Rigosertib, a Ras mimetic, inhibits melanoma cell viability and synergizes with anti-PD1 to promote anti-tumor immune responses

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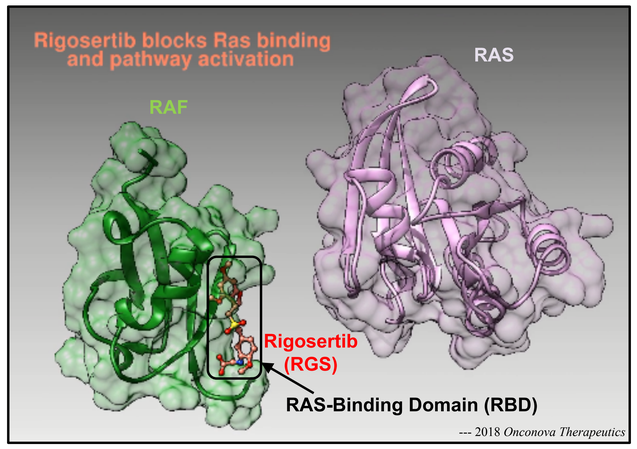
Abstract

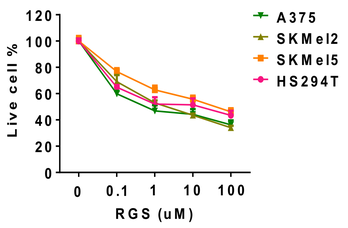
Activating mutations in BRAF or NRAS are present in 40% and 21% of melanoma patients, respectively, leading to enhanced cell survival and proliferation. **Rigosertib (RGS)** is a non-ATP-competitive small molecule RAS mimetic that has the potential to block RAS-RAF-MEK-ERK and PI3K-AKT-mTOR signaling pathways and interfere with CRAF interaction with PLK1 and consequently its centrosomal localization.



Here, we demonstrate that RGS inhibits the cell viability at μM levels of human (including A375/SKMel2/SKMel5/HS294T) and murine (including B16F10 and YUMM2.1/3.3/4.1/5.2/10.1) melanoma cell lines with a variety of somatic mutational backgrounds. We discovered that RGS treatment immediately (<15mins) and constantly (up to 24hrs) suppresses PI3K-AKT^{T308} and mTORC2-AKT^{Ser473} phosphorylation. Using the murine melanoma cell line YUMM3.3 (Braf$^{V600E/wt}$ Cdkn2$^{-/-}$), we showed that RGS monotherapy elevated the production of mitochondrial reactive oxygen species, promoted cellular apoptosis, suppressed mitosis in vitro, and inhibited tumor growth in C57BL/6 mice. The optimal in vivo dose of RGS (300mg/kg), which exhibited >50% inhibition of tumor volume and tumor weight, was well tolerated in mice. RGS-treated tumors exhibited an inflammatory tumor microenvironment (TME) with enrichment of dendritic cells and CD45^{-}MHCII^{+} cells, elevation in frequency and activation of both CD4^{+} and CD8^{+} T cells and NK cells, but a decrease in the level of tumor-infiltrating macrophages. Of note, treatment with RGS plus αPD-1 checkpoint blockade synergistically inhibited tumor growth by ~70%. The RGS + αPD-1 combination treatment, but not the monotherapies, reduced the frequency of exhausted PD-L1^{+}LAG3^{+}TIM3^{+} CD8^{+} T cells at the tumor sites, as well as in the tumor-draining lymph nodes. Conclusion: These results suggest that RGS, which is a Ras mimetic, may be used in combination with anti-PD-1 immunotherapies to enhance anti-tumor immunity and optimize the treatment of melanoma. This combination therapy warrants a clinical study.

