CETSA profiling unveils novel targets engaged by anti-tumor drug rigosertib to inhibit RAS-MAPK signaling and trigger NLRP3 inflammasome activation

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Abstract

- Rigosertib originally described as a non-ATP-competitive inhibitor of Polo-like kinase 1 (PLK1)
- Induces mitotic arrest and inhibits cancer cells growth
- Disruptor of multiple signaling pathways including RAS-MAPK signaling through multiple mechanisms
- Rigosertib is in the late stage of clinical development for treatment of many cancers
- Challenge: Although RAS/RAF/MEK signaling inhibition by rigosertib contributes to its effect on tumor cells, the upstream target of rigosertib remains unknown
- Aim: Decipher the molecular mechanism responsible for the antitumor properties of rigosertib and to consolidate the mechanism of action by identifying unknown targets in cancer cells

Rigosertib does not compete with RAS for binding to CRAF-RBD

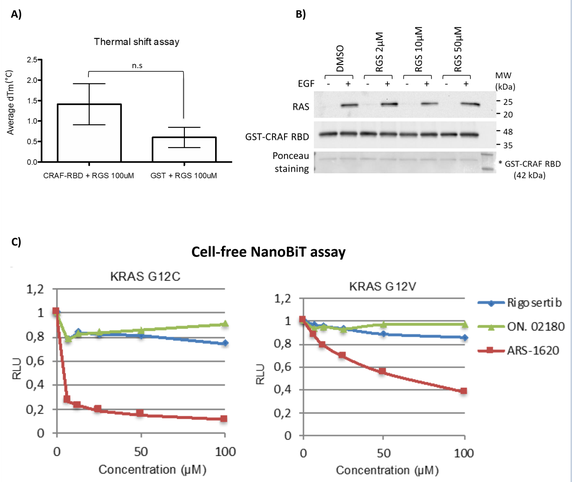


Figure 1. (A) Difference of denaturation temperature in presence of rigosertib 100 µM compared to DMSO assessed by Thermal Shift Assay for purified GST-CRAF RBD and GST control. **(B)** Competition assay between RGS and active RAS from HeLa cell lysates for binding to purified GST-CRAF RBD. **(C)** A cell-free NanoBiT assay in lysates of HEK-293T transfected with SmBiT-CRAF RBD and either LgBiT-KRAS G12V or G12C. Cell lysates were incubated for 4 h with increasing concentrations of ARS-1620, rigosertib or ON02180.Na.

Rigosertib inhibits indirectly RAS-MAPK signaling

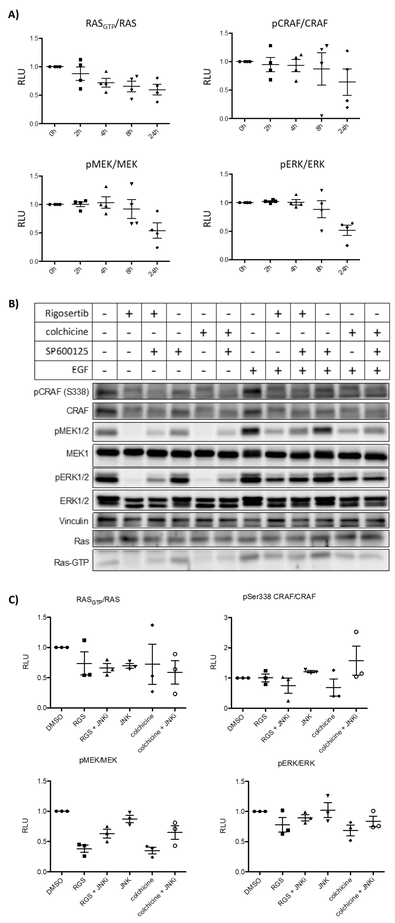
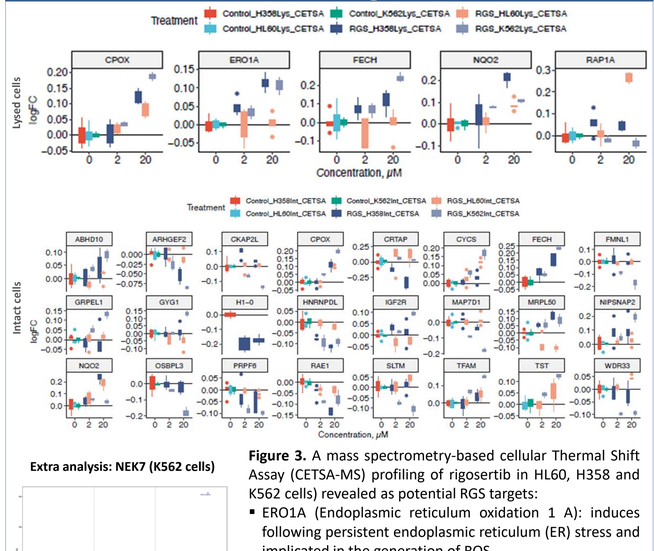


Figure 2. (A) Quantifications of HeLa cells serum starved and treated with rigosertib 2 µM for EGF-stimulated cells relative to DMSO. **(B)** HeLa cells treated for 18 h with DMSO, rigosertib (RGS, 2µM), colchicine (100 nM), or SP600125/JNKi (10 µM) in serum-free DMEM then stimulated with EGF at 100 ng/ml for 5 min. **(C)** Quantification of (B) for EGF-stimulated cells relative to DMSO.

