A BIFUNCTIONAL TUMOR ACTIVATED IMMUNOMODULATOR (TRACIr) TARGETING PD-L1 AND CD28 IS A POTENT ENHANCER OF T CELL-MEDIATED ANTI-TUMOR ACTIVITY

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Background While PD-(L)1 blocking antibodies have demonstrated unprecedented clinical response rates, most patients fail to respond. Preclinical studies have shown that CD28 costimulatory pathway is essential for effective PD-(L)1 therapy. However, the first phase 1 clinical trial of the CD28 agonistic antibody TGN1412 failed due to an unexpected and rapid systemic proinflammatory cytokine response. To overcome the limitation of PD-(L)1 blockade, toxicity of systemic CD28 agonism, and potential healthy tissue toxicity, we engineered a Tumor Activated Immunomodulator (TRACIr). The TRACIr is a tri-specific protein that contains PD-L1- and CD28-binding domains, an albumin-binding domain that extends circulating half-life, and an inhibitory peptide mask bound to the CD28-binding domain via a tumor protease cleavable linker. Only when tumor-resident proteases cleave the TRACIr and enable mask separation can the resulting active molecule bind CD28. This cleavage-dependent CD28 agonism can potentially limit systemic toxicity while enhancing the activity of T cells in the tumor.

Methods Peptide masks against the CD28 binding domain were identified via phage display. Mask efficiency was evaluated using CD28-specific ELISAs. The functional engagement of TRACIr binding arms was evaluated in bioluminescent PD-L1/CD28 cell reporter assays. TRACIr-induced T cell activation was evaluated in human PBMC/tumor cell co-culture assays. Functional activity was confirmed in human renal (RCC) and non-small cell lung cancer (NSCLC) patient-derived TILs. In a mouse model of triple-negative breast cancer (TNBC; MDA-MB231), the cleavage-dependent antitumor activity of TRACIr was demonstrated in combination with CD3 stimulation. The pharmacokinetic and safety profile of TRACIr was evaluated in non-human primate studies.

Results Non-masked PDL1xCD28 bispecific molecule exhibited potent binding to PD-L1 (1 nM KD) and CD28 (3 nM KD). While the presence of the mask decreased binding to CD28 by >1,000x, PD-L1 binding remained unaffected. PD-L1 blocking activity was comparable with atezolizumab, avelumab, nivolumab, and pembrolizumab. In contrast, CD28 agonistic activity was significantly compromised by the presence of the mask. Moreover, the TRACIr enhanced activation of peripheral blood T cells and TILs was signal 1- and cleavage-dependent and superior in magnitude compared to anti-PD-(L)1 and anti-CD28 monoclonal antibodies. In the TNBC tumor model, TRACIr dramatically enhanced the antitumor activity of a CD3 targeted bispecific antibody in a cleavage-dependent manner. Finally, TRACIr was well tolerated in NHPs at high doses and exhibited half-life extended pharmacokinetics.

Conclusions Preclinical activity and safety profiles of PDL1xCD28 TRACIr support its further development as an attractive bifunctional T cell modulator.

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