ES009, a LILRB2 specific blocking antibody, potently reprograms myeloid cells into pro-inflammation phenotypes and potentiates T cell activation

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BACKGROUND

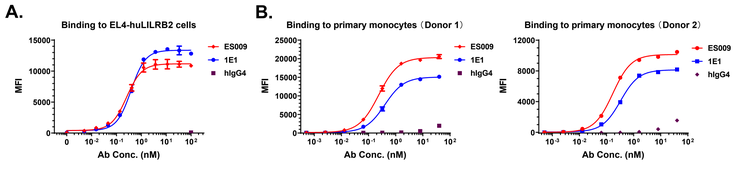
The inhibitory receptor leukocyte immunoglobulin-like receptor B 2 (LILRB2, also known as ILT4), an immunoreceptor tyrosine-based inhibitory motif (ITIM)-containing molecule predominantly expressed in myeloid lineage cells, is emerging as a key immune checkpoint for tumor immunotherapy. Human LILRB2 broadly binds to multiple ligands like classical MHC I, HLA-G, angiopoietin-like (ANGPTL) family members, myelin-associated glycoprotein (MAG), and contributes to immune suppression in the tumor microenvironment (TME). There is increasing evidence that blocking LILRB2 reprograms tumor-associated myeloid cells and promotes anti-tumor efficacy of other immune checkpoint inhibitors. We have developed ES009，a high affinity LILRB2 specific blocking antibody, which demonstrates superior effects in converting anti-inflammation myeloid cells into pro-inflammation phenotypes in *in vitro* and *ex vivo* models.

METHODS

LILR family homologue binding properties were evaluated by ELISA and FACS. Antigen binding affinity was determined by surface plasmon resonance system (Biacore). Blocking activity was determined by competition assay. *In vitro* function activity was evaluated by monocyte activation assay, dendritic cell (DC) differentiation assay, macrophage polarization assay, M2 macrophages-T cells (M2-T) co-culture assay. Epitope analysis was performed by Octet and hydrogen deuterium exchange mass spectrometry (HDX-MS). Lead clone was humanized via CDR grafting and back mutation screening.

RESULTS

ES009 specifically binds human LILRB2 with high affinity



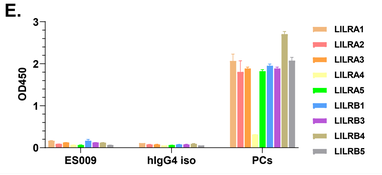
C.

Antibody	EC_{50} (nM)		
	EL4-huLILRB2	Monocytes (Donor 1)	Monocytes (Donor 2)
ES009	0.26	0.24	0.19
1E1	0.39	0.44	0.35

D.

Antibody	Affinity (KD, nM)
ES009	1.71
1E1	8.07

E.



(A-C) Cellular binding of ES009 and 1E1 to LILRB2 overexpressing cell line EL4-huLILRB2 (A), primary monocytes (B) and the EC_{50} summary (C). (D) Binding affinity of ES009 and 1E1 to human LILRB2 measured by Biacore. (E) Binding of ES009 to the recombinant proteins of LILRA and LILRB family members. PCs: positive controls.

RESULTS

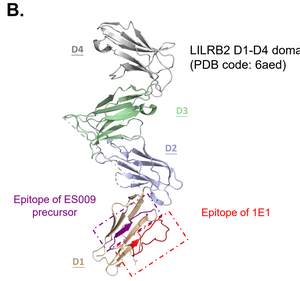
ES009 binds to a unique epitope on D1 domain of LILRB2

A.

Competitor \ Antibody	J-19	1E1	ES009 precursor
J-19	+	+	-
1E1	+	+	-
ES009 precursor *	-	-	+

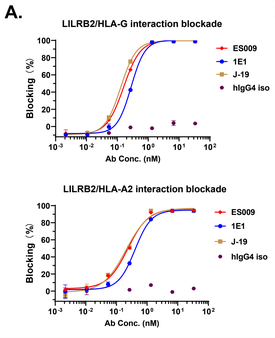
(A) Epitope binning of ES009 precursor and benchmark antibodies by Octet. The plus symbol is used to represent that the testing antibodies compete to bind to LILRB2. The minus symbol represents no competition between the testing antibodies. (B) Epitopes of ES009 precursor (purple) and 1E1 (red) on LILRB2 D1 domain identified by HDX/MS. *ES009 was humanized from ES009 precursor, which is a chimeric human IgG4.

B.

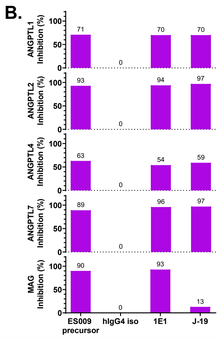


ES009 potently blocks LILRB2 binding to multiple ligands

A.



B.



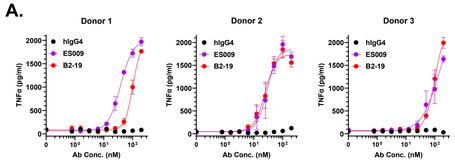
C.

