Targeting the RAS/RAF/PI3K pathway for CD40 induction in melanoma cells to overcome resistance to anti-PD1 immunotherapies

Chi Yan[1,2*], Weifeng Luo[1,2], Jinming Yang[1,2], Sheau-Chiann Chen[3], Bergdorf Kensey[1,2], Douglas B. Johnson[4], Gregory D. Ayers[3], Ann Richmond[1,2]

[1]Tennessee Valley Healthcare System, Department of Veterans Affairs, Nashville, TN; [2]Vanderbilt University School of Medicine, Department of Pharmacology, Nashville, TN;
[3]Division of Cancer Biostatistics, Department of Biostatistics, Vanderbilt University Center for Quantitative Sciences, Nashville, TN;
[4]Division of Hematology and Oncology, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN. * Correspondence: chi.yan@vanderbilt.edu (C.Y.)

Abstract

The first-line treatment for metastatic melanoma is immune checkpoint blockade (ICB), but it fails in many patients and may have serious adverse events. We showed that rigosertib (RGS), a RAS-pathway inhibitor, promotes CD40 upregulation on melanoma cells and synergizes with ICB in preclinical melanoma models. However, residual disease still occurred with uncontrolled ERK activation. Our present study explores the efficacy of RGS plus trametinib (T), a MEK1/2 inhibitor, to overcome ICB resistance. The concurrent RGS+T+αPD1 resulted in an additive effect to suppress ICB-resistant $NRAS^{mut}$ 1014 tumor growth, and 44.4% (4/9) of the tumors completely regressed after 2 weeks of treatment. The RGS+T regimen promoted $CD8^+$ T cell responses in the tumor microenvironment (TME) and extended the time to αPD1 resistance ($p<0.0001$). Combining RGS+T with agonist CD40 (aCD40) resulted in increased $CD8^+$ T cells, natural killer cells, and M1 macrophages in the TME, with a reduction of myeloid-like $CD11b^+PD\text{-}L1^+$ regulatory B cells in the tumors (~70%, $p<0.0001$) and tumor-draining lymph nodes (~40%, $p<0.0001$). CRISPR/dCas9-based overexpression of CD40 (CD40-OE) in 1014 melanoma cells reduced *in vivo* tumor growth and successfully turned the ICB-resistant tumors into responders to αPD1 ($p=0.025$), which further regressed with aCD40+αPD1 ($p<0.001$). Our preclinical data support the therapeutic use of RAS/RAF/PI3K inhibition plus CD40 agonism for metastatic melanoma patients who do not respond to ICB.

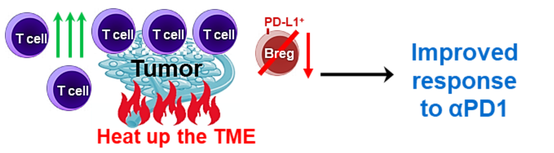
2nd-line treatment for ICB-refractory melanoma

Rigosertib +/- Trametinib

RAS/PI3K/AKT inhibition | RAF/MEK/ERK inhibition

CD40 induction on melanoma cells
Immunogenic cell death

Lead-in αPD1 | **+/- CD40 agonist (aCD40)**

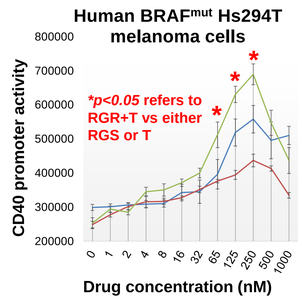


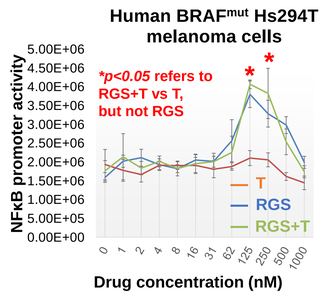
Improved response to αPD1

Preclinical mouse models of *$NRAS^{mut}$* and *$NRAS^{WT}$/$BRAF^{WT}$* melanoma

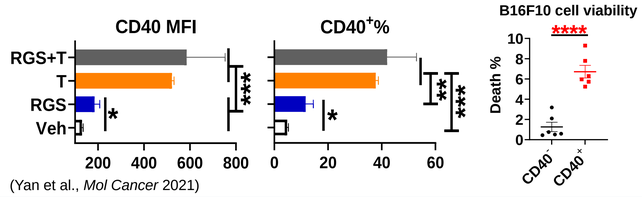
RAS/RAF/PI3K inhibition triggers CD40 induction in melanoma cells

