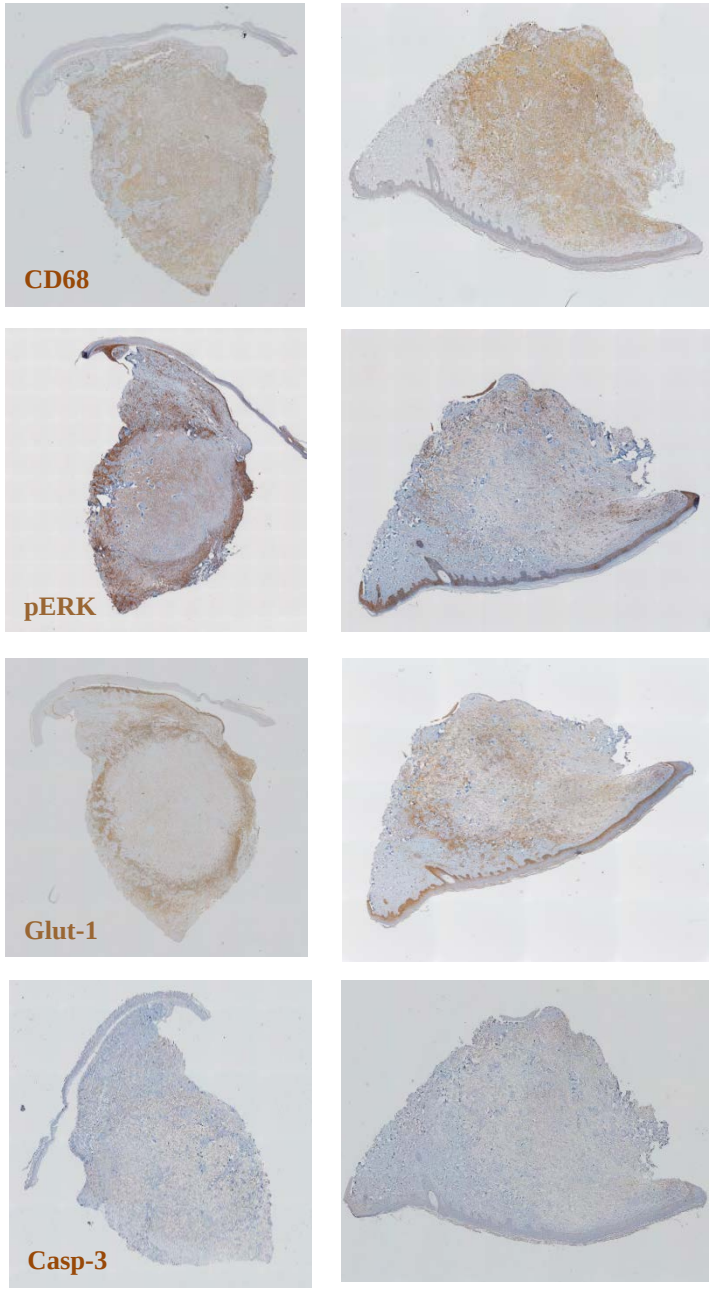


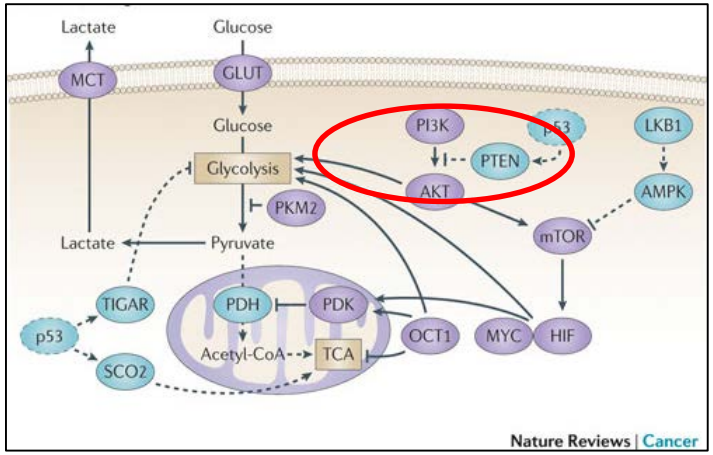


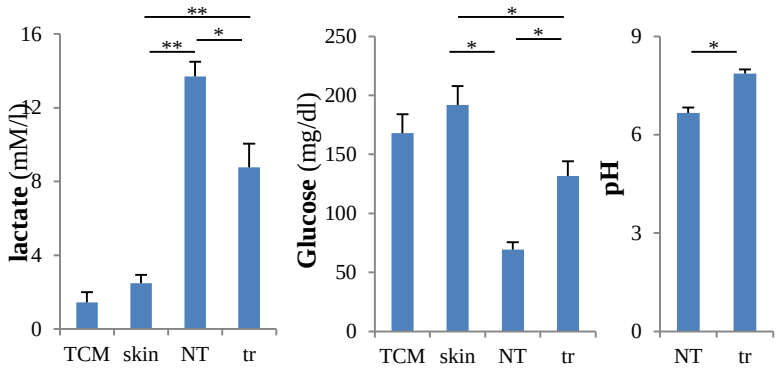


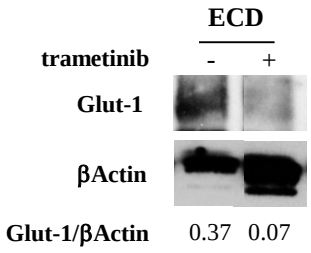


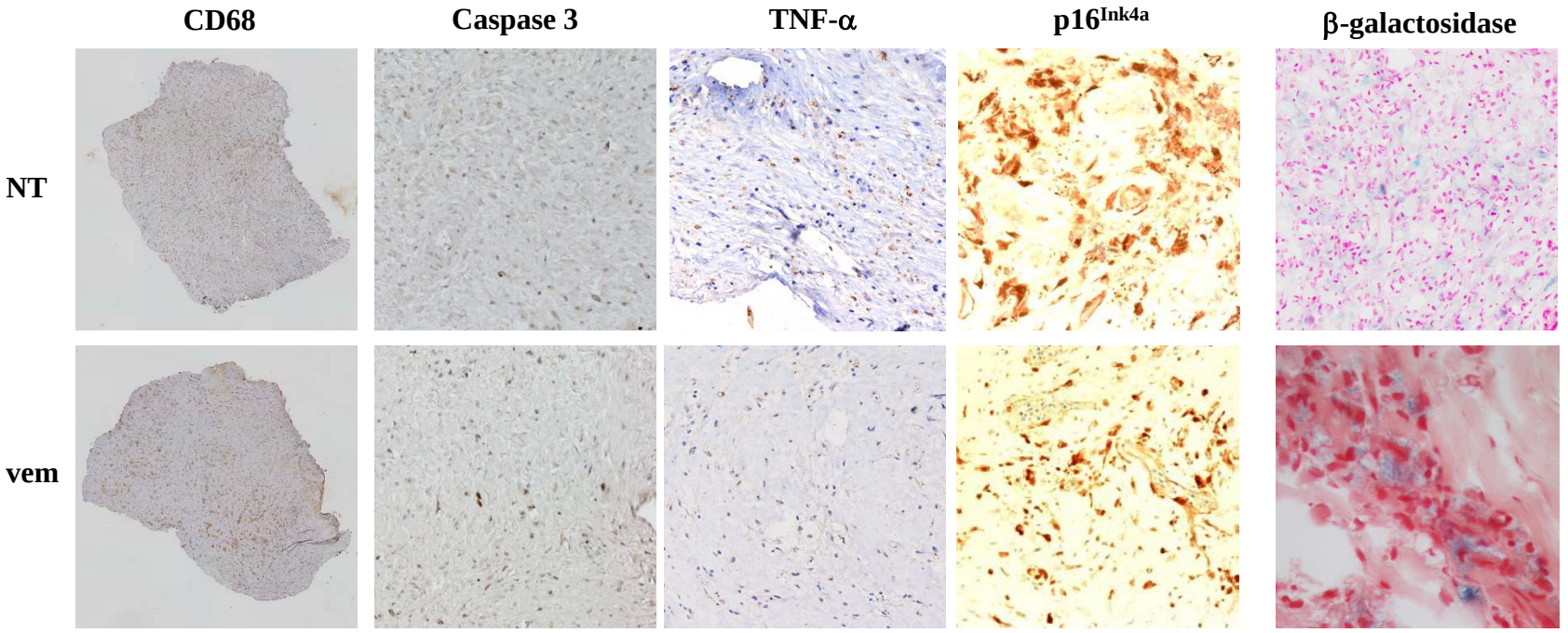


