The science behind combinations with immunotherapy in melanoma
Saturday, 27 September 2014, 16:00-17:30 ; 20 min

Which systemic therapies should be combined to immunotherapy?

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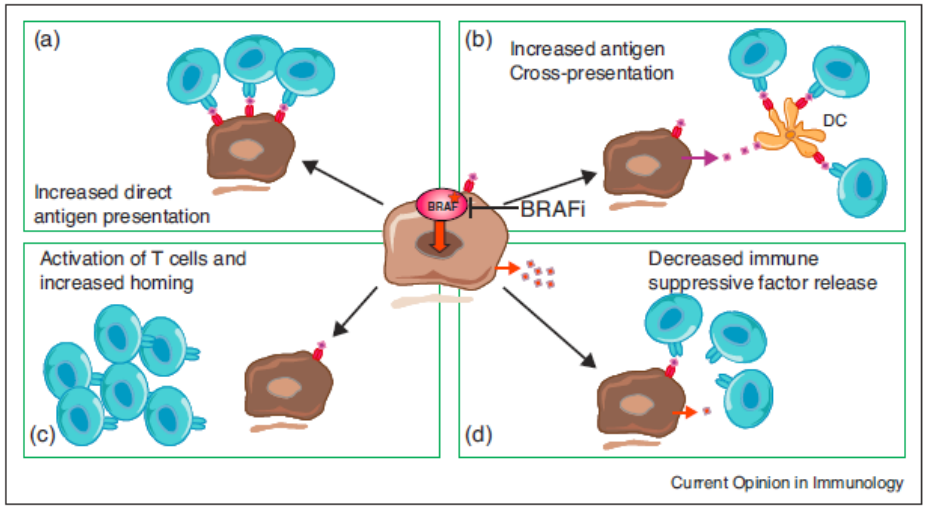
University of California Los Angeles (UCLA)
Chair, Melanoma Committee at SWOG

BRAF inhibitors as immune sensitizing agents

- BRAF inhibitors could sensitize the immune system by:
  - Increase tumor antigen and MHC expression
  - Increase tumor infiltrating lymphocytes
  - Improve immune effector cell function by inducing paradoxical MAPK activation on T cells
  - Improve the tumor microenviroment by decreasing expression of immune suppressive cytokines and immune regulatory ligands



1.Kono M. Mol Cancer Res 2006 2. Sapkota B. Oncoimmunology 2013. 3. Boni A. Cancer Res 2010. 4. Frederick DT. Clin Cancer Res 2013. 5. Long GV. Pigment Cell Melanoma Res 2013. 6. Wilmott JS. Clin Cancer Res 2012. 7. Cooper ZA. Oncoimmunology 2013. 8. Comin-Anduix B. Clin Cancer Res 2010. 9. Koya Cancer Research 2012. 10. Sumimoto H. J Exp Med 2006. 11 Khalili JS. Clin Cancer Res 2012. 12. Yamamoto R. Cancer Sci 2009. 13. Berthon C. Cancer Immunol Immunother 2010. 14 Knight DA. J Clin Invest 2013. 15.Liu CCR 2013. 16. Gray-Schopfer VC. Cancer Res 2007. 17. Landsberg, Nature 2012.

Ribas & Wolchok, Curr Opin Immunol 2013
Hu-Lieskovan, Robert, Homet & Ribas JCO 2014

Tumor infiltration by CD8 cells with BRAF inhibitors

Cancer Therapy: Clinical
See commentary by Bajor and Vonderheide, p. 1192

Clinical Cancer Research

Selective BRAF Inhibitors Induce Marked T-cell Infiltration into Human Metastatic Melanoma

James S. Wilmott[1,2], Georgina V. Long[1,2,6,7,8], Julie R. Howle[1,2,8], Lauren E. Haydu[1,2], Raghwa N. Sharma[2,4,8], John F. Thompson[1,2,3], Richard F. Kefford[1,2,6,7,8], Peter Hersey[1,2,5], and Richard A. Scolyer[1,2,3]

Early post-dosing dense intra-tumor infiltrates with CD8+ CTLs →

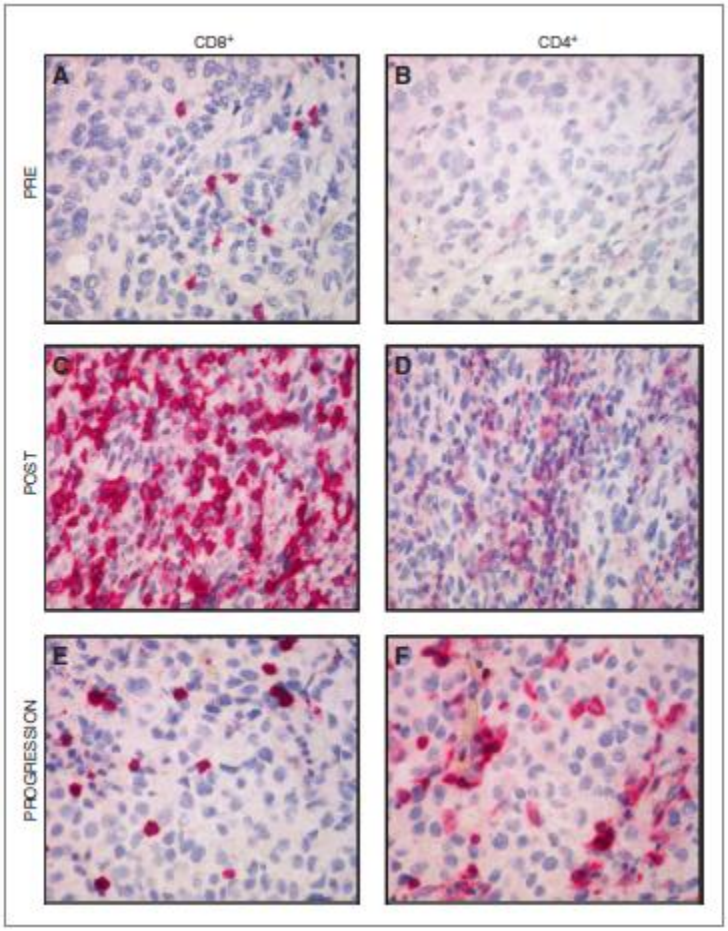
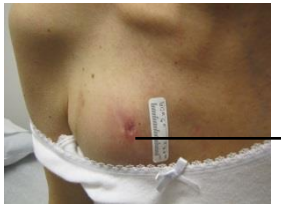
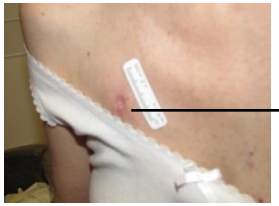


Figure 1. Immunohistochemical staining for $CD8^+$ and $CD4^+$ lymphocytes in biopsies taken from patient 4 at different treatment stages (×20 magnification). A, PRE biopsy showing low density of $CD8^+$ lymphocytes. B, PRE biopsy negative for $CD4^+$ lymphocytes. C, POST biopsy showing high density of $CD8^+$ infiltration. D, POST biopsy showing a high density of $CD4^+$ lymphocytes. E, PROGRESSION biopsy showing low levels of $CD8^+$ infiltration. F, PROGRESSION biopsy showing low levels of $CD4^+$ infiltration.

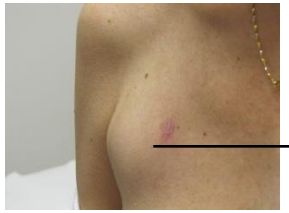
RNASeq analysis of baseline, on BRAFi therapy and progressive melanoma metastases