CD40 transcription



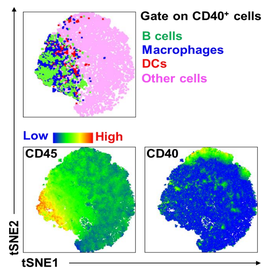


CD40 protein at cell surface level (B16F10 cells)

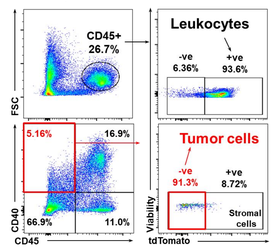


(Yan et al., *Mol Cancer* 2021)

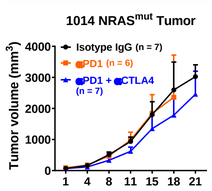
Tumor Microenvironment

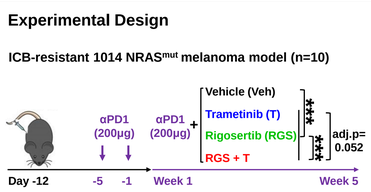


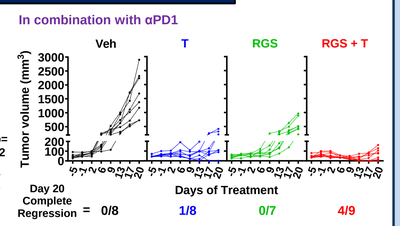
ROSA reporter mice



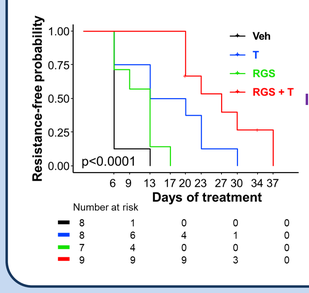
RAS/RAF/PI3K inhibition overturns αPD1-resistance in $NRAS^{mut}$ melanoma



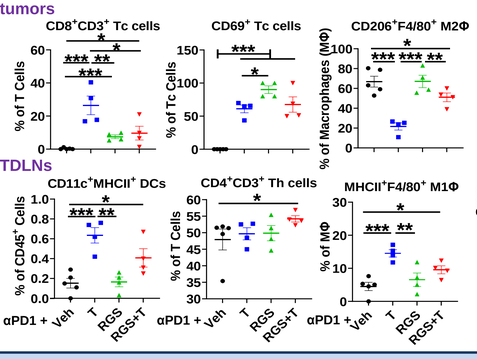




αPD1-resistance-free probability reaches 50% at
Day 6 13 13 27 ($p<0.001$)



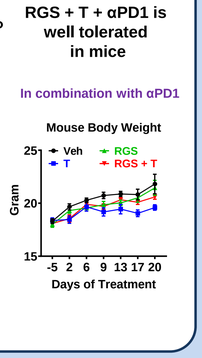
In tumors



In TDLNs

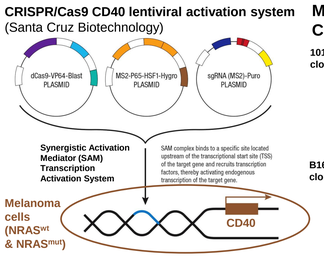
RGS + T + αPD1 is well tolerated in mice

In combination with αPD1

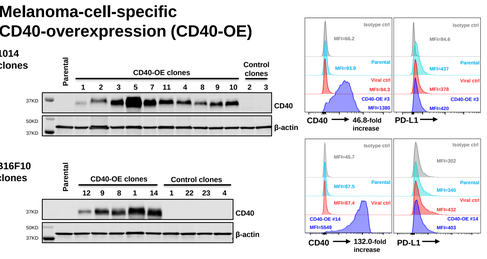


CD40 overexpression in melanoma cells shifted αPD1-non-responding melanomas into responders

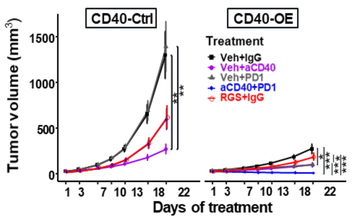
CRISPR/Cas9 CD40 lentiviral activation system (Santa Cruz Biotechnology)



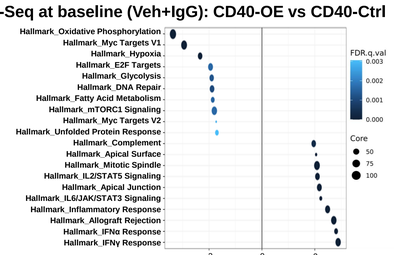
Melanoma-cell-specific CD40-overexpression (CD40-OE)