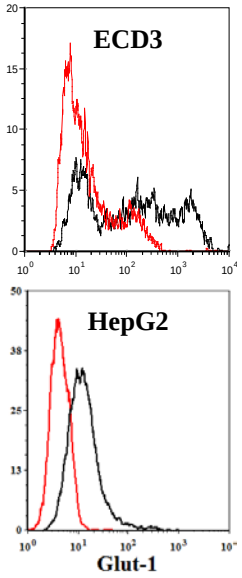

NT
trametinib
CD68
pERK
Glut-1
Casp-3

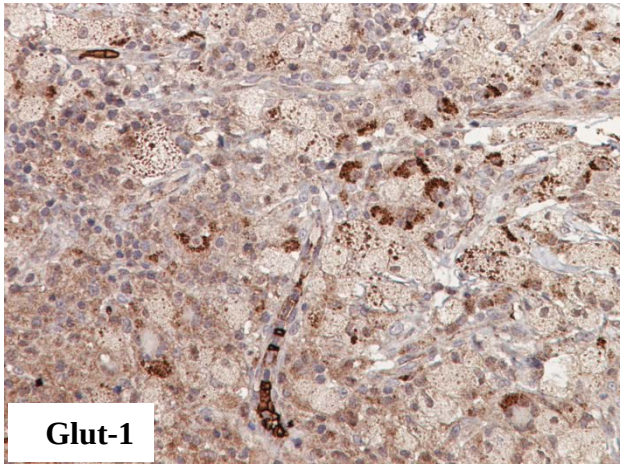

Lactate
Glucose
MCT
GLUT
Glucose
Glycolysis
PKM2
Lactate
Pyruvate
PI3K
PTEN
AKT
LKB1
AMPK
mTOR
TIGAR
PDH
PDK
p53
SCO2
Acetyl-CoA
TCA
OCT1
MYC
HIF
Nature Reviews | Cancer

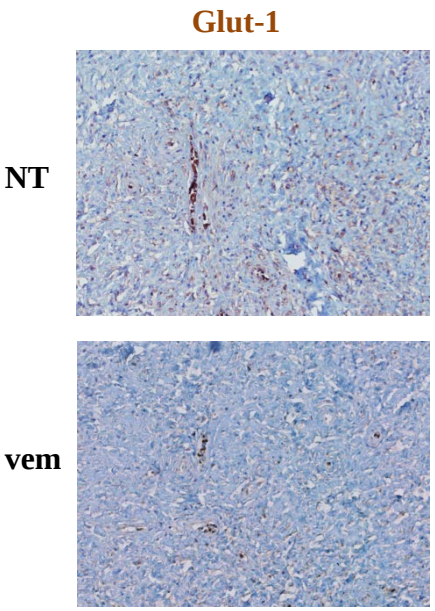

lactate (mM/l)
16
12
8
4
0
TCM
skin
NT
tr
Glucose (mg/dl)
250
200
150
100
50
0
pH
9
6
3
0