RGS inhibits human melanoma cell viability *in vitro*

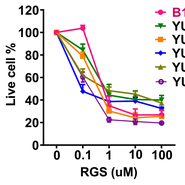
CellTiter-Blue Viability Assay



Melanoma	NRAS (21%)	BRAF (40%)	PTEN (12%)	TP53 (16%)	CDKN2A (43%)	IC50 (uM)
HS294T	WT	V599E	-	++ WT	-	29.12
SKMel5	WT	V599E	+ WT	++ WT	-	60.05
SKMel2	Q61R	WT	+ WT	+++ G245S	+	3.85
A375	WT	V600E	+ WT	+ WT	+	7.06

RGS inhibits murine melanoma cell viability and induces ROS production and apoptosis *in vitro*

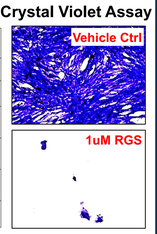
CellTiter-Blue Viability Assay

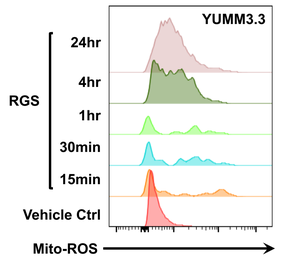


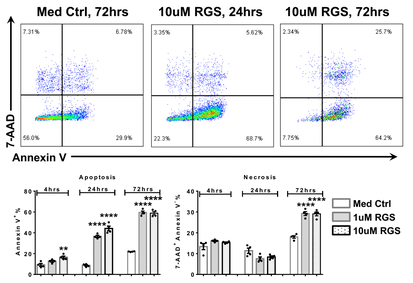
	IC50 (uM)
B16F10	1.416
YUMM4.1	4.184
YUMM5.2	0.6317
YUMM2.1	0.01693
YUMM3.3	1.782
YUMM10.1	0.1468

	NRAS (21%)	BRAF (40%)	PTEN (12%)	TP53 (16%)	CDKN2A (43%)
B16 F10	WT	WT	WT	WT	-/-
YUMM 4.1	WT	WT	-/-	WT	-/-
YUMM 5.2	WT	V600E	WT	-/-	WT
YUMM 2.1	WT	V600E	-/-	WT	+/-
YUMM 3.3	WT	V600E	WT	WT	-/-
YUMM10.1	Q61R	WT	WT	-/-	WT