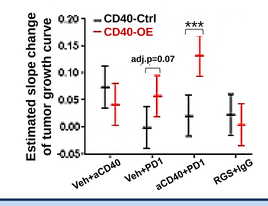
***In vivo* tumor growth and therapy responses**



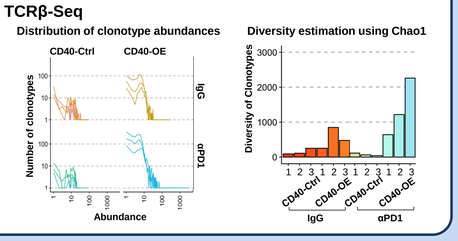
RNA-Seq at baseline (Veh+IgG): CD40-OE vs CD40-Ctrl



Treatment effect on the reduction of tumor growth rate compared to Veh+IgG

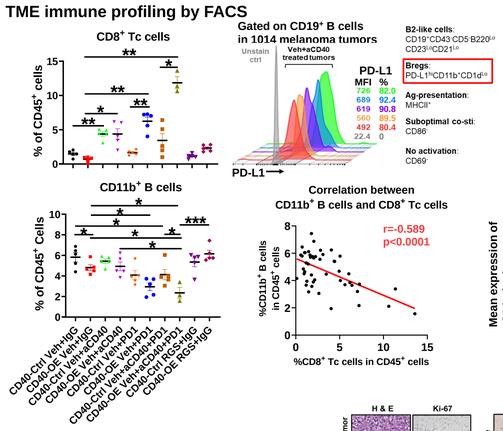


TCRβ-Seq

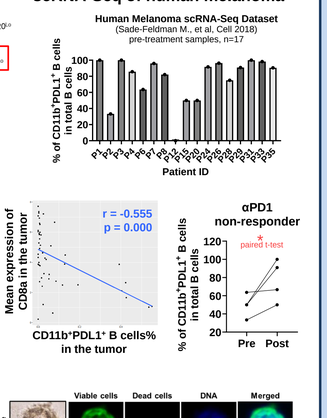


Regulatory B cells, αPD1-resistance and PDO model

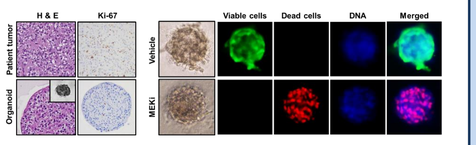
TME immune profiling by FACS

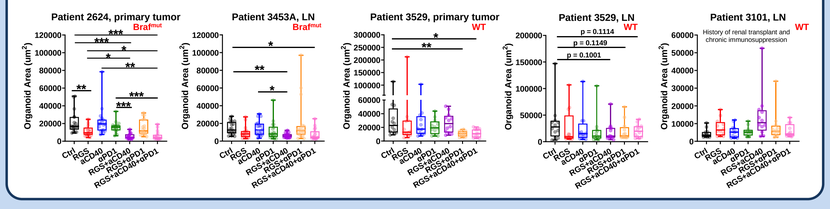


scRNA-Seq of human melanoma



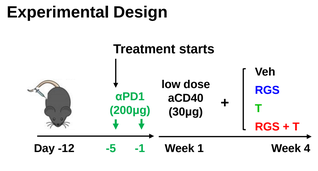
Patient-derived organoid 3D cultures
(Vilgelm et al., *iScience* 2020)

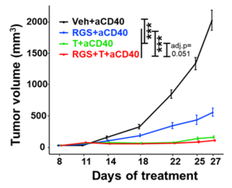


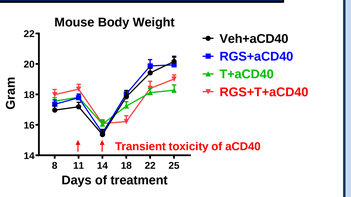


Combine agonist CD40 (aCD40) and RAS/RAF/PI3K inhibition to overcome αPD1-resistance in $NRAS^{mut}$ melanoma

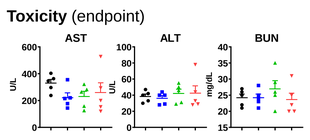
Experimental Design



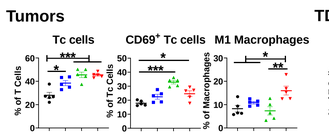




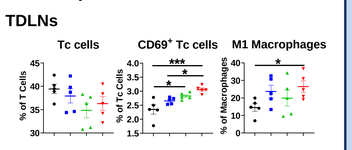
Toxicity (endpoint)



