Adenosine and TGFβ are two key immune suppressors in tumor microenvironment ("TME") that cause broad immune suppression resulting in resistance to current CPI immunotherapies. Cancers frequently express transforming growth factor-β (TGFβ), which drives immune dysfunction in the tumor microenvironment by inducing regulatory T cells (Tregs), inhibiting CD8+ activation and infiltration into TME, and promoting epithelial–mesenchymal transition (EMT). We observed that TGFβ inhibits the expression of CD39, a critical enzyme that regulates adenosine generation. CD39 is highly expressed in Tregs within TME, it drives the production of adenosine, an immunoinhibitory molecule that also partly mediates Treg inhibitory function. To inhibit CD39, adenosine and TGFβ simultaneously to create an immune-favorable tumor microenvironment, we designed a bi-specific antibody targeting both CD39 and TGFβ (ES014), which aims to neutralize the inhibitory effect of adenosine and TGFβ to the immune system in TME. The immuno-stimulating effect of ES014 was demonstrated in a PD-1-unresponsive mouse model where tumor growth was significantly inhibited after the treatment of the bi-specific antibody.

**RESULTS**

- **Figure 1.** Structure of ES014 Which Simultaneously Binds to CD39 and TGF-β
  - Binding target KD (M) kon(1/Ms) kdis(1/s) = 25
  - Simultaneous binding

- **Figure 2.** ES014 Binds to CD39 And Neutralizes CD39 ATPase Activity
  - Blocking Adenosine (CD73 KO) prevents radiation-induced TGFβ accumulation

- **Figure 3.** ES014 Binds to TGF-β And Neutralizes TGF-β Activity
  - CD39 up-regulation on CD8+ T cells upon anti-CD3/anti-CD28 stimulation

**METHODS**

The bifunctional antibody–ligand trap ES014 was created by fusing two copies of TGFβ receptor II ectodomain to an antibody targeting CD39. ES014 molecule could simultaneously inhibit CD39 enzymatic function to prevent extracellular ATP from degradation and neutralizes autocrine/paracrine TGFβ in the target cell microenvironment. The immunological function of ES014 was studied in an in vitro Elpiscience proprietary ImmunoShine platform which includes T cell activation and apoptosis assay, iTreg differentiation and suppression assay, NK cell activation assay and DC maturation activity. The in vivo animal efficacy of ES014 was investigated in a human PBMC engrafted cancer model.

**CONCLUSION**

We find that by simultaneously targeting CD39 and TGFβ by a novel bispecific molecule ES014, a synergistic anti-tumor effects can be achieved. Our pre-clinical data demonstrate that ES014 counteracts TGFβ-mediated inhibitory effect and adenosine induced immune tolerance and has a unique ability in protecting T cell from apoptosis. ES014 demonstrated strong efficacy in vivo in tumor growth inhibition.