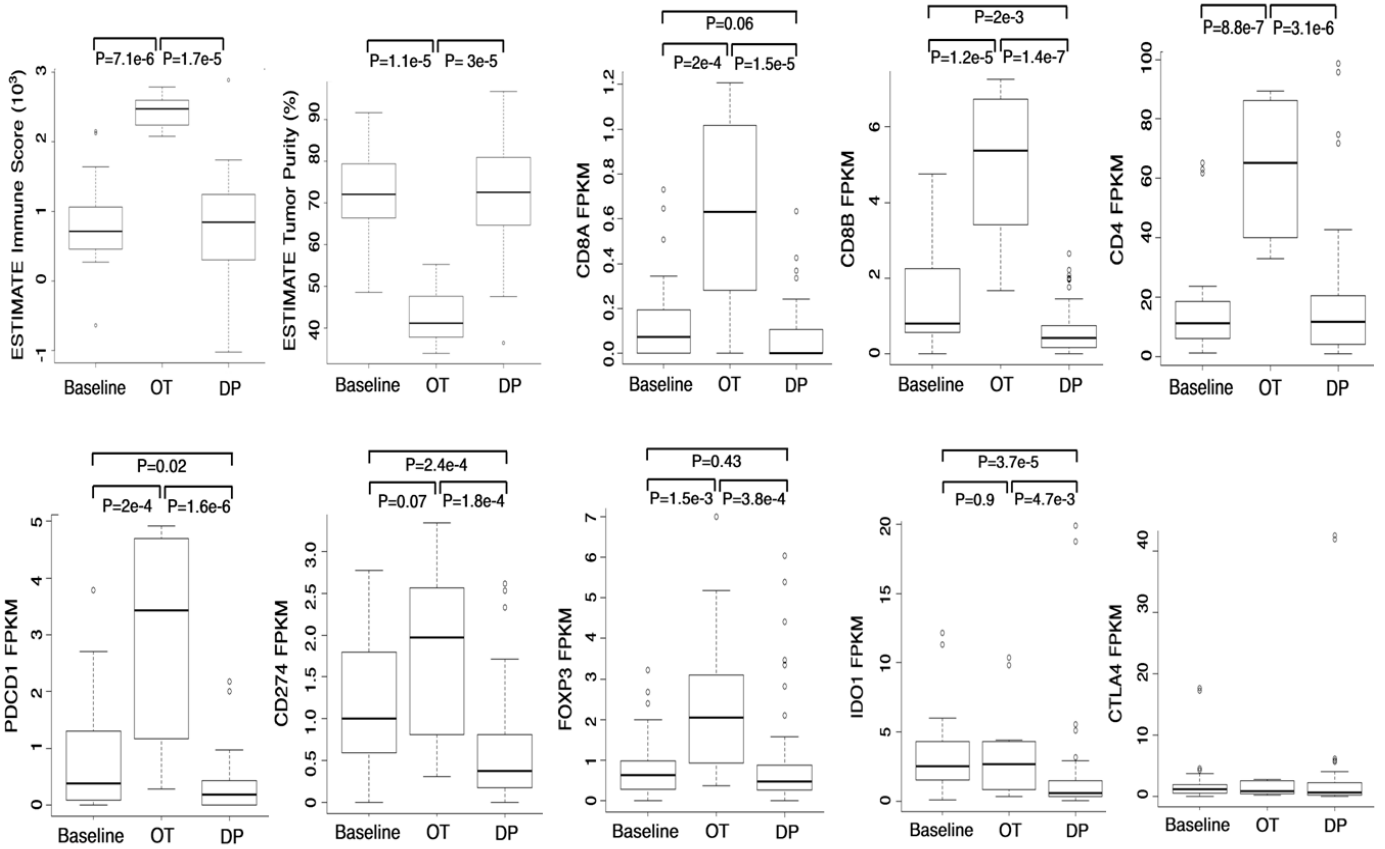
Baseline biopsy



On treatment biopsy (OT)



Disease progression (DP)

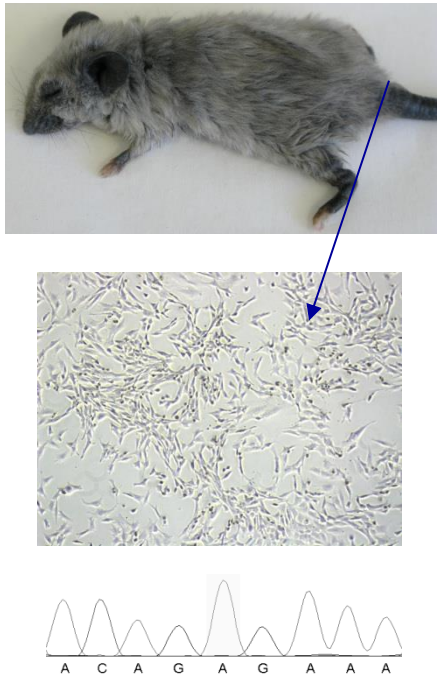


RNASeq analysis of 31 baseline biopsies, 15 on-treatment biopsies, and 57 disease progression (DP) biopsies.

Roger S. Lo, MD, PhD
Willy Hugo, PhD
Sun Lu
Xiangju Kong

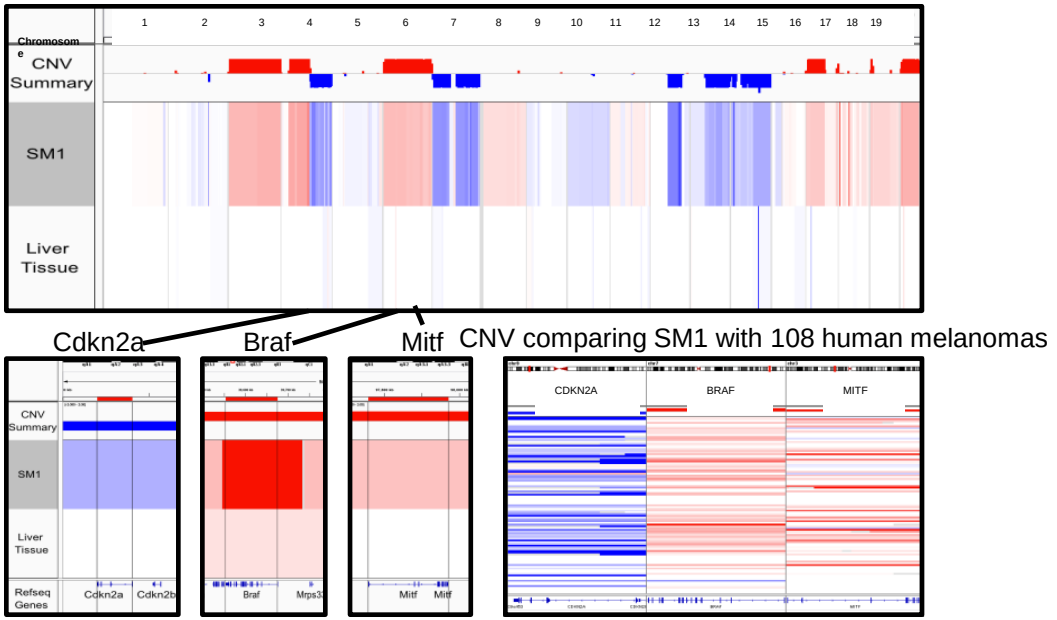
SM1: A BRAF^{V600E}-driven melanoma syngeneic to immunocompetent C57BL/6 mice