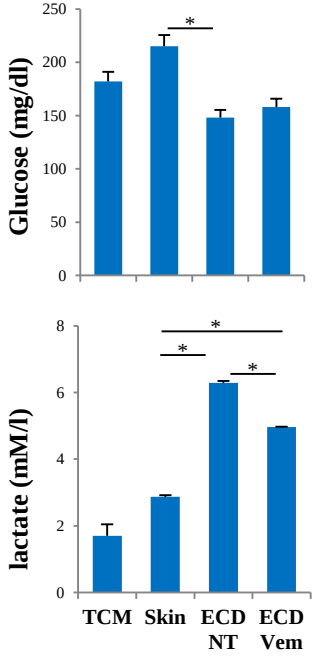

ECD
trametinib
-
+
Glut-1
βActin
Glut-1/βActin
0.37
0.07


CD68
Caspase 3
TNF-α
p16Ink4a
β-galactosidase
NT
vem


ECD3
HepG2
Glut-1


Glut-1

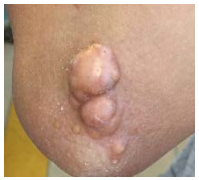

Glut-1
NT
vem

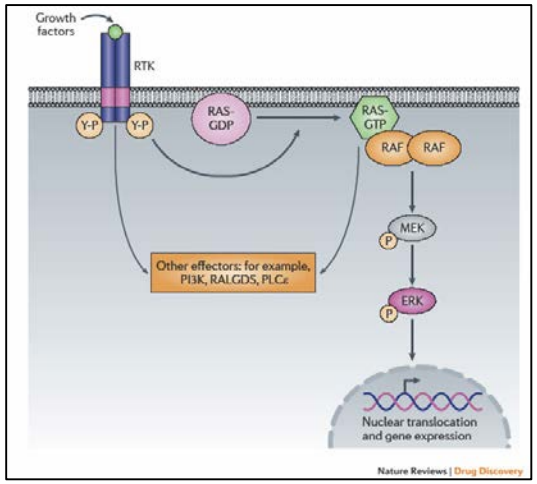

Glucose (mg/dl)
lactate (mM/l)
TCM
Skin
ECD NT
ECD Vem

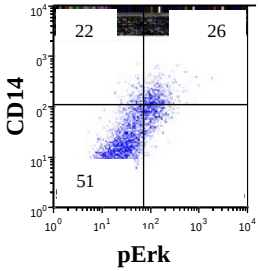