Crystal Violet Assay

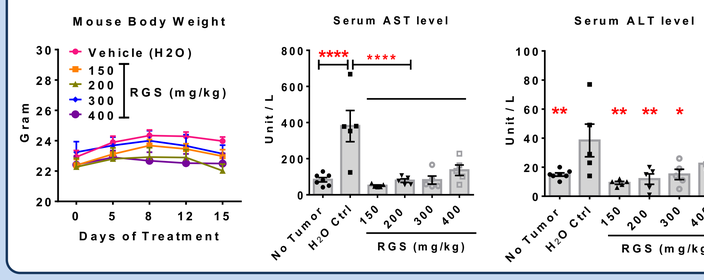




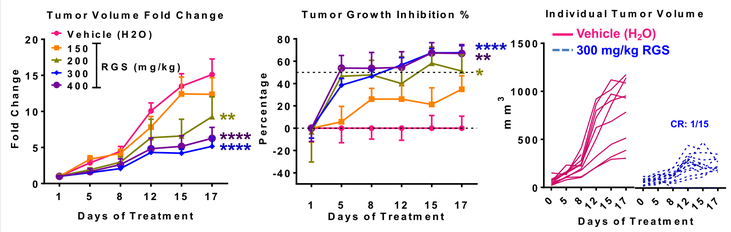


Dosage and toxicity of RGS *in vivo*

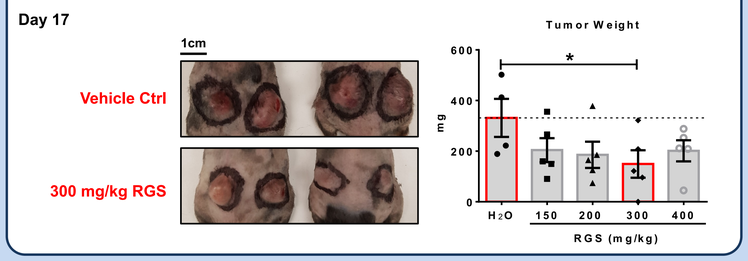
YUMM3.3 cells in C57BL/6 mice



RGS inhibits tumor growth *in vivo*

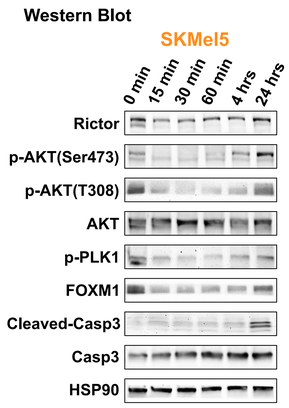


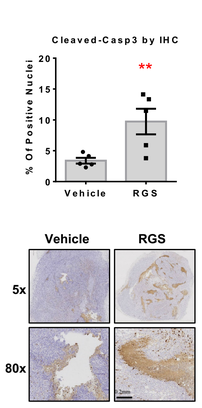
Day 17

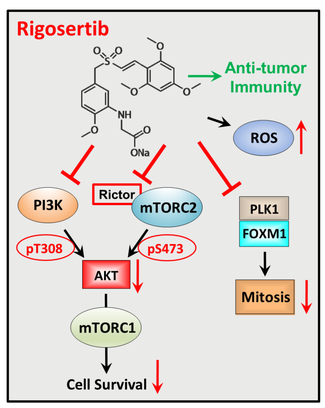


RGS inhibits PI3K/mTOR/AKT and cancer cell survival

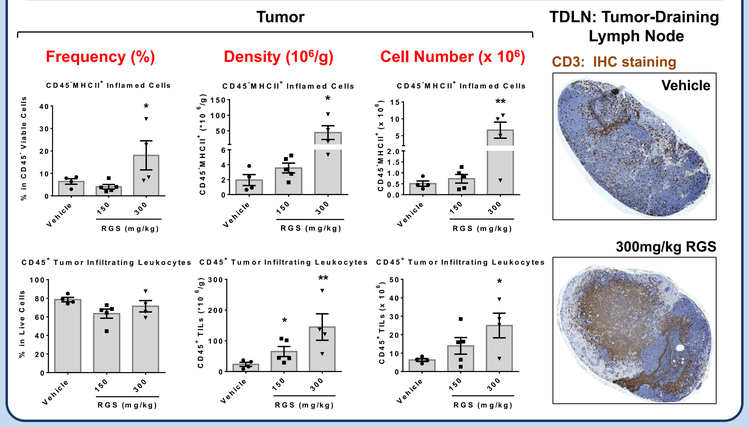
Western Blot





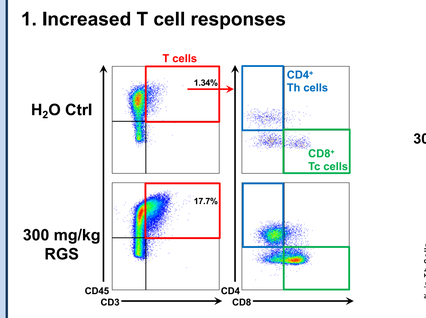


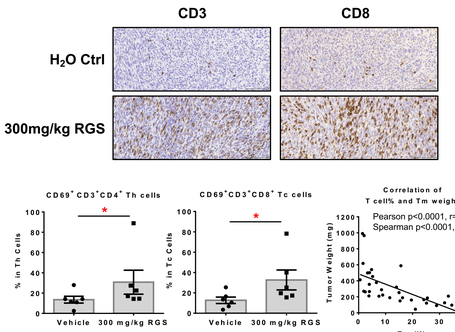
RGS turns the cold tumor/LN hot (immunogenic)



