

CHARACTERIZATION OF HZ-G206: A POTENT AND ORAL SMALL MOLECULE PD-L1 INHIBITOR

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Background Blocking of PD-1/PD-L1 protein-protein interaction has been proven as an effective therapeutic strategy in multiple types of cancer. More than ten PD-1 or PD-L1 antibody drugs have been approved worldwide. Contrasting to antibody drug, small molecule PD-L1 inhibitor has shown potential advantages, including increased tissue penetration, less immune-related adverse effect and better clinical compliance. In recent years, several small molecule PD-L1 inhibitors entered clinical stage study. Herein, we described our state-of-the-art work in discovery and identification of HZ-G206, which is a oral small molecule PD-L1 inhibitor and exhibited the potential of best-in-class.

Methods In vitro PD-1/PD-L1 inhibition potency was determined using ALPHAlisa assay. Surface PD-L1 internalization and degradation was carried out in hPD-L1-MC38 cell line and human PBMC through flow-cytometry. Immune-activation effect after PD-1/PD-L1 inhibition was evaluated in NFAT-reporter assay and cytokine production assay. The in-vivo anti-tumor efficacy was assessed in C57BL/c mice which were engrafted with hPD-L1-MC38 cell line and the tumor-bearing mice were administrated with different dosage of HZ-G206 followed by monitoring of tumor volume.

Results HZ-G206 binds and strongly blocks PD-1/PD-L1 interaction in in-vitro ALPHAlisa assay with the IC₅₀ value of 0.35nM. Binding of HZ-G206 in cell surface PD-L1 results in dimerization and destabilization of PD-L1 and followed by internalization and degradation in endosome, which is a distinct pharmacology effect comparing with traditional PD-L1 mAb drugs. HZ-G206 shows stronger PD-L1 internalization and degradation potency than clinical stage compounds INCB86550 and INCB99318 in hPD-L1-MC38 cell line and IFN- γ stimulated human PBMC with IC₉₀ values of 18.1nM and 105.6nM respectively. HZ-G206 also exhibits enhanced T-cell activation effect after stimulation with CD3/CD28 in presence of PD-L1 overexpressed cells. In in-vivo anti-tumor evaluation, oral administration of HZ-G206 BID can significantly suppress the growth of tumor in a dose dependent manner, the TGI of middle dose group is comparable to Atezolizumab. In the end of experiment, the tumor tissue was also collected. The PD-L1 level in tumor cell and T cell infiltration were analyzed by flow-cytometry. For the mice treated with HZ-G206, the MFI of PD-L1 is significantly decreased. The ratio of CD3+T cells in tumor tissue is enhanced which is in accordance with Atezolizumab group.

Conclusions HZ-G206 is a novel small molecule PD-L1 inhibitor with good drug-like prosperities. The compound is identified with potent in-vitro activity which translates to anti-tumor efficacy in pre-clinical animal study. In conclusion, HZ-G206 is an excellent drug candidate for further clinical development.

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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: 19885. All participants gave informed consent before taking part.

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