**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/SKMel28/SKMel5/H5294T) and murine (including B16F10 and YUMM2/3.3.4.1/5.2/10.1) melanoma cell lines with a variety of somatic mutational backgrounds. We discovered that RGS treatment immediately (<15mins) and constantly (up to 24hrs) suppresses PI3K-AKT⁷³⁸ and mTORC2-AKT焐焐 phosphorylation. Using the murine melanoma cell line YUMM3.3 (Braf⁷³⁸⁶R), 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. RGS-treated tumors exhibited an inflammatory tumor microenvironment (TME) with enrichment of dendritic cells and CD4⁵ CD8⁵ 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 aPD-1 checkpoint blockade synergistically inhibited tumor growth by >70%. The RGS + aPD-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**

**RGS modulates immune responses in the TME**

1. Increased T cell responses
2. Increased NK and DC responses
3. Reduced tumor-associated macrophages (TAMs)

**RGS and aPD-1 synergistically inhibit tumor growth**

**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. Inhibits T cell frequency in the TDLNs;
2. Increases MHCII⁺ and CD4⁺ 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.

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