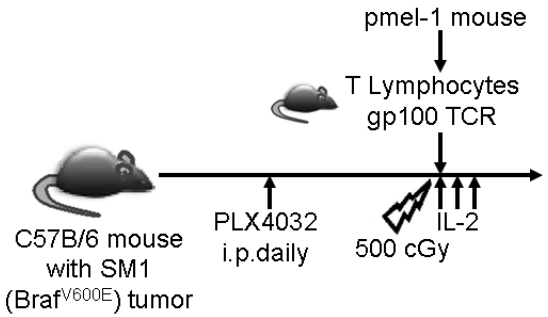
Goel, Haluska *et al*. Oncogene. 2009

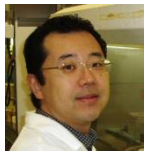


BRAF^{V600E} mutation

SM1 has genomic alterations similar to human melanoma





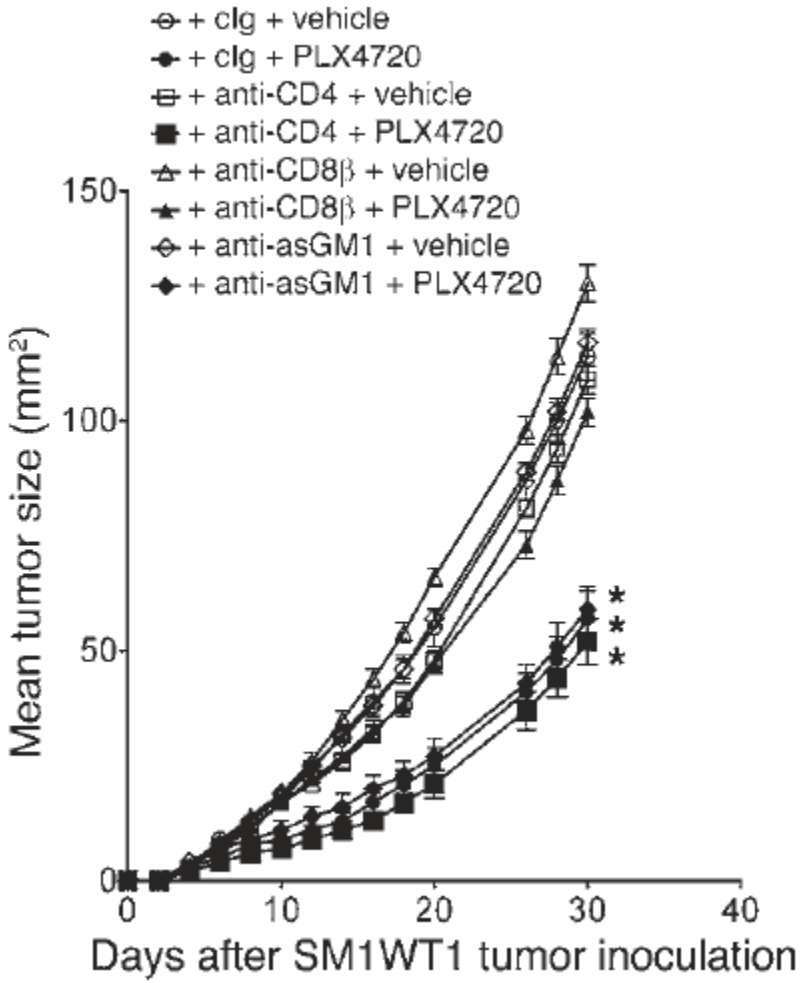


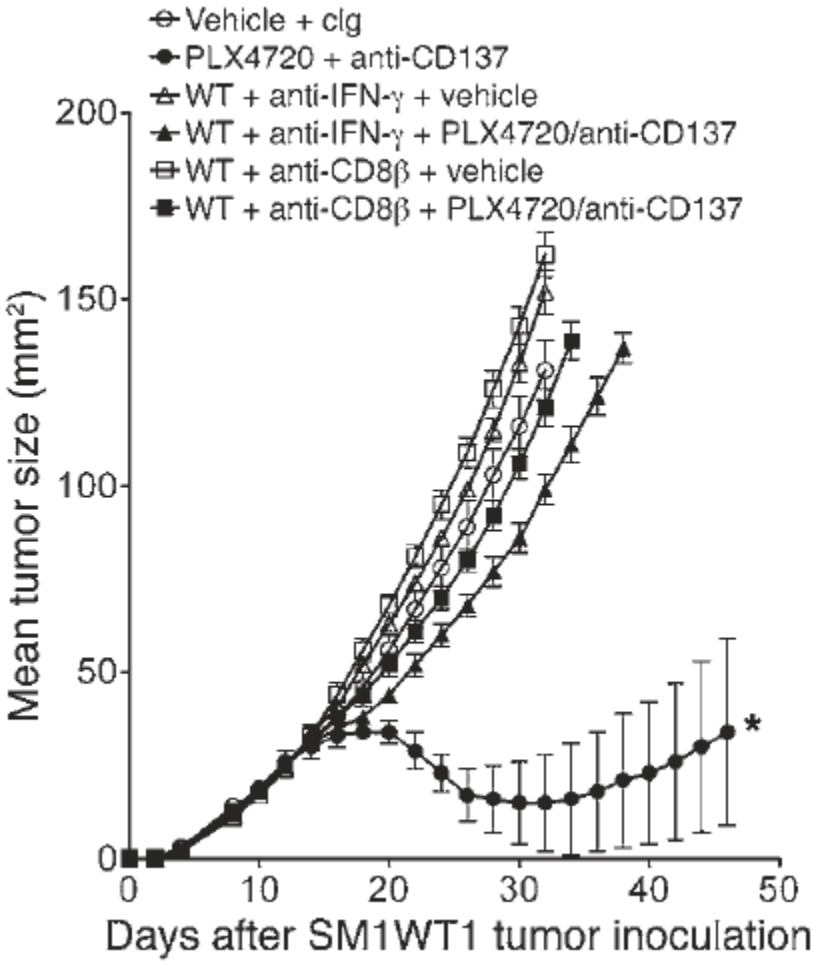
Richard Koya, MD, PhD

Stephen Mok

Koya, Mok *et al*. Cancer Res 2012

Role of CD8 on BRAFi responses alone or in combination with anti-CD137



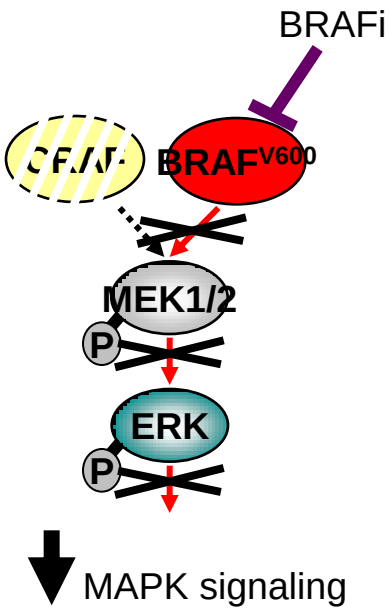


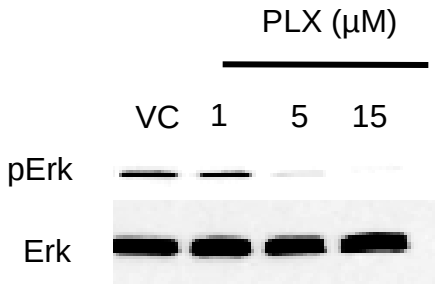
Knight … Ribas, McArthur, Smyth. JCI 2013

Mark Smyth, PhD
Grant McArthur, MD
PeterMac, Melbourne

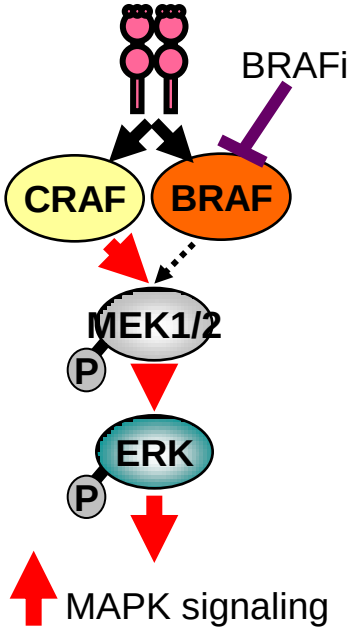
Paradoxical activation of pERK with exposure of lymphocytes to vemurafenib

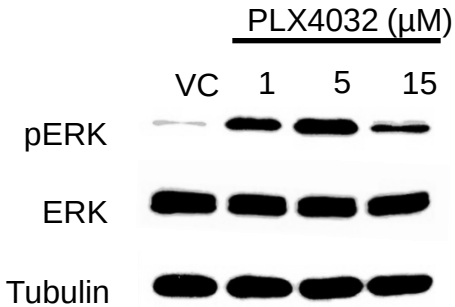
SM1





pmel-1





Koya, Mok *et al*. Cancer Res 2012

Paradoxical activation of pERK with exposure of lymphocytes to vemurafenib

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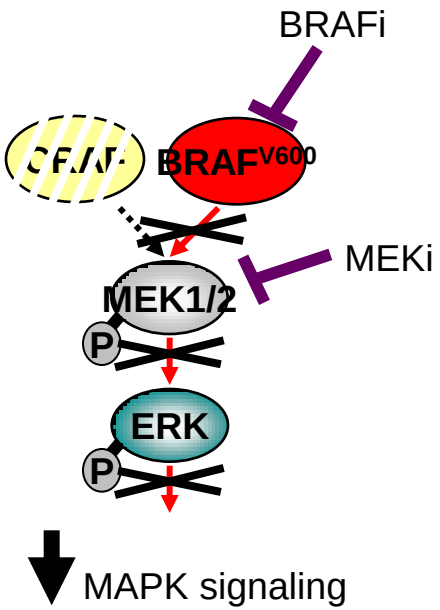
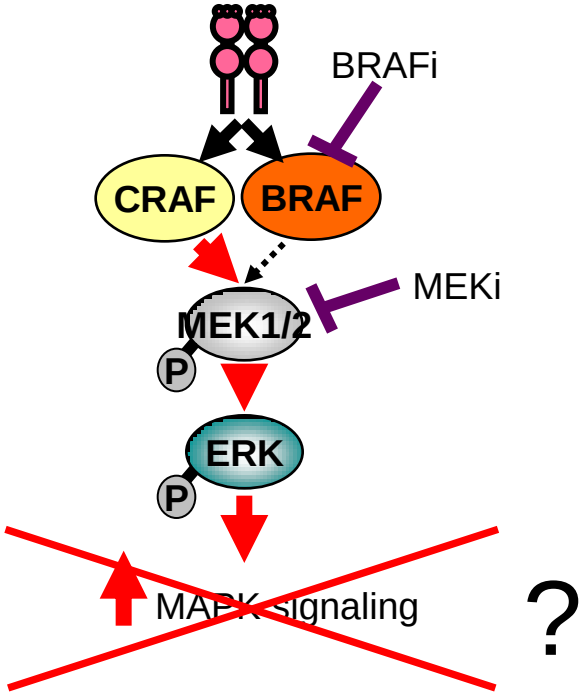
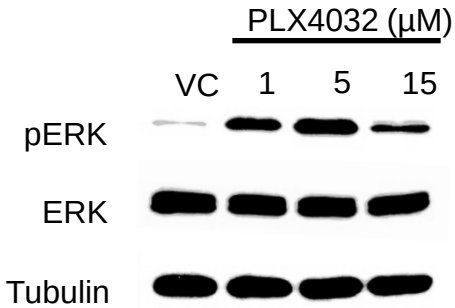


