Immune-Stimulants and Immune Modulators

1056 ZG033, A NOVEL CD137 AGONIST, INDUCES SUPERIOR ANTI-TUMOR ACTIVITY WITHOUT HEPATOTOXICITY RELY ON FC-MEDIATED CROSSLINKING

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Background CD137 (TNFRSF9, 4-1BB) is a member of the tumor necrosis factor receptor superfamily that functions as a costimulatory molecule of immunocytes. Agonistic antibodies against CD137 have shown promising therapeutic activity in mouse tumor models. However, molecules in clinical development have shown limitations due to either dose-dependent severe liver toxicity or modest antitumor activity. We developed ZG033, a fully human IgG4 agonist of CD137 that engages with an exclusive epitope, to achieve a better efficacy and safety profile for immunotherapy.

Methods The biophysical properties and activities were determined using multiple in vitro assays, including enzyme-linked immunosorbent assay (ELISA), surface plasmon resonance (SPR), cell-based and reporter gene assays. In vivo antitumor activities were assessed in human 4-1BB transgenic mice transplanted with human colon cancer cell line MC38. The pharmacokinetic (PK) behavior and safety profiles of ZG033 were characterized in cynomolgus monkeys.

Results ZG033 is a safe and potent antibody injection associated with a differentiated pharmacology and toxicology profile. The structure of the ZG033/CD137 complex was determined by X-ray crystallography. The Fab of ZG033 binds CD137 at an obvious competitive epitope with the CD137 ligand, which differs from the currently known agonist antibodies of CD137. The residues T61A and I64R play a vital role in the formation of the complex, which was demonstrated by affinity assays. The binding affinity to human 4-1BB of ZG033 determined by surface plasmon resonance (SPR) was moderate (KD=10 nM). ZG033 increased CD137-driven NFκB reporter gene activation and the production of IFN-γ by human T cells in an FcγR-dependent manner. In human 4-1BB transgenic mice, ZG033 showed robust single-agent antitumor activity and induced durable antigen-specific immunological memory that prevented the tumor formation in the rechallenged mice. To determine the safety of ZG033, we incubated ZG033 with peripheral blood mononuclear cells (PBMCs) from healthy donors (n=4) and proved that it does not induce nonspecific production of proinflammatory cytokines. The results in 5-week I.V. repeated-dose (3, 10 and 30 mg/kg) and single-dose toxicity studies (60 and 180 mg/kg) suggested that ZG033 was well tolerated in cynomolgus monkeys with no abnormal hepatic or renal function and hematological index.

Conclusions These data demonstrate that ZG033 acts as an FcγR crosslinking-dependent CD137 agonist that displays a favorable safety profile and has potential in either mono- or combinational immunotherapies.