DUAL TARGETING OF INNATE AND ADAPTIVE IMMUNE CHECKPOINTS WITH A PD-L1/SIRPα BISPECIFIC MACROPHAGE ENAGER TO PROMOTE ANTI-TUMOR ACTIVITY

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**Background** Tumor-associated macrophages are major components of immune cells in the tumor micro-environment that express an array of effector molecules leading to the inhibition of anti-tumor immune responses. Signal regulatory protein α (SIRPα) is a myeloid-lineage inhibitory receptor that restricts phagocytosis through engagement of its ligand CD47 expressed on tumors and normal tissues. Compared to anti-CD47 therapeutics, targeting myeloid-restricted SIRPα may provide a differential pharmacokinetic, safety, and efficacy profile. Here, we report the construction of a SIRPα antagonist-based bispecific macrophage engager (BiME) called ES019, which uses PD-L1 antibody as a tumor associated antigen (TAA) targeting arm and also as a tool to relieve the inhibition of T cell. The PD-L1/SIRPα bispecific macrophage engager aims to promote macrophage phagocytosis against PDL1 expressing tumor cells, and to activate T cell adaptive immunity resulting in further tumor cell killing.

**Methods** Through Elpiscience proprietary BiME platform, we have generated a panel of single domain antibody (sdAb) based anti-PDL1/SIRPα bispecific antibodies, including different orientations, ratios, and IgG isotypes of anti-PDL1 arm and anti-SIRPα arm. These bispecific antibodies were evaluated for PDL1, SIRP family homologue binding, PD1-PDL1 and CD47-SIRPα blocking properties by ELISA and FACS. In vitro function activity was determined by phagocytosis assay using human monocyte derived macrophage and mouse bone marrow derived macrophage. In vivo anti-tumor efficacy was tested in a syngeneic tumor model with hSIRPα knock-in mice. The pharmacokinetic (PK) and safety profile were assessed in hSIRPα knock-in mice or cynomolgus monkeys.

**Results** In this study, we demonstrated that anti-PDL1/SIRPα bispecific antibodies bound to PD-L1 expressing tumor cell and macrophage simultaneously, effectively inhibited CD47-SIRPα signal and triggered strong macrophage phagocytosis. ES019 showed potent in vitro macrophage and T cell tumor killing activity in the presence of peripheral blood mononuclear cells, but without nonspecific killing in PDL1 negative cells. Remarkably, ES019 showed almost 100% tumor growth inhibition in in vivo SIRPα knock-in syngeneic models. On the other hand, ES019 did not induce phagocytosis of normal immune cells like activated T cells. In summary, we demonstrated that ES019 exhibited super anti-cancer effects, evidenced by potent phagocytosis in vitro and almost complete tumor regression in vivo.

**Conclusions** Using our bispecific macrophage engager (BiME) platform, we have developed a PD-L1/SIRPα bispecific antibody that is capable of activating both macrophages and T cells to kill cancer cells with the potential to overcome the limitations of traditional anti-PD1 therapies.