Ab code	Blocking (IC_{50}, nM)	
	LILRB2/HLA-G	LILRB2/HLA-A2
ES009	0.16	0.24
1E1	0.28	0.40
J-19	0.13	0.20

(A, C) Activity of ES009 and benchmark antibodies to block LILRB2 binding to HLA-G and HLA-A2 measured by competitive FACS (A) and the IC_{50} summary (C). (B). Activity of ES009 and benchmark antibodies to block LILRB2 binding to non-HLA ligands: ANGPTL1, ANGPTL2, ANGPTL4, ANGPTL7 and MAG measured by competitive FACS.

ES009 promotes monocytes and monocytes derived DCs into a pro-inflammatory status

A.



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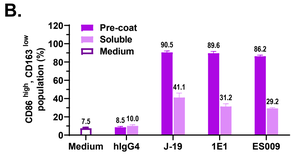
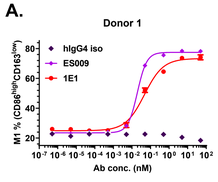


(A) Effects of ES009 and B2-19 to upregulate TNFα secretion of LPS primed primary monocytes. (B) Effects of ES009 and benchmark antibodies to promote human monocyte derived DCs into a maturation status (CD86 high, CD163 low) under plate coating or soluble format.

ES009 effectively reprograms human monocyte derived M2 macrophages into pro-inflammation M1 phenotypes

A.



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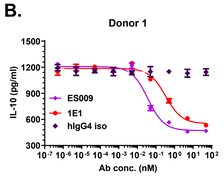


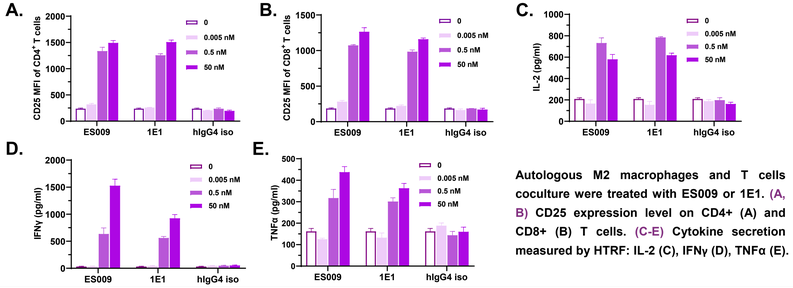
C.

Antibody	M1 switch (EC_{50}, nM)		
	Donor 1	Donor 2	Donor 3
ES009	0.020	0.040	0.031
1E1	0.047	0.187	0.041
Antibody	**IL-10 (IC_{50}, nM)**		
	Donor 1	**Donor 2**	**Donor 3**
ES009	0.044	0.270	0.084
1E1	0.350	0.960	0.240

Human monocyte derived M2 macrophages were treated with ES009 or 1E1. (A) M1 macrophages (CD86 high, CD163 low) population switch and (B) IL-10 secretion by a representative donor. (C) EC_{50} and IC_{50} summary of three different donors.

RESULTS

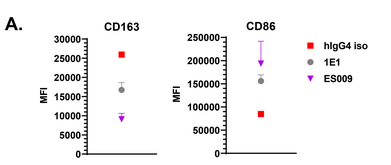
ES009 effectively relieves T cells from M2 macrophages mediated suppression



Autologous M2 macrophages and T cells coculture were treated with ES009 or 1E1. (A, B) CD25 expression level on CD4+ (A) and CD8+ (B) T cells. (C-E) Cytokine secretion measured by HTRF: IL-2 (C), IFNγ (D), TNFα (E).

ES009 converts primary macrophages in malignant ascites in ovarian cancer patients into a pro-inflammatory status

A.



B.

Fold change after been treated with ES009

Marker \ Sample	Patient 1	Patient 2	Patient 3
CD163	0.35	0.47	0.90
CD86	2.30	2.80	2.30

After been treated with ES009 or 1E1 *ex vivo*, CD11b positive cells in malignant ascites in ovarian cancer patients exhibited decreased CD163 and increased CD86 levels. (A) Representative data from the sample of patient 1. (B) Changes in CD163 and CD86 expression levels in samples from 3 different patients.

SUMMARY

ES009 specifically recognizes human LILRB2 with high affinity. It binds to a unique epitope on LILRB2 that is distinct from known competitor molecules. ES009 potently blocks LILRB2 binding to multiple HLA ligands (HLA-A2, HLA-G) as well as non-HLA ligands (ANGPTL1, ANGPTL2, ANGPTL4, ANGPTL7, MAG). Through blocking ligand(s) interaction and receptor activation, ES009 can promote human monocytes and human monocytes derived DCs into a pro-inflammatory status, reprogram human monocyte derived M2 macrophages into pro-inflammation M1 phenotype, and relieve T cells from M2 macrophages mediated suppression. Most importantly, in an *ex vivo* study, ES009 can also potently convert primary macrophages in malignant ascites in ovarian cancer patients into a pro-inflammatory status.