CETSA-MS profiling of rigosertib – Identification of cellular targets



Extra analysis: NEK7 (K562 cells)

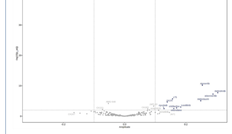


Figure 3. A mass spectrometry-based cellular Thermal Shift Assay (CETSA-MS) profiling of rigosertib in HL60, H358 and K562 cells) revealed as potential RGS targets:

- ERO1A (Endoplasmic reticulum oxidation 1 A): induces following persistent endoplasmic reticulum (ER) stress and implicated in the generation of ROS
- NQO2 (NRH: quinone oxidoreductase 2): detoxifying enzyme which is involved in ROS generation
- NEK7 (NIMA Related Kinase 7): involves in microtubules organization (mitotic cell cycle progression) and NLRP3 inflammasome activation

Rigosertib inhibits RAS-MAPK signaling through ROS induced activation of JNK signaling

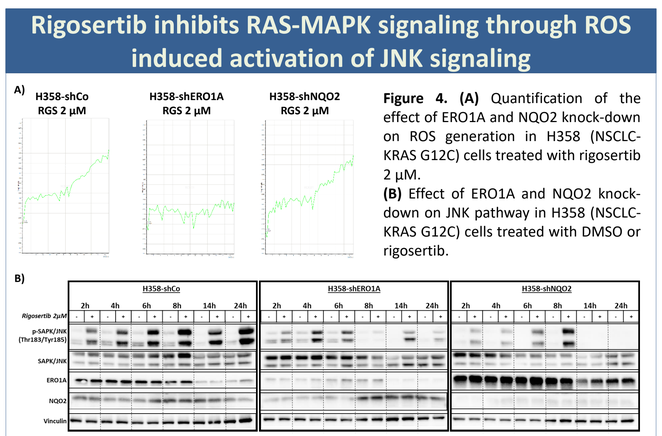


Figure 4. (A) Quantification of the effect of ERO1A and NQO2 knock-down on ROS generation in H358 (NSCLC-KRAS G12C) cells treated with rigosertib 2 µM.
(B) Effect of ERO1A and NQO2 knock-down on JNK pathway in H358 (NSCLC-KRAS G12C) cells treated with DMSO or rigosertib.

Rigosertib affects microtubule polymerization

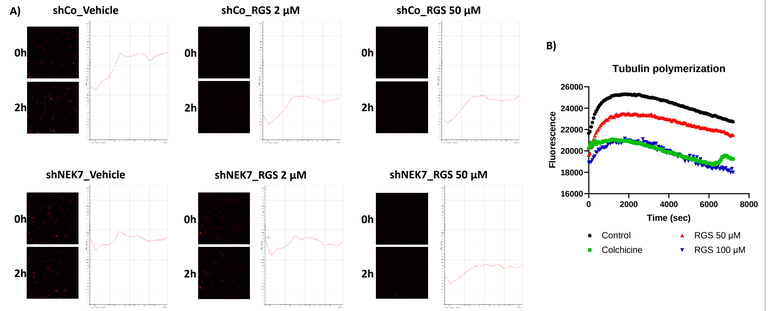


Figure 5. (A) Cell images and quantification of the effect of NEK7 knock-down on the fluorescent staining of polymerized tubulin in H358 (NSCLC-KRAS G12C) cells treated for 2 hours with the indicated concentrations of rigosertib (RGS).
(B) In vitro tubulin polymerization assay in presence of colchicine (3 µM) or indicated concentration of rigosertib.

Rigosertib activates Caspase 1 activity and inflammasome to trigger IL-1β and IL-18 secretion

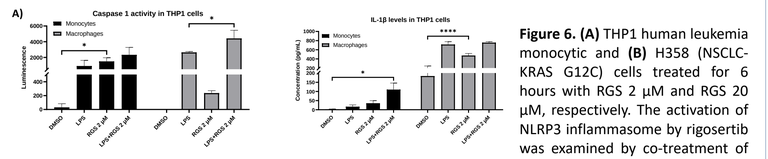
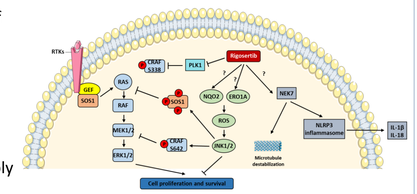


Figure 6. (A) THP1 human leukemia monocytic and **(B)** H358 (NSCLC-KRAS G12C) cells treated for 6 hours with RGS 2 µM and RGS 20 µM, respectively. The activation of NLRP3 inflammasome by rigosertib was examined by co-treatment of H358 cells with 10 µM of MCC950, a selective inhibitor of NLRP3 inflammasome, and Tranilast, an antiallergic drug with inhibitory effect on NLRP3 inflammasome. IL-1β and IL-18 levels were detected in the cells supernatant by ELISA.

Conclusions

a) Rigosertib indirectly inhibits RAS-MAPK signaling through ROS induced activation of JNK signaling.
b) CETSA-MS profiling revealed NQO2, ERO1A and NEK7 as potential rigosertib targets.
c) Rigosertib might affect microtubules polymerization, possibly through NEK7 activation.
d) Rigosertib increases ROS generation, possibly through ERO1A activation.
e) Rigosertib activates inflammasome to trigger IL-1β and IL-18 secretion.



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Acknowledgements

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