Background Hematopoietic progenitor kinase 1 (HPK1, MAP4K1) is emerging as a well-renowned, druggable target for T cell-based immunotherapies. HPK1 is a member of the serine/threonine MAP4K family, predominantly expressed in hematopoietic cell lineages and shown to be a negative regulator of the T cell receptor (TCR) signaling pathway. Upon TCR activation, HPK1 is recruited to the proximity of the cell membrane and phosphorylates an adaptor protein SLP-76 at the Ser376 residue which, in turn, abrogates TCR signaling. Other studies point to a potential role of HPK1 in T cell exhaustion as well as in functional re-programming of regulatory T cells. Moreover, mounting evidence suggest that HPK1 kinase activity suppresses the immune functions of a wide range of other immune cell subsets like B cells and dendritic cells. Taken together, these observations support small-molecule HPK1 inhibitors as an attractive modality in cancer immunotherapy either as single agents or in combination with immune checkpoint inhibitors.

Methods Activity of compounds against HPK1 and selected off- and anti-targets was assessed in biochemical assays. Phosphorylation of SLP-76 was measured either by flow cytometry or TR-FRET. Jurkat and primary T cells were activated and cultured in the presence of tested compounds and immunosuppressive agents. Impact on TCR selectivity and T cell function was measured by AlphaLISA and flow cytometry. Target engagement was measured in splenocytes of mice administered orally with tested compounds followed by IP injection of aCD3 antibody. Anti-tumor efficacy of HPK1 inhibitors was assessed in a syngeneic tumor model.

Results Ryvu's proprietary small molecule HPK1 inhibitors exhibit sub-nanomolar activity against human and mouse HPK1 proteins and good selectivity against other TCR pathway kinases. Tested compounds efficiently block phosphorylation of SLP-76 upon TCR engagement. TCR selectivity of Ryvu's inhibitors, measured as a ratio between CD69 and pSer376 SLP-76 inhibition, is on par or superior to reference molecules. Tested compounds are not only able to overcome PGE-2 induced resistance following TCR activation in human PBMCs, inducing elevated IL-2 release but also affect T cell function in co-culture assay. Developed molecules have favorable PK profiles, allowing for sustained target coverage in proposed dosing regimens and demonstrate efficacy in a mammary carcinoma syngeneic model.

Conclusions Ryvu has developed potent and selective HPK1 inhibitors with favorable PK and PD profiles, whose activity in vitro translates to in vivo efficacy. Further preclinical work is warranted to select a lead candidate for IND-enabling studies and subsequently clinical studies across a variety of solid tumors.

http://dx.doi.org/10.1136/jitc-2021-SITC2021.752