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Preclinical Efficacy and Immunological Safety of FR104, an Antagonist Anti-CD28 Monovalent Fab' Antibody

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Antagonist anti-CD28 antibodies prevent T cell costimulation and differentiate from CTLA4Ig since they cannot block CTLA-4 and PDL-1 coinhibitory signals. They demonstrated efficacy in suppressing effector T cells while enhancing regulatory T cells function and immune tolerance. However, anti-CD28 antibodies devoid of immunotoxicity and with a good pharmacokinetic profile have not yet been developed. Here, we describe FR104, a novel humanized pegylated anti-CD28 Fab' antibody fragment presenting a long elimination half-life in monkeys. *In vitro*, FR104 failed to induce human T cell proliferation and cytokines secretion, even in the presence of anti-CD3 antibodies or when cross-linked with secondary antibodies. Furthermore, in humanized NOD/SCID mice adoptively transferred with human PBMC, whereas superagonist and divalent antibodies elicited rapid cytokines secretion and human T cell activation, FR104 did not. These humanized mice developed a florid graft-versus-host disease, which was prevented by administration of FR104 in a CTLA4-dependent manner. Interestingly, administration of high doses of CTLA4-Ig was ineffective to prevent GVHD, whereas administration of low doses was partially effective. In conclusion, we demonstrated that FR104 is devoid of agonist activity on human T cells and thus compatible with a clinical development that might lead to higher therapeutic indexes, by sparing CTLA-4, as compared to CD80/CD86 antagonists.

Key words: CD28, costimulation, CTLA-4, GVHD, immunotoxicity

Abbreviations: CNI, calcineurin inhibitor; ED50, effective dose 50%; IC50, inhibitory dose 50%; GVHD, graft versus host disease; iTreg, inducible regulatory T cell; MLR, mixed lymphocyte reaction; NOD/SCID, nonobese diabetic severe combined immunodeficiency; PBMC, peripheral blood mononuclear cells; TCR, T cell receptor; PEG, polyethylene glycol.

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Introduction

T cells are a major culprit in the immune response directed against self-antigens or transplanted organs, therefore a number of immunosuppressive drugs target these cells, and certain newer approaches target the process by which T cells become activated. This process is induced by antigen binding to the T cell receptor and reinforced by costimulatory molecules—CD80 and CD86—binding to the T cell CD28 receptor. CD80/86 can bind also to the CTLA-4 receptor, resulting in T cell inhibition. CTLA-4 is also required for the function of regulatory T cells (Tregs) (1), which suppress aberrant immune responses and are important for inducing tolerance toward transplanted tissue (2). CTLA-4 is also required for the induction of peripheral T cell tolerance to soluble antigens (3–6), tumors (7) and allografts (8–10). Further, selective agonistic ligation of CTLA-4 attenuates *in vivo* T cell responses and prevents development of autoimmunity (11,12), while the absence (1,13) or blockade (14–16) of CTLA-4 is associated with autoimmunity.

Targeting the CD28–CD80/86 pathway in patients with CTLA-4-Ig reagents (Abatacept, Belatacept) is a promising alternative to current immunosuppressive treatments in autoimmunity (17,18) and renal transplantation allowing CNI avoidance (19–21). However, CD80/86-directed blocking strategies may deprive the evolving immune responses of CTLA-4-driven signals crucial to the function of Tregs and do not reproducibly induce transplant tolerance (22,23). Moreover, the recent discovery of the inhibitory interaction between PDL-1 and CD80 (24,25) and the costimulatory interaction between CD28 and ICOSL (26), reinforce the theoretical superiority of targeting CD28 instead of CD80/86. In order to preserve physiological regulatory pathways of the immune system, blocking selectively CD28 without affecting CTLA-4 and PDL-1 might represent a better and long-lasting strategy for modulating immune responses by preventing the maturation of pathogenic effectors while preserving the function of Tregs (27,28). While the function of Tregs would likely be preserved and even enhanced by