pmel-1





Koya, Mok *et al*. Cancer Res 2012

Priority Report

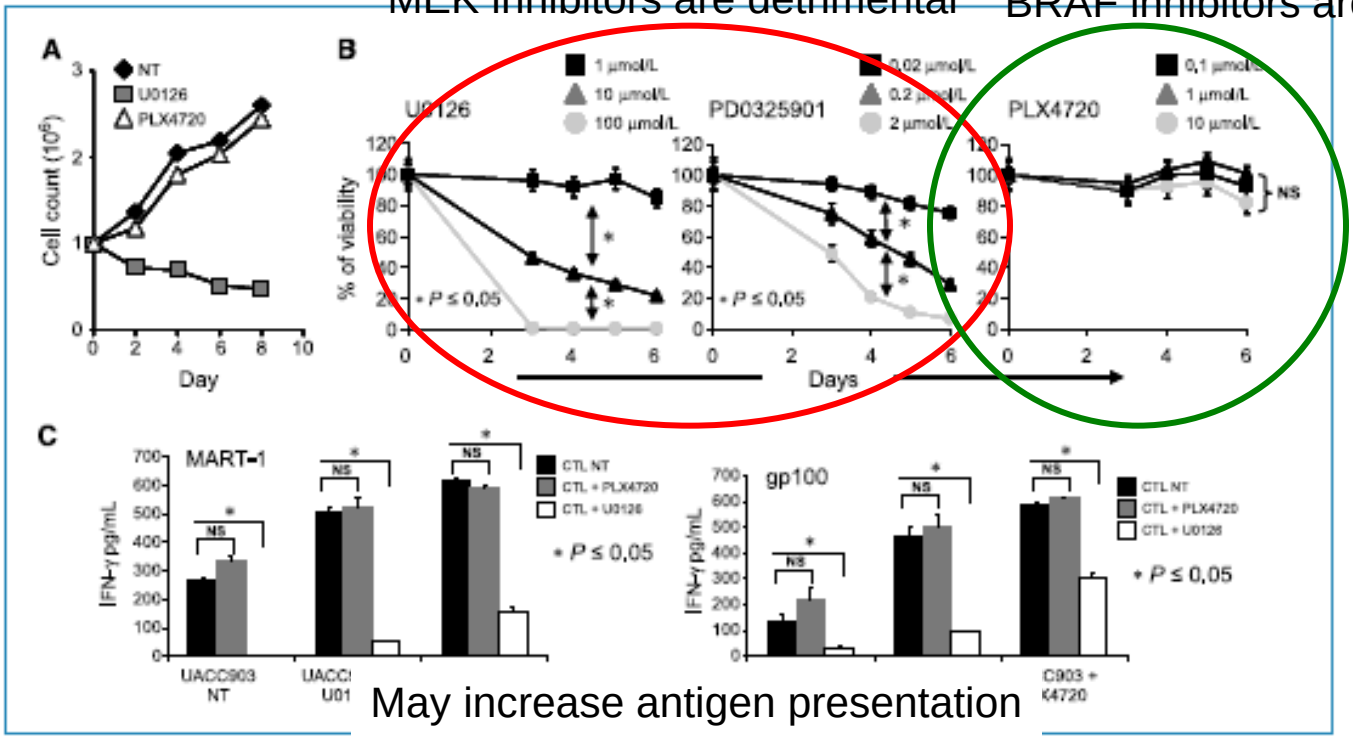
Cancer Research

Selective BRAF^{V600E} Inhibition Enhances T-Cell Recognition of Melanoma without Affecting Lymphocyte Function

Andrea Boni, Alexandria P. Cogdill, Ping Dang, Durga Udayakumar, Ching-Ni Jenny Njauw, Callum M. Sloss, Cristina R. Ferrone, Keith T. Flaherty, Donald P. Lawrence, David E. Fisher, Hensin Tsao, and Jennifer A. Wargo

MEK inhibitors are detrimental

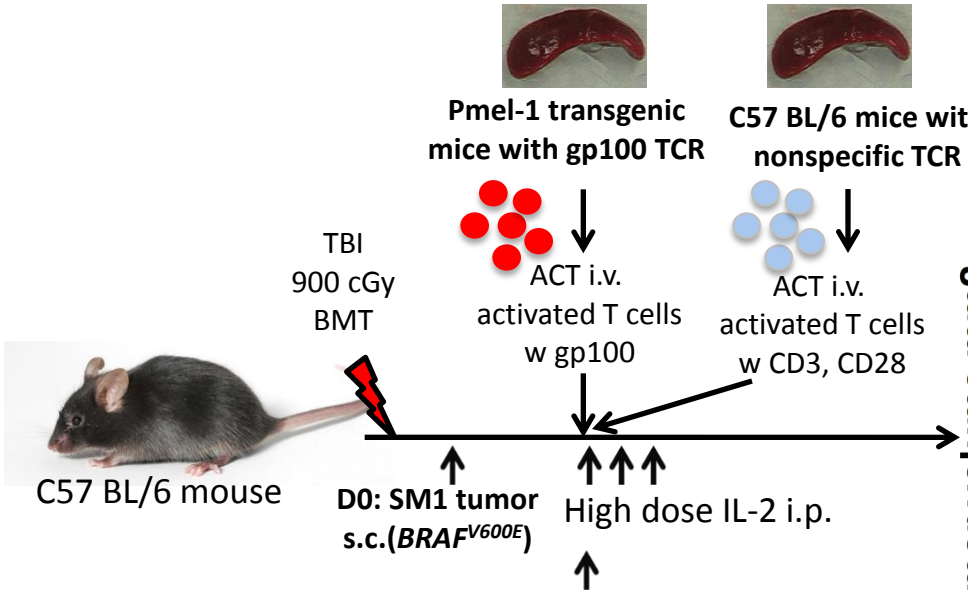
BRAF inhibitors are fine



May increase antigen presentation

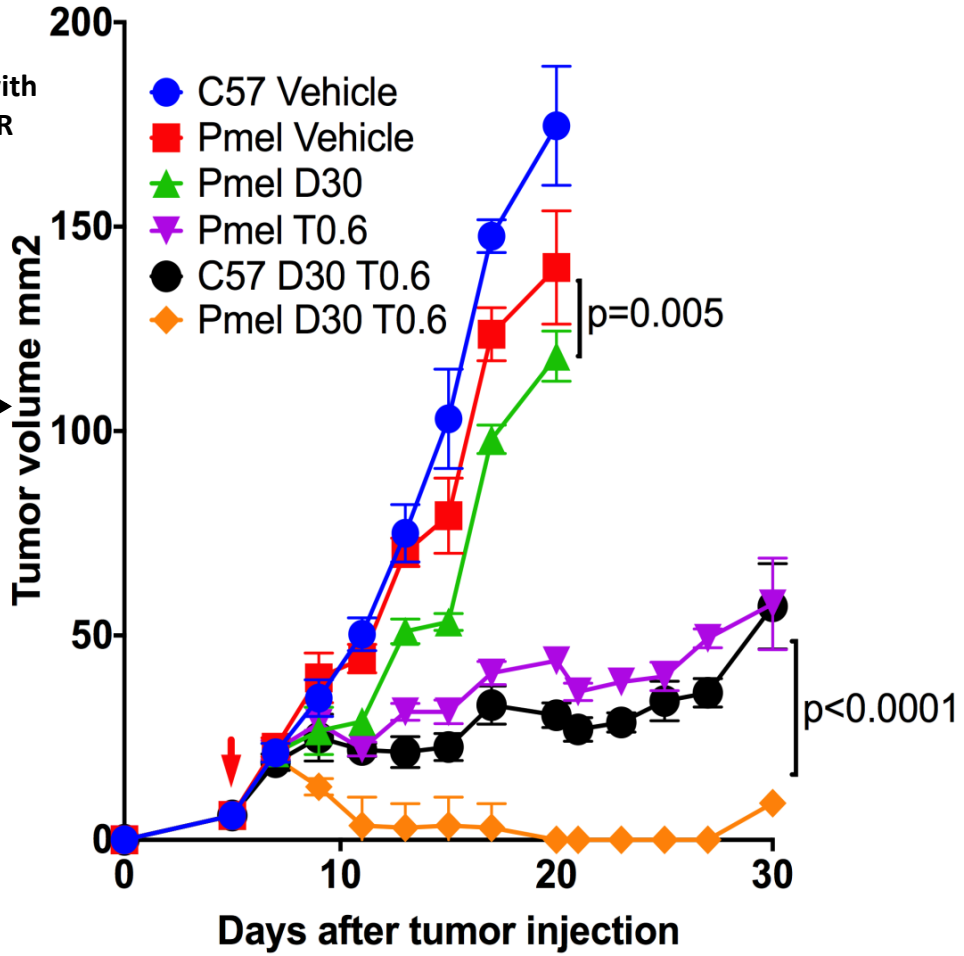
Figure 4. MEK inhibition (but not selective BRAF^{V600E} inhibition) impairs T-cell function. A, PBLs were stimulated alone (control; NT) or with U0126 or PLX4720. Absolute number of live cells was calculated using trypan blue exclusion following incubation at standard drug concentrations (U0126, 10 μmol/L; PLX4720, 1 μmol/L). B, cell viability was assessed by CellTiter-Glo viability assay after treatment at three separate drug concentrations of U0126 (1, 10, and 100 μmol/L), PD0325901 (0.02, 0.2, and 2 μmol/L), and PLX4720 (0.1, 1, and 10 μmol/L). C, treated and untreated UACC903 melanoma cells (treatment group = *X* axis) were cultured with CTL specific for either MART-1 (left) or gp100 (right) that had been cultured with U0126 (10 μmol/L; white columns), PLX4720 (1 μmol/L; gray columns), or media alone (NT; black columns). Experiments were repeated at least three times with similar results.

