Discovery of A Highly Potent HPK1 Inhibitor That Augments Immune Activation and Anti-tumor Immunity

Hao Liu[1], Lei Wu[2], Song Feng[2], Wei Huang[2], Huijuan Li[2], Jing Lin[2], Yangyang Liu[2], Liufeng Mei[2], Baoqi Ren[2], Julie Xie[1], Lili Yao[2], Wenge Zhong[2]
[1] Regor Pharmaceuticals Inc., 245 Main Street, Second Floor, Cambridge, MA 02142, USA
[2] Regor Therapeutics Inc. 1206 Zhangjiang Road, Building C, Pu Dong New District, Shanghai 201210, China

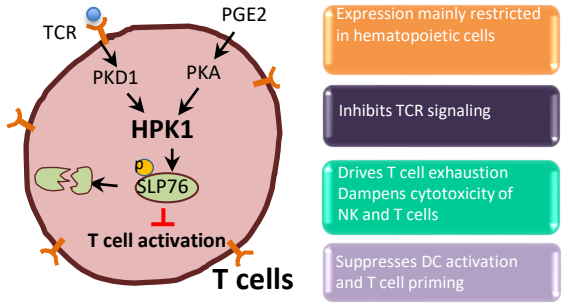
Background

HPK1 (MAP4K1) is a serine/threonine Ste20-related protein kinase that belongs to the mitogen-activated protein kinase (MAPK) family. HPK1 is mainly expressed in hematopoietic cells and serves as a negative regulator of anti-tumor immunity through modulating the activation of lymphocytes and dendritic cells. Upon TCR activation, HPK1 phosphorylates the adaptor protein SLP76 at Ser376 to destabilize the SLP76 microclusters, which leads to the attenuation of the TCR signaling. The reported anti-tumor efficacy data from HPK1 knockout and kinase-dead knock-in mouse models support HPK1 as a novel intracellular I/O target.

Methods

Regor CARD platform (Computer Accelerated Rational Discovery) was deployed to identify potent and selective inhibitors of HPK1. Biochemical assays and primary human pan T cell-based cellular assays were utilized to support the structure-activity relationship (SAR) analysis and inhibitor optimization. *In vitro* and *in vivo* target engagement studies were conducted in Jurkat T cells and mouse splenocytes, respectively. *In vivo* efficacy study data were generated using CT-26 syngeneic tumor mouse model.

Figure 1. HPK1 serves as immune checkpoint

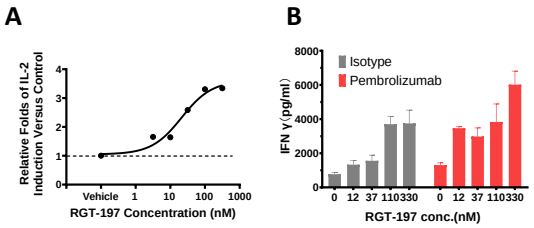


Results

Table 1. RGT-197 is a subnanomolar HPK1 inhibitor with selectivity against immune liable kinases and super cellular activity

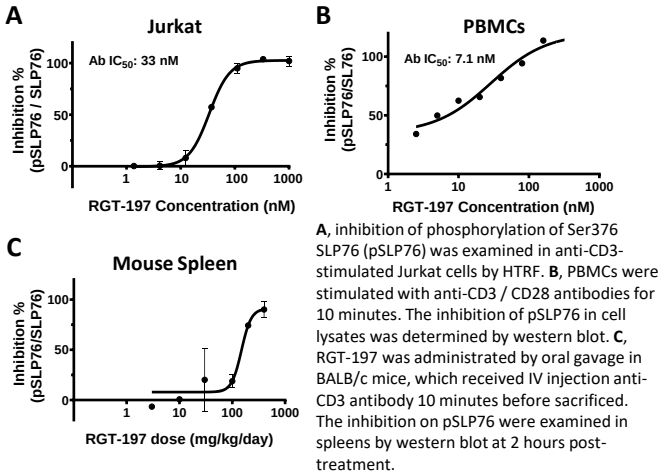
	RGT-197
HPK1 Abs IC_{50} (nM)	0.23
Human primary pan T cells IL-2 EC_{50} (nM)	19
IEC6 cytotoxicity IC_{50} (mM)	3.1
Selectivity against HPK1 IC_{50} (Folds)	
JAK3	>100
LCK	>500
PKC-theta	>100
ZAP70	>10000
TBK1	>10000

