Background Siglec15 (S15) is a glycan-recognition protein belonging to the Siglec family, preferentially recognizing the Neu5Aca2–6GalNAca– structure. Siglec15 is induced by MCSF and is highly expressed on TAM and many tumor cells, which inhibits T cell activity. The detailed mechanism of S15 function on T cells and myeloid cells, however, remains unclear. In this study, we seek to investigate Siglec15 biology and generate anti-Siglec15 antibodies for therapeutic use.

Methods The binding and function of Siglec15 on different cell subsets from healthy donor PBMC were evaluated. Siglec15 antibodies were screened and characterized by antigen binding, ligand blocking assay, rescue of monocyte apoptosis, reversion of monocyte inhibition and PBMC T cell activation assay. Selected leads underwent epitope binning by Bio-layer interferometry (BLI) and mapping by hydrogen deuterium exchange mass spectrometry (HDX-MS), they were then evaluated in MC38/hS15 bearing human S15 knock-in B6/C57 mice for PK profile and in vivo efficacy. Finally, the selected chimeric antibody (ES012) was humanized and undergone preclinical assessment, including potential acute toxicity study in cynomolgus monkeys and the pharmacokinetics (PK) study.

Results S15 expresses on human monocyte cells, M2 macrophage and DC cells but not on M1 macrophage. S15 also mainly binds to myeloid cells (including monocytes, M1 and M2 macrophage and THP-1 cell lines). S15-Fc dose-dependently inhibited OKT3-driven T cell proliferation in a PBMC culture, but not T cell proliferation driven by either PMA plus ionomycin, PHA or CD3/CD28 beads, all of which imply that S15-Fc probably does not directly suppress T cell activity; instead, this T cell inhibitory effect is probably through the inhibition on monocyte. We found that S15 dose-dependently induced the apoptosis of subsets of monocyte and down-regulated the surface expression of CD86, HLA-DR and CD14.

ES012 is a S15 humanized monoclonal antibody with strong blocking activity between S15 and multiple ligands including SiaTn and LRRC4C. ES012 dose-dependently rescued S15-mediated monocyte apoptosis, reversed S15-mediated monocyte inhibition, and rescued S15-mediated T cell inhibition. ES012 exhibited a good PK profile and showed excellent anti-tumor activity in a hS15 KI syngeneic mice model with 100% CR at dose of 5mg/kg.

Conclusions In summary, we found that siglec15 has a major effect on myeloid cells (inducing monocyte apoptosis and inhibiting monocyte activation) and an indirect inhibitory effect on T cell function. Based on this newly discovered S15 biology, we have developed a potent, functional anti-Siglec15 mAb ES012 that has great potential to reverse immune suppression in the TME to promote anti-tumor immunity.