Enhanced *in vivo* antitumor activity pmel-1 ACT + dabrafenib and/or trametinib



Six groups (N=6):

Pmel ACT+Vehicle (V)
Pmel ACT+D30
Pmel ACT+T0.6
Pmel ACT+D30+T0.6
C57 ACT +V
C57 ACT +D30+T0.6



Dabrafenib and trametinib were kindly provided by Drs. Tona Gilmer, Li Liu and Jeff Legos through an MTA with GSK

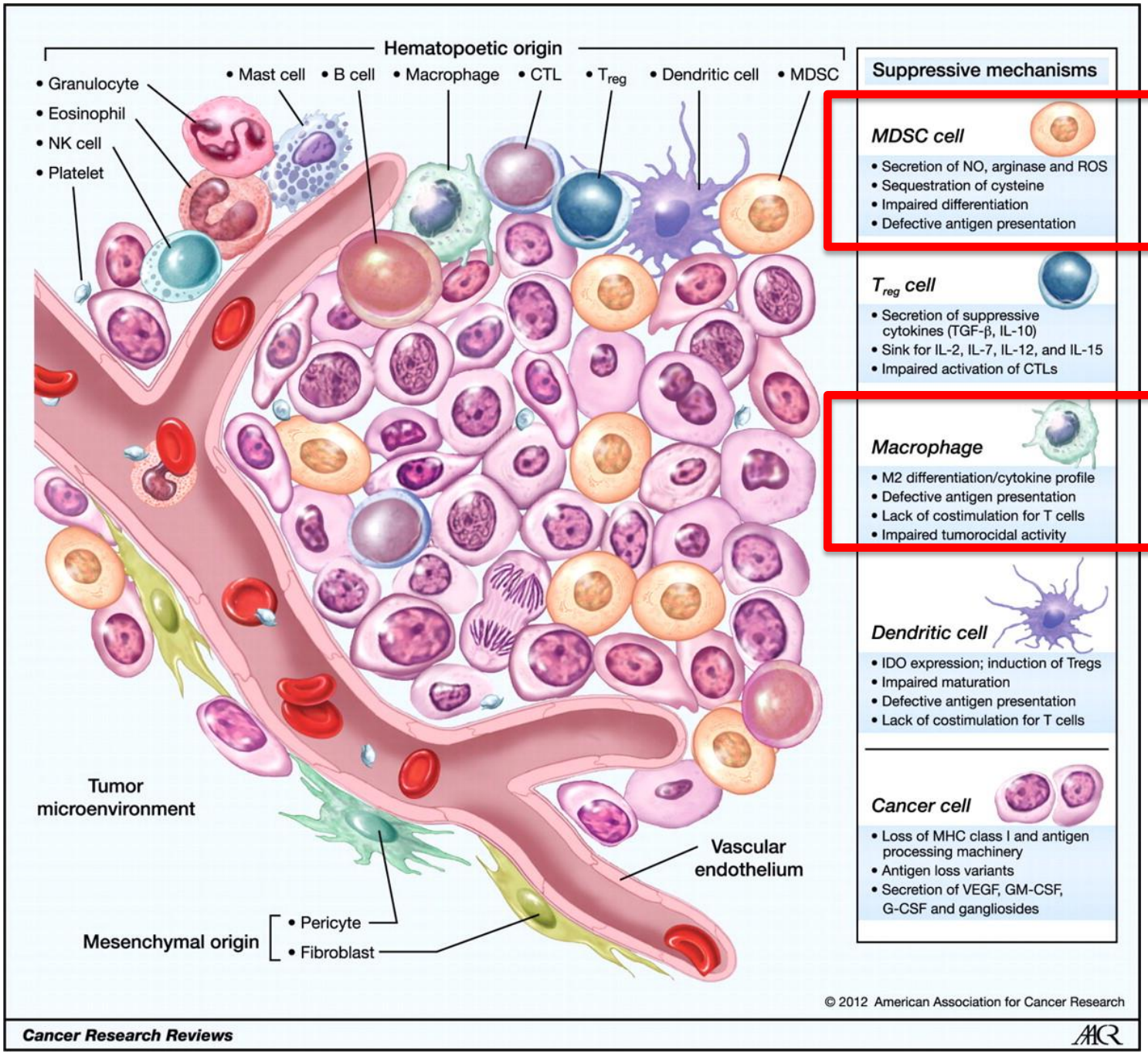
Siwen Hu-Lieskovan, MD, PhD

Stephen Mok

Ongoing clinical trials combining targeted therapy + immunotherapy for melanoma

Clinical Trial	NCT number	Status	Phase	Intervention
Systemic Therapy With Interferon, Interleukin-2 and BRAF Inhibitor	NCT01603212	Recruiting	Phase I/II	Vemurafenib + IL-2 + Interferon Alpha-2b
Safety and Efficacy Study of Vemurafenib and High-dose Interferon Alfa-2b in Melanoma	NCT01943422	Active	Phase I/II	Vemurafenib + High-dose Interferon alfa-2b
Vemurafenib With Lymphodepletion Plus Adoptive Cell Transfer & High Dose IL-2 Metastatic Melanoma	NCT01659151	Recruting	Phase II	Vemurafenib + Lymphodepletion + ACT with TIL Infusion + High Dose Interleukin-2 (IL-2)
Ph I/II Ipilimumab Vemurafenib Combo	NCT01400451	Active	Phase I	Vemurafenib + Ipilimumab (BMS-734016)
Combined BRAF-Targeted Therapy & Immunotherapy for Melanoma	NCT01754376	Recruting	Phase II	Vemurafenib + Aldesleukin
Ipilimumab With or Without Dabrafenib, and/or Trametinib in Treating Patients With Melanoma That is Metastatic or Cannot Be Removed By Surgery	NCT01940809	Recruiting	Phase I	ARM1- Ipilimumab ARM2 - Ipilimumab +Dabrafenib ARM3 - Ipilimumab + Trametinib ARM 4 - Ipilimumab+ Dabrafenib + Trametinib
Vemurafenib and White Blood Cell Therapy for Advanced Melanoma	NCT01585415	Recruting	Phase I	Vemurafenib + Lymphodedeplition + Drug: Young TIL + Aldesleukin
Ipilimumab and Imatinib Mesylate in Advanced Cancer	NCT01738139	Recruiting	Phase I	Imatinib mesylate + Ipilimumab
A Phase 1b Open Label, Dose Escalation Study of PLX3397 in Combination With Vemurafenib in V600-mutated BRAF Melanoma	NCT01826448	Recruiting	Phase I	Vemurafenib + PLX3397
A Study of The Safety and Pharmacology of MPDL3280A Administered in Combination With Vemurafenib (Zelboraf®) in Patients With Previously Untreated BRAFV600-Mutation Positive Metastatic Melanoma	NCT01656642	Recruiting	Phase I	Vemurafenib + MPDL3280A
Phase II Safety Study of Vemurafenib Followed by Ipilimumab in Subjects With V600 BRAF Mutated Advanced Melanoma	NCT01673854	Recruiting	Phase II	Vemurafenib 6 weeks followed by switch to Ipilimumab (sequential)