Figure 2. RGT-197 enhances T cell activation *in vitro*



A, anti-CD3 / CD28 stimulated human primary pan T cells were treated with RGT-197. IL-2 levels were examined in supernatant after 48 hours. **C**, anti-CD3 stimulated PBMCs were incubated with RGT-197 in the presence of 2.5 μg/ml anti-PD-1 or isotype antibody. IFN-g levels were analyzed after 96 hours.

Figure 3. RGT-197 inhibits TCR Activation-induced pSLP76 *In vitro* and *In vivo*



A, inhibition of phosphorylation of Ser376 SLP76 (pSLP76) was examined in anti-CD3-stimulated Jurkat cells by HTRF. **B**, PBMCs were stimulated with anti-CD3 / CD28 antibodies for 10 minutes. The inhibition of pSLP76 in cell lysates was determined by western blot. **C**, RGT-197 was administrated by oral gavage in BALB/c mice, which received IV injection anti-CD3 antibody 10 minutes before sacrificed. The inhibition on pSLP76 were examined in spleens by western blot at 2 hours post-treatment.

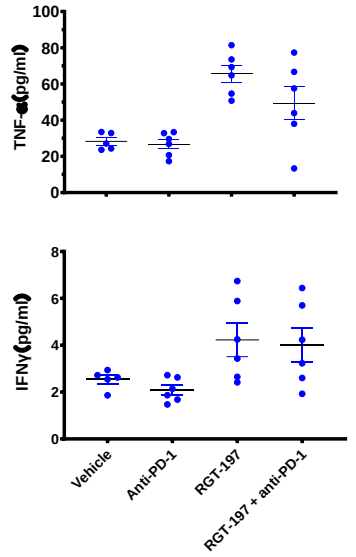
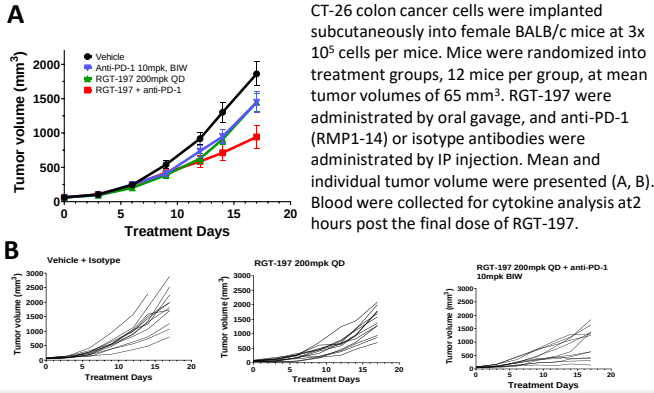


Figure 4. RGT-197 delays tumor progression in CT26 syngeneic mouse model



CT-26 colon cancer cells were implanted subcutaneously into female BALB/c mice at 3x 10^5 cells per mice. Mice were randomized into treatment groups, 12 mice per group, at mean tumor volumes of 65 mm^3. RGT-197 were administrated by oral gavage, and anti-PD-1 (RMP1-14) or isotype antibodies were administrated by IP injection. Mean and individual tumor volume were presented (A, B). Blood were collected for cytokine analysis at2 hours post the final dose of RGT-197.

Conclusion

RGT-197, a potent and selective HPK1 inhibitor, provides a potential opportunity as a small molecule immunotherapy to boost anti-tumor immunity either as monotherapy or in combination with immune checkpoint inhibitors.

Regor Therapeutics Inc.