RGS modulates immune responses in the TME

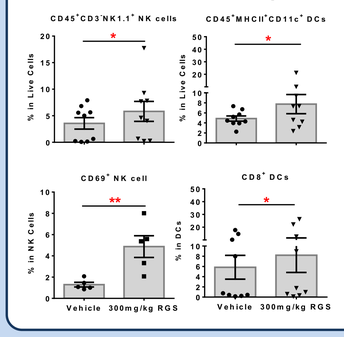
1. Increased T cell responses



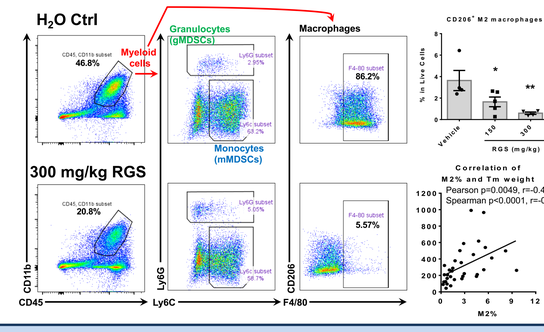


Th cells: CD4^{+} T helper cells; Tc cells: CD8^{+} Cytotoxic T cells

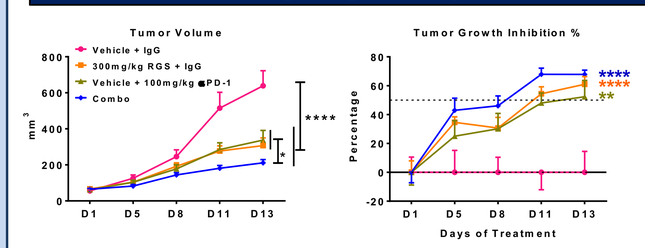
2. Increased NK and DC responses

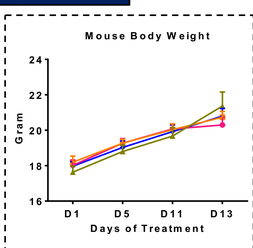


3. Reduced tumor-associated macrophages (TAMs)

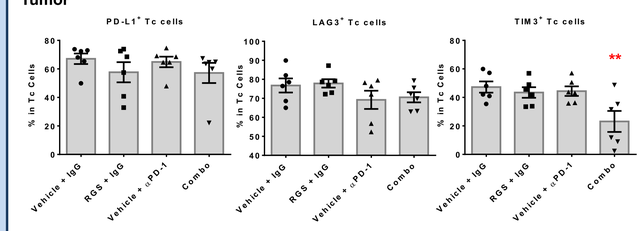


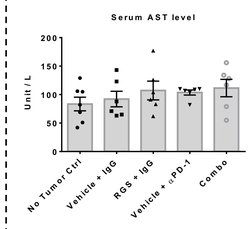
RGS and αPD-1 synergistically inhibit tumor growth



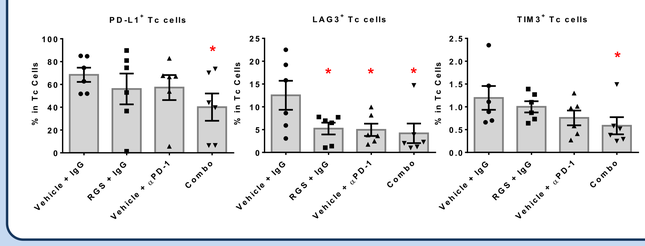


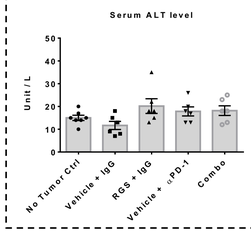
Tumor





TDLN





Summary of key findings

***In vitro*, RGS will:**

1. Inhibit melanoma cell viability;
2. Suppress PI3K/AKT/mTOR and PLK1 activities;
3. Induce ROS-dependent cytotoxicity;
4. Induce cellular apoptosis.

***In vivo*, RGS turns the cold tumor/LN hot (immunogenic):**

1. Promotes T cell frequency in the TDLNs;
2. Increases MHCII^{+}CD45^{-} inflamed cells;
3. Increases the frequency and activation of T cells and NK cells;
4. Reduces the frequency of myeloid cells (e.g., TAMs);
5. Increases the frequency of DCs, especially CD8^{+} DCs.

***In vivo*, RGS + αPD-1 reduces the frequency of exhausted PD-L1^{+}LAG3^{+}TIM3^{+} Tc cells**

Acknowledgements

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