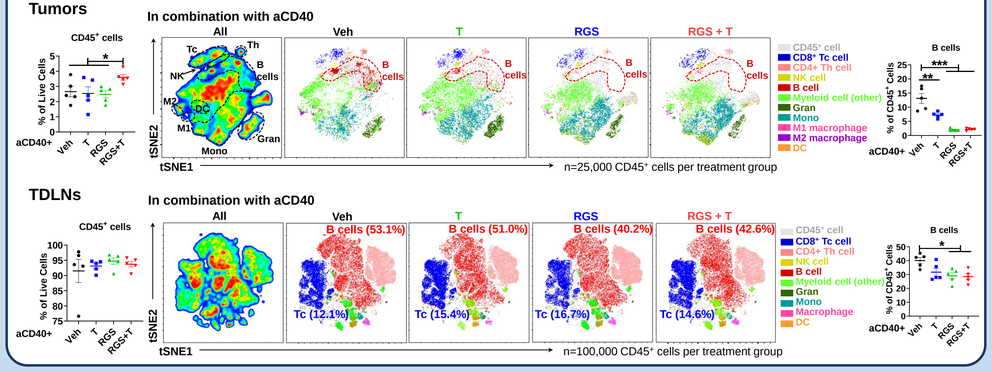
Tumors



TDLNs

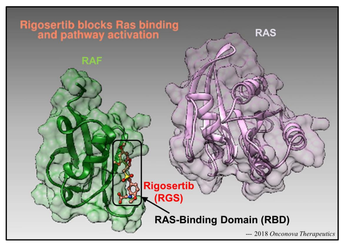


Tumors

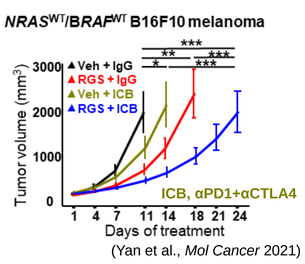


TDLNs

RGS synergizes with ICB *in vivo*

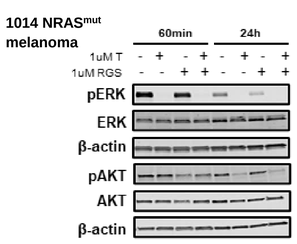


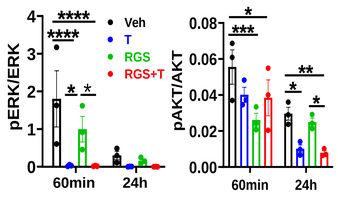
***$NRAS^{WT}$/$BRAF^{WT}$* B16F10 melanoma**



(Yan et al., *Mol Cancer* 2021)

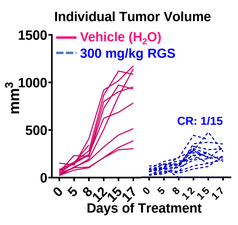
1014 $NRAS^{mut}$ melanoma

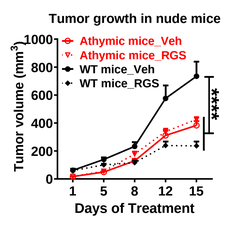


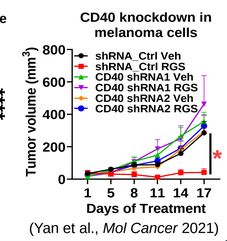


RGS-induced anti-tumor immunity depends on T cells and CD40 in melanoma cells

YUMM3.3 $BRAF^{mut}$ melanoma







(Yan et al., *Mol Cancer* 2021)

Conclusions and future works

- **Inhibition of RAS/RAF/PI3K pathway triggers CD40 upregulation in melanoma cells.**
- **Melanoma-cell-selective induction of CD40** successfully shifted αPD1-non-responding $NRAS^{mut}$ melanomas into responders.
- **Combining RAS/RAF/PI3K targeted inhibition and agonist CD40** offers considerable promise for ICB-resistant $NRAS^{mut}$ and $NRAS^{WT}$ melanoma treatment.
- In collobration with OncoNova and Merck, we have initiated a **Phase IIb clinical trial at Vanderbilt** of rigosertib plus pembrolizumab for treatment of patients with unresectable/ metastatic melanoma that is ICB-refractory. Correlative lab studies are ongoing. **(ClinicalTrials.gov Identifier: NCT05764395, RECRUITING)**

Acknowledgements

- **This work was supported by grants from the Lloyd Foundation Melanoma Research Grant (CY), NCI R01-CA116021 (CY, AR), the Department of Veterans Affairs SRCS Award IK6BX005225 (AR) and the MERIT Award 101BX002301 (AR), as well as the VICC Cancer Center Support Grant, P30 CA068485 (AR), for Core Facilities.**
- **Rigosertib was kindly provided by Onconova Therapeutics, Newtown, PA 18940.**