Patient 4

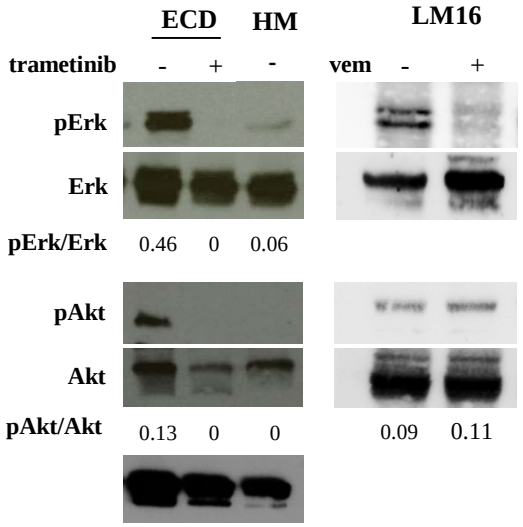
BRAFwt, KRAS^{G12V}

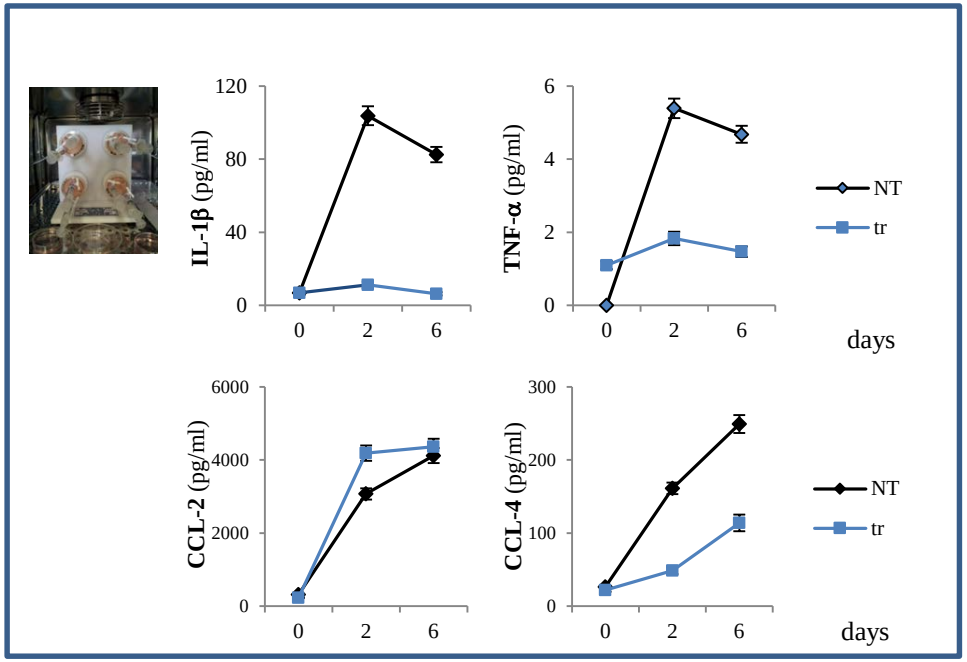
xanthelasma

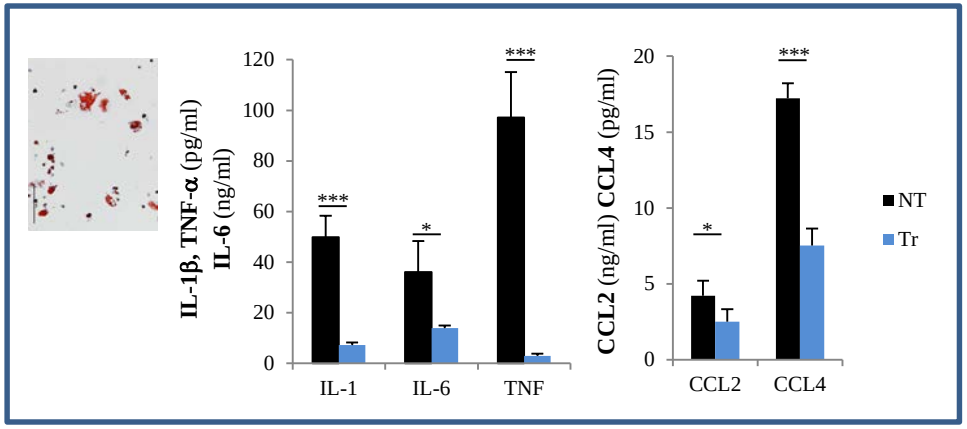






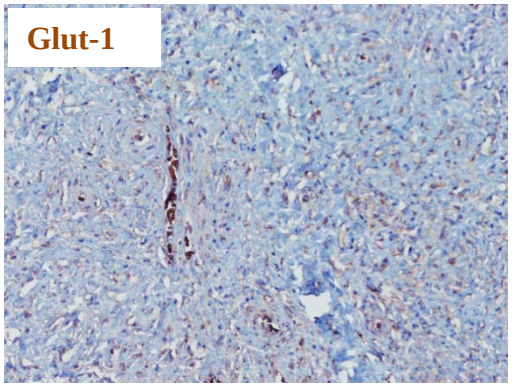




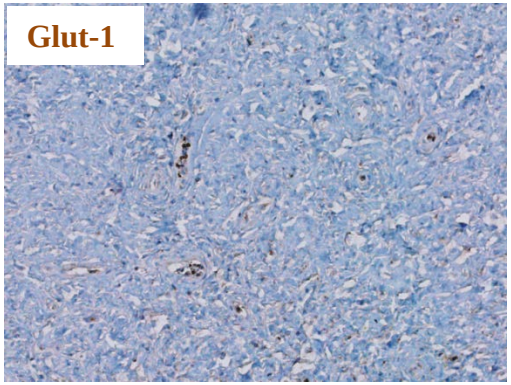


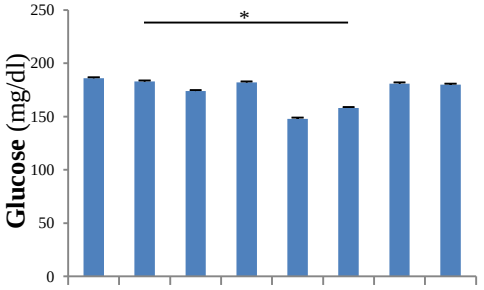
Effects of vemurafenib on BRAF-mutated ECD histiocytes: metabolism

