Creating an Immune-favorable Tumor Microenvironment By A Novel Anti-CD39/TGFβ-Trap Bispecific Antibody

Dawei Sun, Jun Sun, Yanan Geng, Jinhui Zhang, Lily Tang, Rui Gao, Lei Li, Zhihao Wu, Yangsheng Qiu, Zheng Song, Hongtao Lu

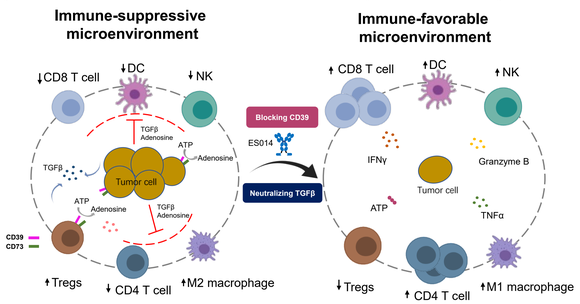
Elpiscience Biopharma, Ltd., 987 Cailun Road, 3rd Floor, Shanghai, China 201203

Poster abstract ID: 792

Elpiscience

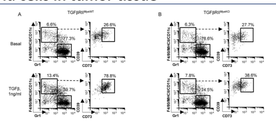
BACKGROUND

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 induces 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.

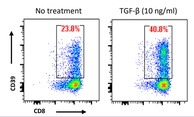


BACKGROUND

TGF-β promotes the generation of CD39+/CD73+ myeloid cells in tumor tissue

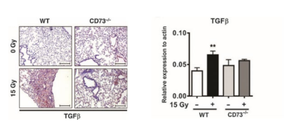


J Immunol. 2014



In house data

Blocking Adenosine (CD73 KO) prevents radiation-induced TGFβ accumulation



Cancer Res. 2016

➢ **TGFβ pathway can crosstalk with CD39-CD73-adenosine pathway**

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 inhibits 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.

RESULTS

Figure 1. Structure of ES014 Which Simultaneously Binds to CD39 and TGF-β

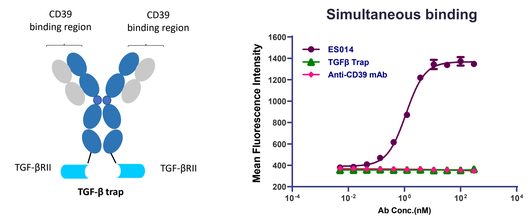


Figure 2. ES014 Binds to CD39 And Neutralizes CD39 ATPase Activity

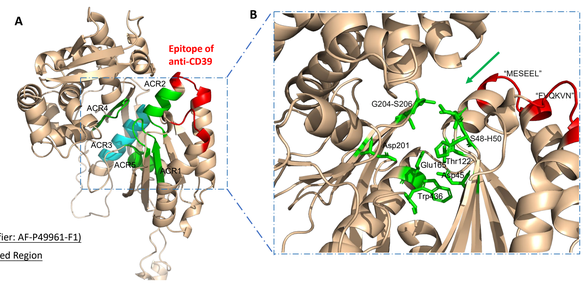
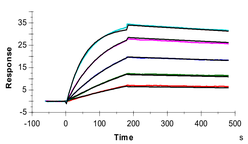
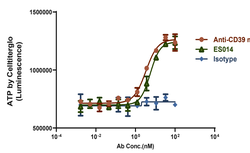


Figure 2: **A** The anti-CD39 epitope (red) locates at the entry of ATP catalytic cleft on CD39 extracellular domain, and is away from ATP catalytic active site (five conserved ACRs are highlighted by green or blue color); **B** Key residues of CD39 ATP catalytic active site (green) and anti-CD39 binding amino acids (red).