Hu-Lieskovan, Robert, Homet, Ribas, J Clin Oncol 2014

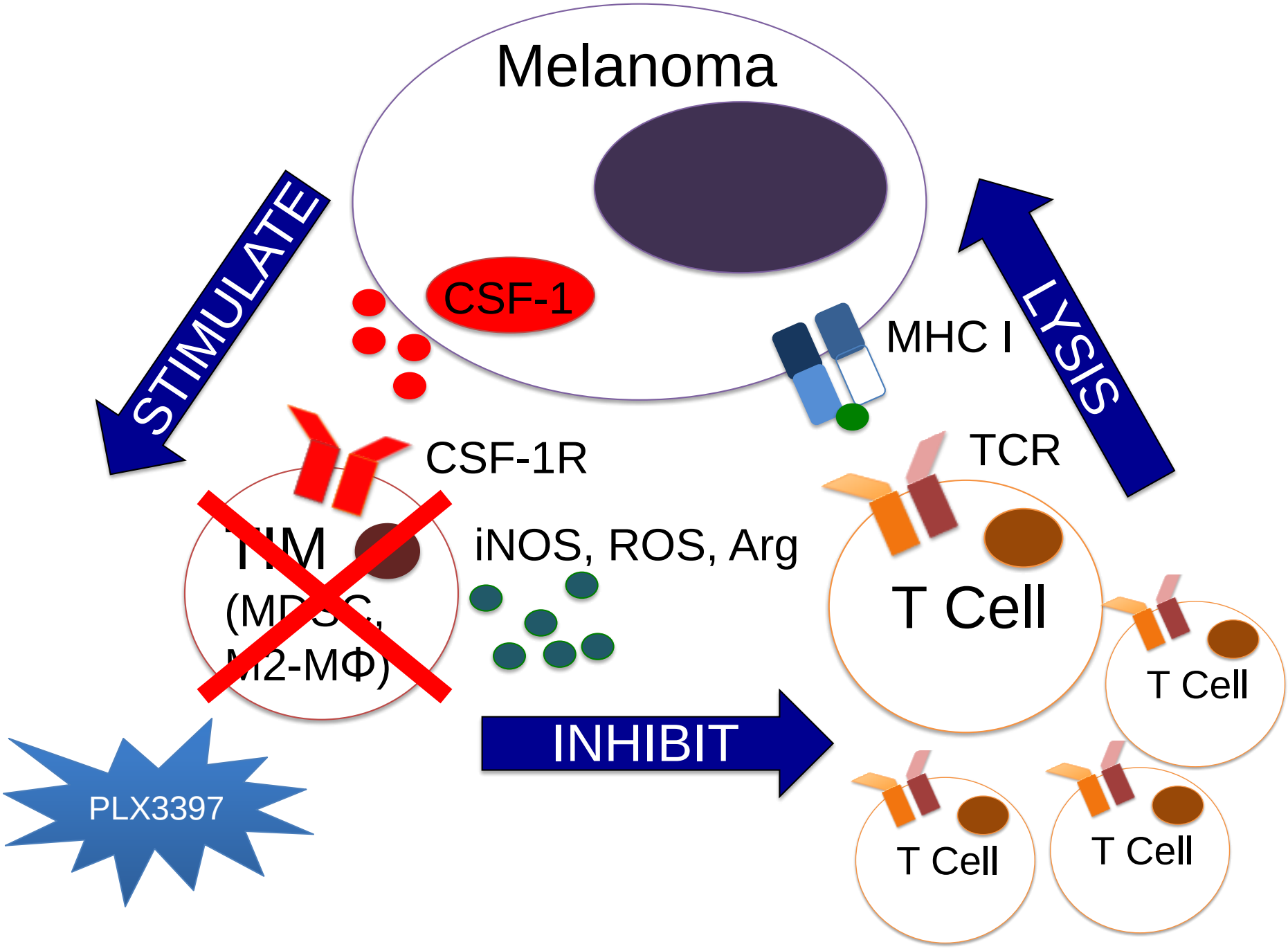


Kerkar SP. et al. 2012

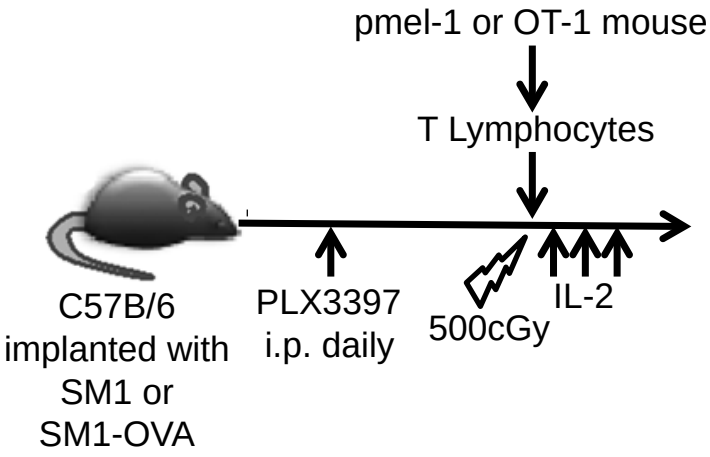
Secreted proteins expressed by SM1

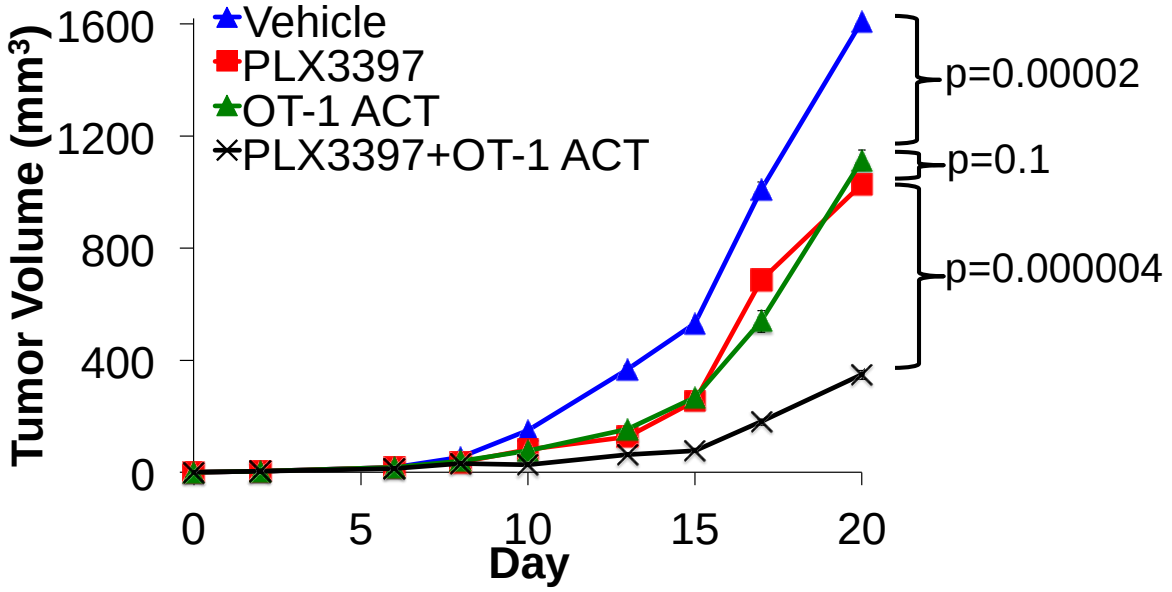
1. S100a6	16. Gmfb	31. Ltbp2	46.Pdgfb	61.Tgfb3	76.Figf	91.Ngf	106.Il17re
2. Cyr61	17. Slit2	32.Cmtm7	47.Rabep1	62.Vegfc	77.Pthlh	92.Pdgfra	107.Ccl9
Spp1	Ereg	Cx3cl1	Sema7a	Ogn	Cxcl10	Ptn	108. Bmp4
Mif	Inhba	Il6st	Bdnf	Tor2a	Oxt	Sema3f	
Hdgf	Cxcl1	Cmtm3	Vegfa	Ccl7	Il13ra1	Insl6	
Tnc	Cxcl12	Lif	Cklf	Edn1	Il17ra	Artn	
Hbegf	Sbds	Cat	Il17rc	Il11ra1	Lrsam1	Pdgfd	
Csf1	Pdgfa	Igf2	Cmtm6	Sema6d	Sema4b	Btc	
Fgf7	Nampt	Jag1	Il1r1	Apln	Stc2	Igf1	
Grn	Pdgfrb	Vegfb	Il15ra	Il17rd	Tnfsf12	Sema3c	
Ltbp3	Rabep2	Hdgfrp3	Ccl5	Nppb	Sema4c	Nrtn	
Ctgf	Clcf1	Txlna	Tgfb2	Gdnf	Il7	Ctf1	
Pdgfc	Il1rl1	Bmp1	Bmp2	Ccl20	Ltbp4	Il34	
14. Ccl2	29.Cd320	44.Il10rb	59.Plau	74.Sema3a	89.Il1rap	104.Il6ra	
15. Ltbp1	30.Il18rap	45.Nenf	60.Areg	75.Ccl25	90.Il24	105.Cmtm5	