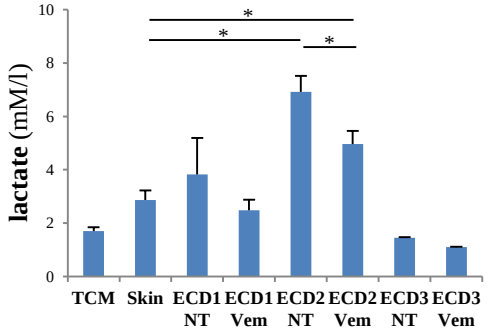
NT

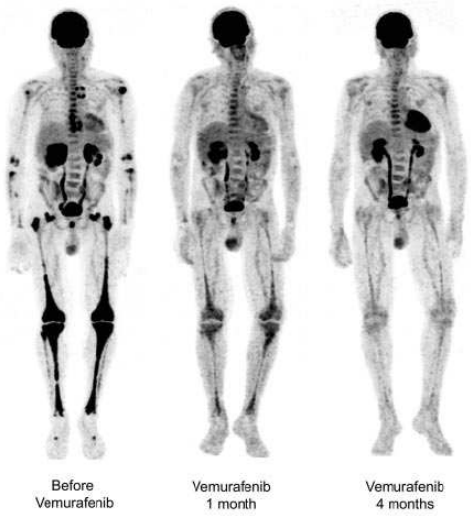


vem

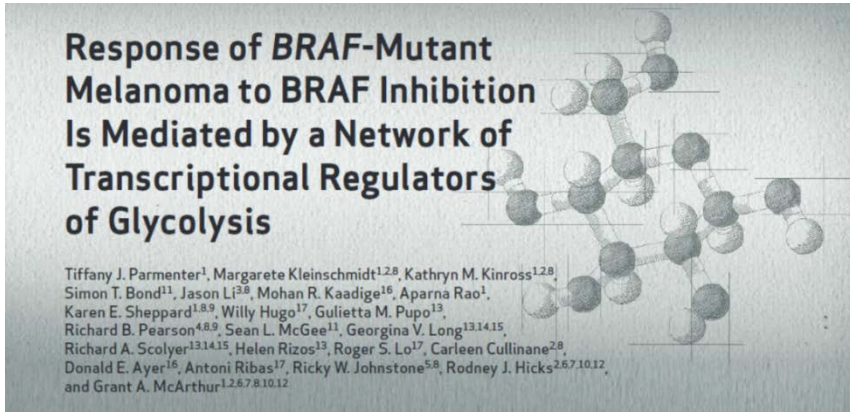








BRAF-mutation and metabolism in ECD histiocytes



Response of *BRAF*-Mutant Melanoma to BRAF Inhibition Is Mediated by a Network of Transcriptional Regulators of Glycolysis

Tiffany J. Parmenter[1], Margarete Kleinschmidt[1,2,8], Kathryn M. Kinross[1,2,8], Simon T. Bond[11], Jason Li[3,8], Mohan R. Kaadige[16], Aparna Rao[1], Karen E. Sheppard[1,8,9], Willy Hugo[17], Gulietta M. Pupo[13], Richard B. Pearson[4,8,9], Sean L. McGee[11], Georgina V. Long[13,14,15], Richard A. Scolyer[13,14,15], Helen Rizos[13], Roger S. Lo[17], Carleen Cullinane[2,8], Donald E. Ayer[16], Antoni Ribas[17], Ricky W. Johnstone[5,8], Rodney J. Hicks[2,6,7,10,12], and Grant A. McArthur[1,2,6,7,8,10,12]

ECD2