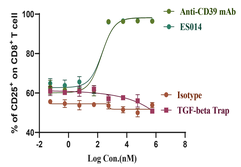
CD39 Binding Affinity by Octet



CD39 ATPase Neutralizing Activity



Reverse ATP Metabolite Driven Inhibition of T Cell Activation in the Presence of Anti-CD3/CD28 and ATP

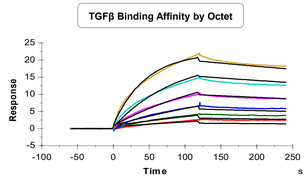


Binding target	KD (M)	kon(1/Ms)	kdis(1/s)
Human CD39	7.42E-10	3.71E+05	2.75E-04

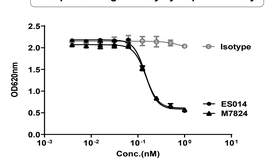
✓ **ES014 binds to hCD39 (ENTPD1) with sub-nM level affinity and neutralizes ATPase activity.**

Figure 3. ES014 Binds to TGF-β And Neutralizes TGF-β Activity

TGFβ Binding Affinity by Octet



TGFβ Blocking Efficacy by Reporter Assay

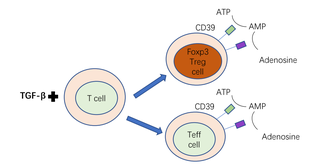


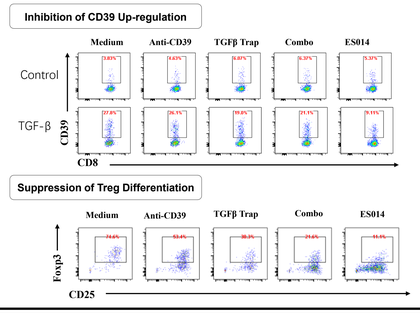
Binding target	KD (M)	kon(1/Ms)	kdis(1/s)
TGF-β1	1.17E-09	8.48E+05	9.93E-04

✓ **ES014 binds to hTGFβ with nM level affinity and neutralizes TGFβ activity by SMAD2/3 reporter assay.**

RESULTS

Figure 4. ES014 Inhibits Treg Differentiation and TGF-β–induced CD39 expression in T cells



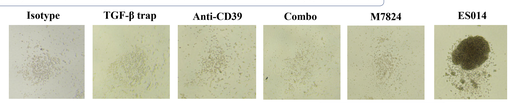


✓ **TGF-β induced T_{reg} cell differentiation and CD39 up-regulation on $CD8^+$ T cells upon anti-CD3/anti-CD28 stimulation**

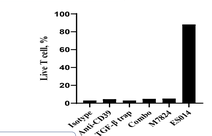
✓ **ES014 vs. mono or combination treatment with anti-CD39 and TGFβ trap showed resulted in superior reversal of CD39 expression and T_{reg} cell differentiation**

Figure 5. ES014 Promotes T Cell Survival

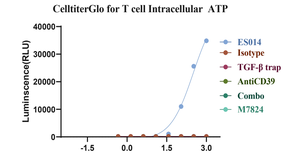
T cell expansion by anti-CD3/CD28 tetramer stimulation from StemCell for 5 days, 5E4 cell/well



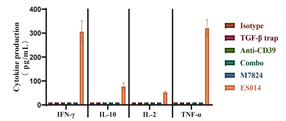
T cell survival By FACS PI staining



Intracellular ATP



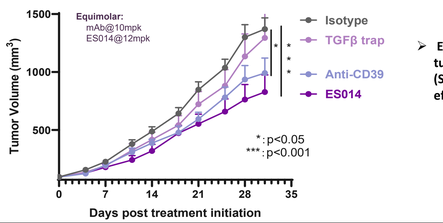
Cytokine production



➢ **In conclusion, ES014 is very effective in preventing T cell apoptosis and promoting T cell survival and activation as compared to anti-CD39, TGFβ trap or their combination which have no effect, indicating that this is a unique property of ES014.**

Figure 6. ES014 significantly Inhibits Tumor Growth in PBMC Humanized Model

SK-MEL 5 in PBMC Engraft Model



➢ **ES104 has demonstrated significant anti-tumor activity in an in vivo efficacy model (SK-Mel-5) where PD-1 antibody had no effect.**

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 in vivo tumor growth inhibition.