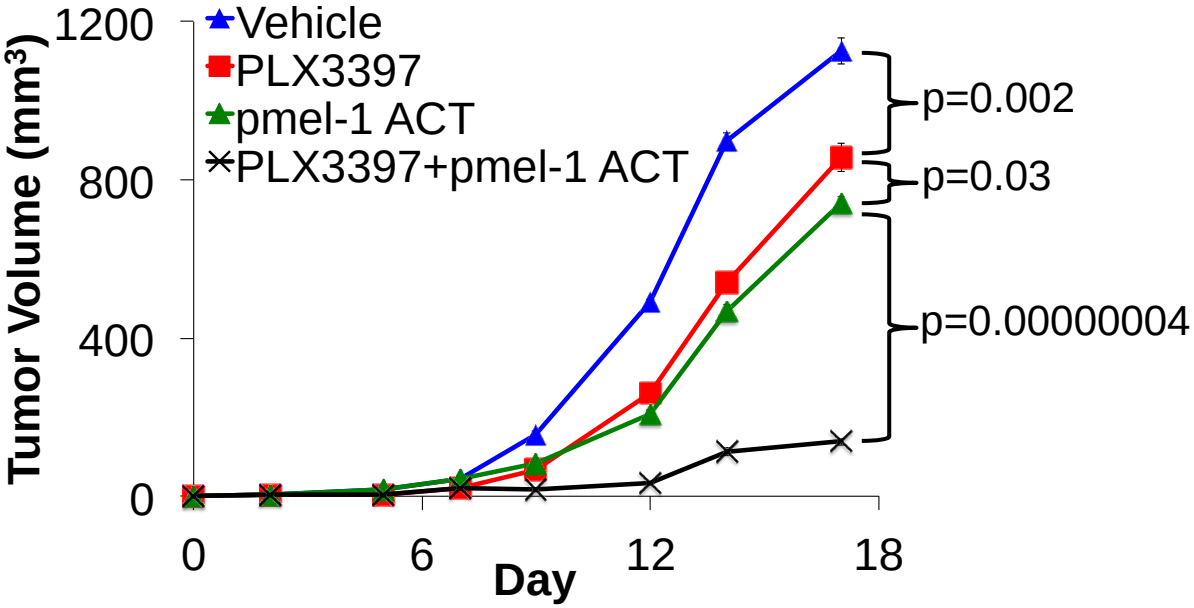
Stephen Mok, Ashley Cass, Tom Graeber


Melanoma
STIMULATE
LYSIS
CSF-1
MHC I
CSF-1R
TCR
TIM
(MDSC,
M2-MΦ)
iNOS, ROS, Arg
T Cell
T Cell
T Cell
T Cell
INHIBIT
PLX3397

Combined anti-tumor activity of adoptive cell transfer (ACT) immunotherapy and PLX3397

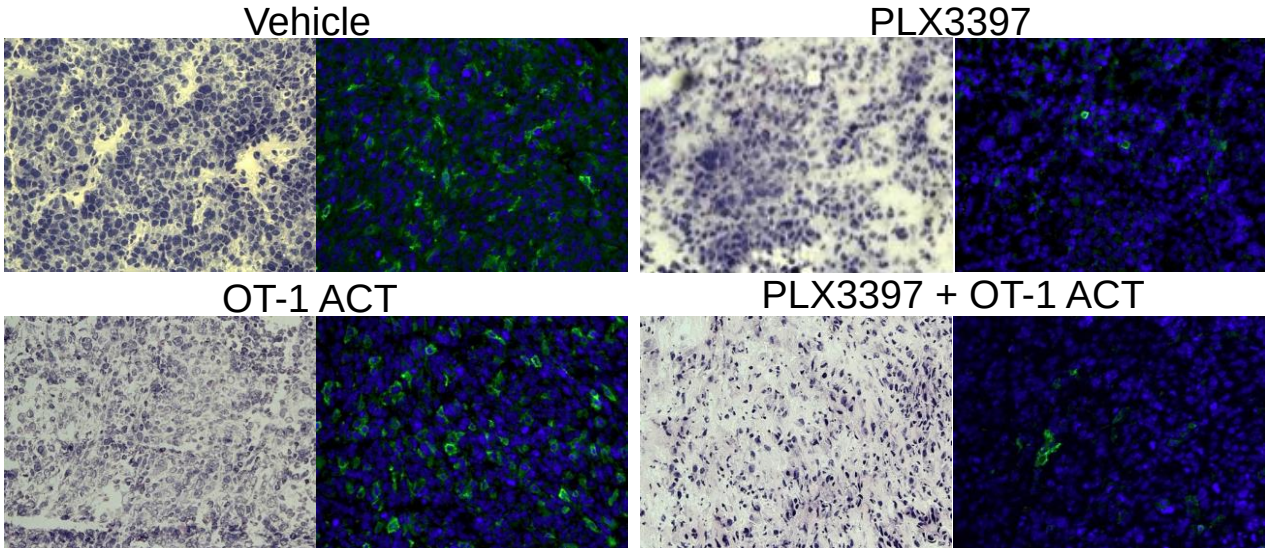


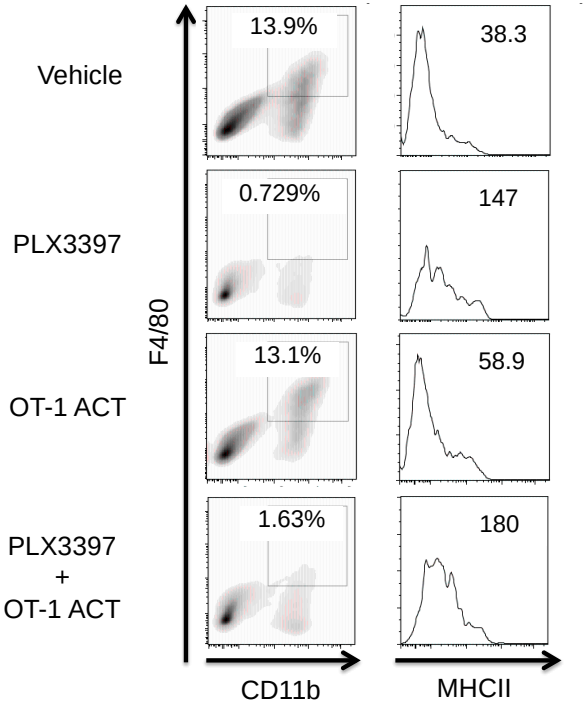


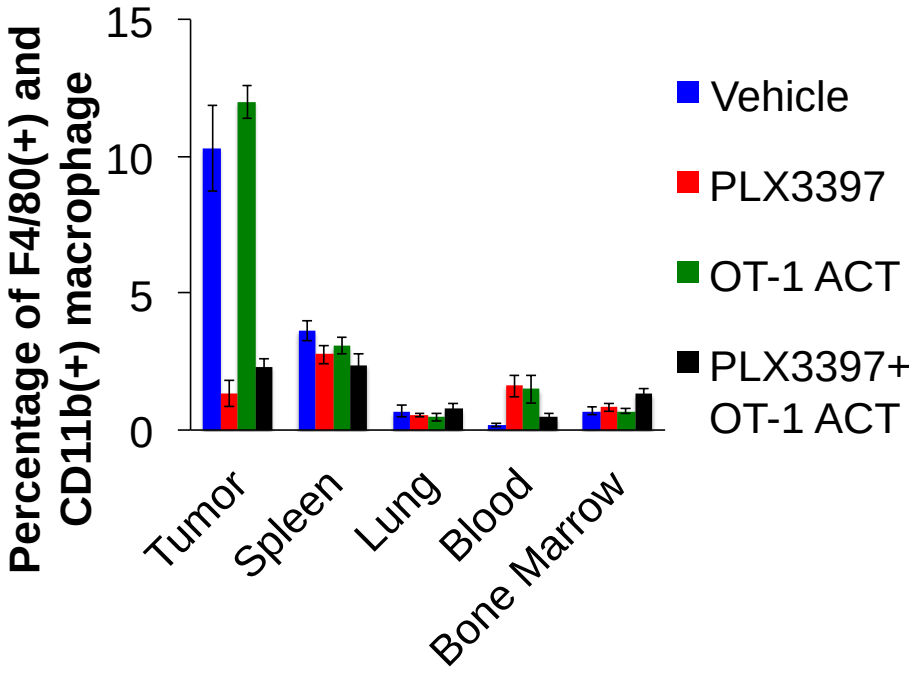


S. Mok *et al.* Cancer Research 2014

Changes in intratumoral macrophages in responses to PLX3397



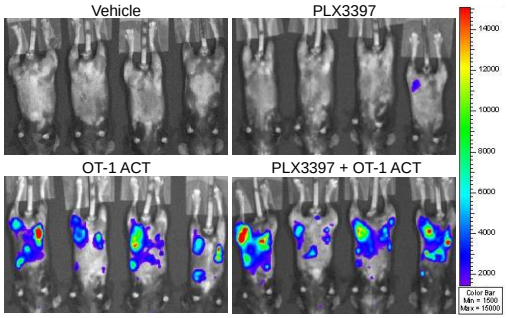


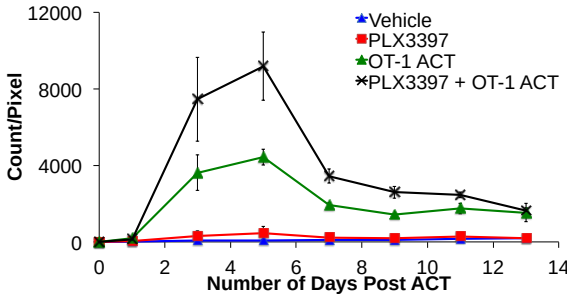


Effects of PLX3397 on expansion, distribution and cytokine production by adoptively transferred lymphocytes

