HZ-S506, A SELECTIVE AND ORALLY BIOAVAILABLE HPK1 DEGRADER, IS EFFICACIOUS AS A SINGLE AGENT OR IN COMBINATION WITH PD-1 ANTIBODY

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Background Hematopoietic Progenitor Kinase 1 (HPK1/MAP4K1) has been demonstrated to restrain T cell activation through phosphorylation of SLP-76 at Serine 376 leading to TCR signal blocking. The negative feedback role of HPK1 in TCR signaling makes it a promising target for immunooncology therapy. Several HPK1 inhibitors have entered into phase 1 clinical trial. However, the HPK1 selectivity against other MAP4Ks family protein is still challenging, which may lead to the weakening of HPK1-targeted therapy. Recently, PROteolysis TAargeting Chimera (PROTAC) is a bifunctional molecule that can bring the target protein and E3 enzyme closer for ubiquitination labeling, and the target protein can be degraded by UPS system. Therefore, in theory, it can reduce the generation of drug resistance and avoid the problem of off target inhibition to further enhance subtype selectivity.

Methods HZ-S506 was discovered based on our team’s unique DaTProD® platform. Western blot and HPK1 biochemical assay were used to analyze HPK1 degradation and inhibition. T cell activation and SLP-76 phosphorylation were also conducted. The in-vivo anti-tumor efficacy was assessed in C57BL/6 mice which were engrafted with CT-26 cell line.

Results We found that HZ-S506 potently inhibited HPK1 kinase activity (IC50 = 4.6 nM) under Km concentration of ATR and it also had good selectivity against other MAP4K family members. HZ-S506 catalyzed the degradation of HPK1 in Jurkat and PBMC (DC50 < 10 nM). HZ-S506 treatment of Jurkat T cell demonstrated robust HPK1 degradation without significant downregulation of other off-target proteins in the proteomics experiment. HZ-S506 had stimulatory effect on immune response by increasing in IL-2 production (EC50 = 279.1 nM) in Jurkat cell with stimulation with anti-CD3 and anti-CD28, which was more significantly more potent. Treatment with HZ-S506 also can overcome PGE2 mediated immune-suppression. Interestingly, HZ-S506 possessed excellent pharmacokinetic properties, the exposure of which is higher than its parent inhibitor (~2 folds). In efficacy studies, HZ-S506 exhibited anti-tumor activity CT26 tumor model as single agent and in combination with an anti-PD1 antibody, demonstrated robust anti-tumor activities of anti-PD1 efficacy in 4T1 and MC38 syngeneic tumor models.

Conclusions In summary, HZ-S506 is a potent and selective degrader of HPK1 with good ADME properties and efficacy. These results support further development of HZ-S506 as a single-agent or combinational therapy with the current checkpoint inhibitors.

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Ethics Approval All animal studies were performed in strict accordance with the institutional guidelines as defined by the Institutional Animal Care and Use Committee (IACUC), approved by the Animal Care and Use Committee, Zhejiang University Laboratory Animal Center (Hangzhou, China), approval ID: 20210068. All participants gave informed consent before taking part.