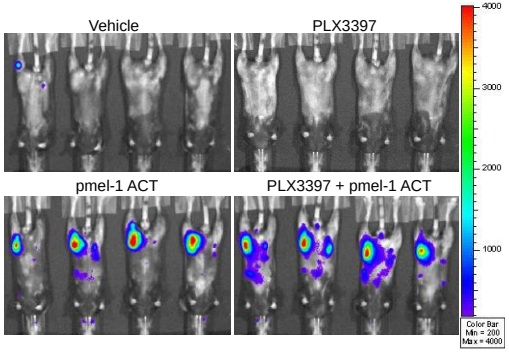
T cell expansion and distribution

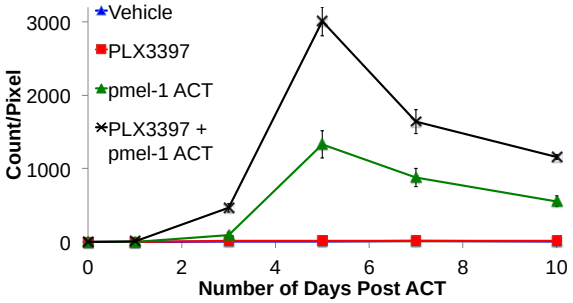
OT-1 ACT model



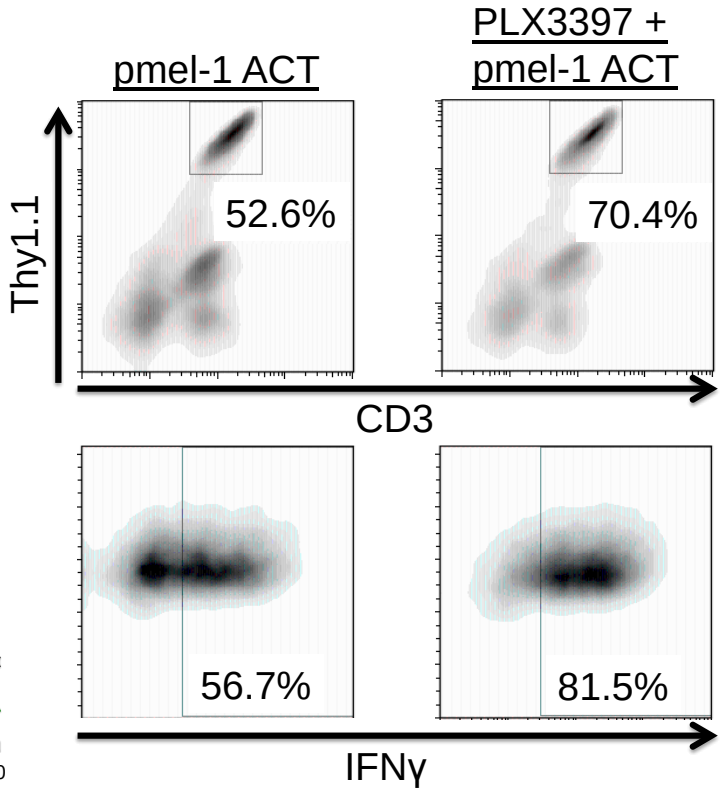


pmel-1 ACT model

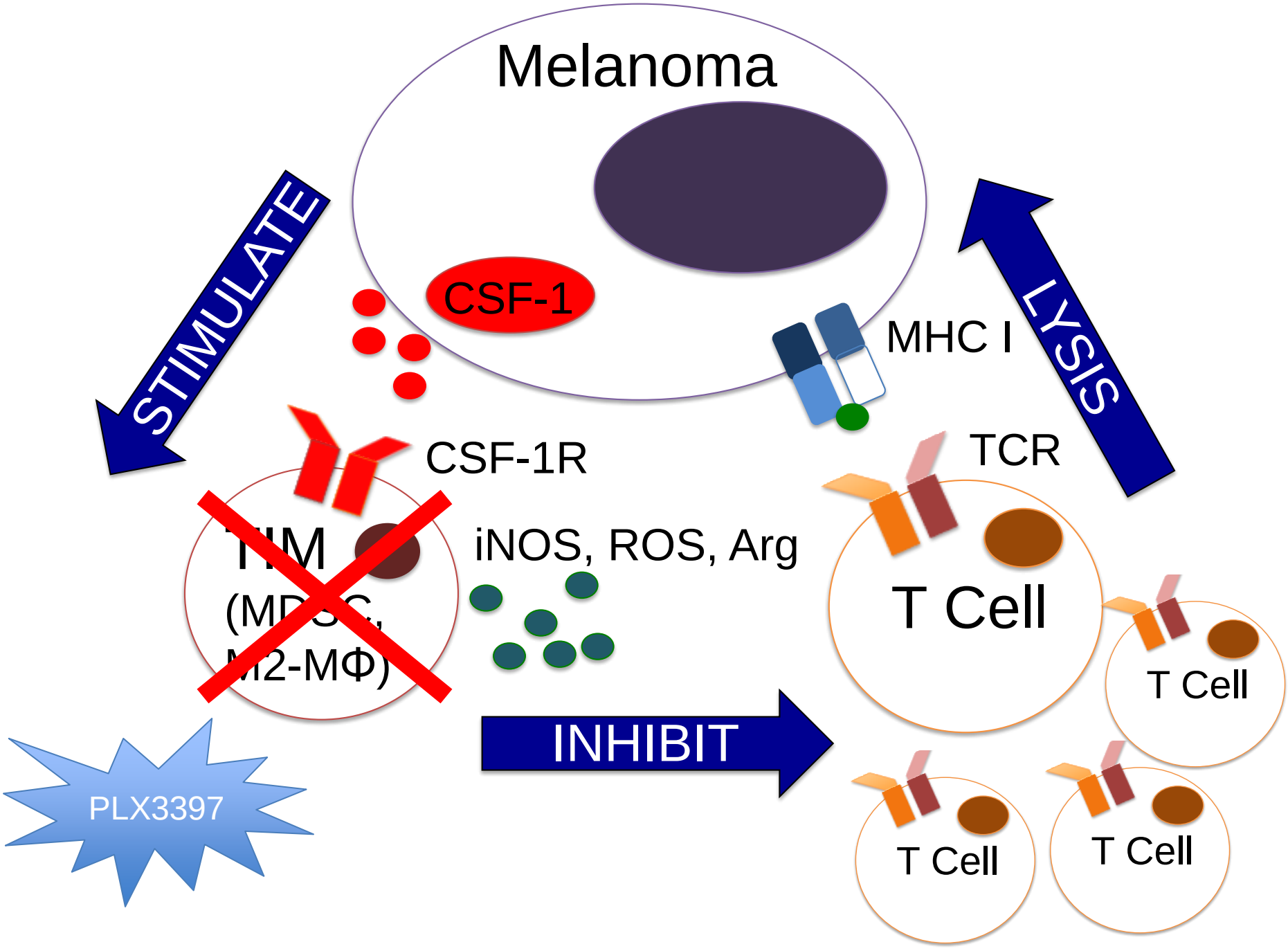




T cell cytokine production by TIL



By depleting macrophages in tumors, PLX3397 increases the expansion and function of T cells


Melanoma
CSF-1
MHC I
STIMULATE
LYSIS
CSF-1R
TCR
TIM
(MDSC,
M2-MΦ)
iNOS, ROS, Arg
T Cell
T Cell
T Cell
T Cell
INHIBIT
PLX3397

Conclusions

- There is a strong scientific rationale for combining oncogenic $BRAF^{V600E}$ blockade and immunotherapy:
  - Improving direct antigen presentation by tumor cells
  - Sensitizing cancer cells to apoptotic death resulting in antigen cross-presentation
  - Inhibiting suppressive factors in the tumor
  - Improving lymphocyte function
- Paradoxical MAPK activation needs to be considered with these combinations to interpret combined efficacy and toxicities
- MEK inhibitors may block paradoxical MAPK activation by BRAF inhibitors, their effects on immune cells *in vivo* need to be further explored
- Inhibiting intratumoral macrophages with CSF-1R inhibitors improves the antitumor activity of tumor-specific T cells

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The State Stem Cell Agency

P01 CA168585-01A1 (Ribas)
P01 CA132681 (Baltimore)
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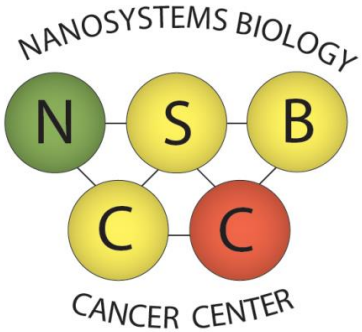

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