ES005, a high affinity anti-LAG3 monoclonal antibody, inhibits the interactions of LAG3 with multiple ligands and enhances anti-tumor activity of T cells in preclinical models

Xiaofeng Niu, Chunnian Wang, Jinfeng Zhao, Yefeng Lu, Yingchao Liu, Zhaohua Zhu, Rui Gao, Zheng Song, Hongtao Lu

Elpiscience Biopharma, BLDG. 3, 998 Halei RD., Pudong, Shanghai, 201203, P.R. CHINA

BACKGROUND

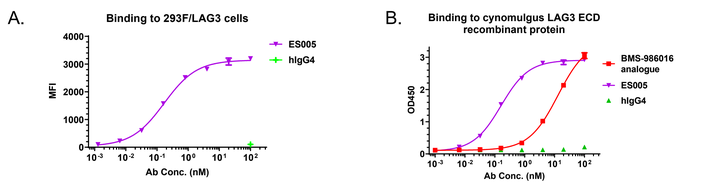
Lymphocyte-activated gene 3 (LAG3) is a cell surface inhibitory receptor expressed by both activated and exhausted CD4+/CD8+ T cells, as well as regulatory T cells (Tregs). It plays an important role in regulating immune homeostasis with multiple biological activities related to T cell functions and is considered a next-generation immune checkpoint after programmed cell death protein 1 (PD-1) and cytotoxic T-cell lymphocyte antigen-4 (CTLA-4). On March 18, 2022, FDA approved anti-LAG3 relatlimab-rmbw (BMS-986016) as part of a combination therapy for melanoma. The first identified LAG3 functional ligand is major histocompatibility complex class II (MHC-II). Recently other LAG3 ligands, like fibrinogen like 1 (FGL1), liver and lymph node sinusoidal endothelial cell C-type lectin (LSECtin), and galectin-3, were also found responsible for the inhibitory function of LAG3, suggesting that blocking these interactions simultaneously may bring greater clinical benefit in cancer treatment. We have developed a high affinity LAG3 blocking antibody ES005 that inhibits the interactions of LAG3 with multiple ligands including FGL1, and enhances anti-tumor activity of T cells in preclinical models.

METHODS

LAG3 binding activity and affinity were evaluated by FACS and surface plasmon resonance system (Biacore). Blocking activity was determined by competition assay. *In vitro* functional activity was determined by NFAT reporter assay and antigen specific T cell activation assay. *In vivo* efficacy was evaluated in a syngeneic mouse breast tumor model with human LAG3 knock-in mice. Epitope analysis was performed by ELISA and hydrogen deuterium exchange mass spectrometry (HDX-MS). Lead clone was humanized via CDR grafting and back mutation screening. Non-human primates (NHPs) models were used to assess the safety and pharmacokinetics of the humanized candidate.

RESULTS

ES005 is a high affinity and cynomolgus reactive anti-LAG3 mAb



C.

Antibody \ EC_{50} (nM)	Human	Cynomolgus
ES005	0.17	0.16
BMS-986016 analogue	/	12.9

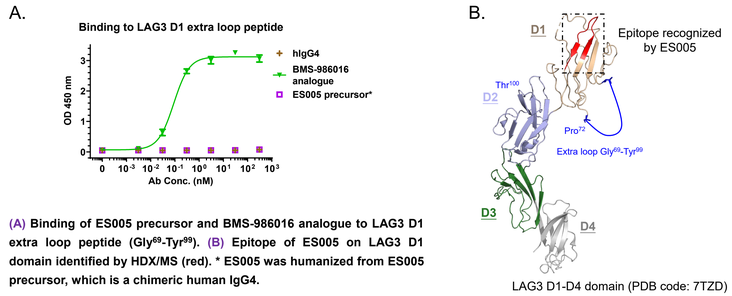
D.

Antibody	Affinity (KD, nM)
ES005	0.021
BMS-986016 analogue	1.61

(A) Cellular binding of ES005 to human LAG3. (B) Binding of ES005 and BMS-986016 analogue to cynomolgus LAG3 ECD recombinant protein. (C) EC_{50} summary for panel A and B. (D) Binding affinity of ES005 and BMS-986016 analogue to human LAG3 measured by Biacore.

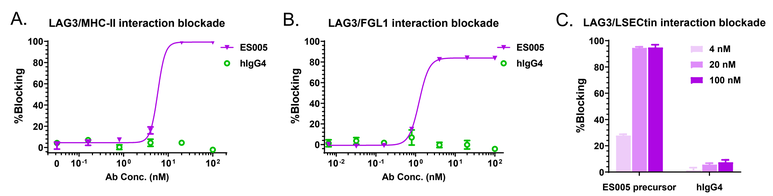
RESULTS

ES005 binds to a unique epitope on LAG3 D1 domain



(A) Binding of ES005 precursor and BMS-986016 analogue to LAG3 D1 extra loop peptide (Gly^{69}-Tyr^{99}). (B) Epitope of ES005 on LAG3 D1 domain identified by HDX/MS (red). * ES005 was humanized from ES005 precursor, which is a chimeric human IgG4.

ES005 potently blocks LAG3 binding to multiple ligands

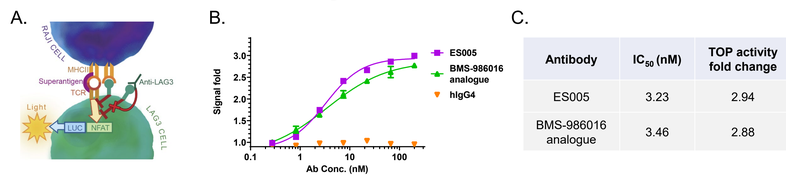


D.

Antibody	Blocking (IC_{50}, nM)	
	LAG3/MHC-II	LAG3/FGL1
ES005	5.8	1.2

(A-C) Activity of ES005 to block LAG3 binding to MHC-II (A), FGL1 (B) and LSECtin (C) measured by competitive FACS (A) or competitive ELISA (B, C). (D) IC_{50} summary for panel A and B.

ES005 effectively upregulates NFAT reporter gene transcription via blocking LAG3/MHC-II interaction

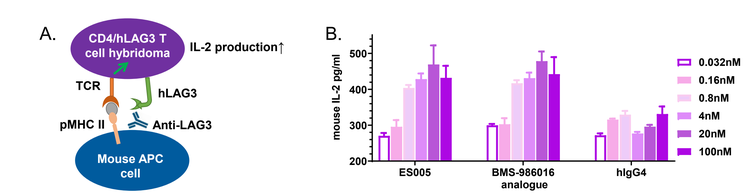


C.

Antibody	IC_{50} (nM)	TOP activity fold change
ES005	3.23	2.94
BMS-986016 analogue	3.46	2.88

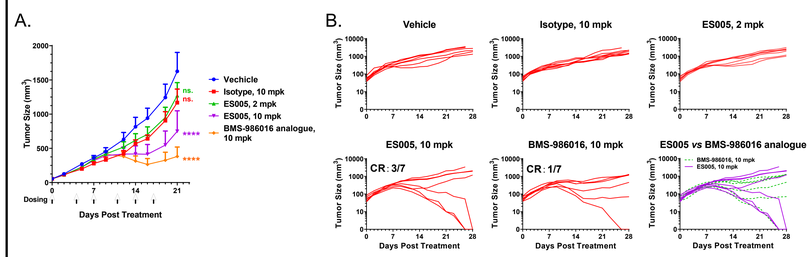
(A) Schematic diagram of the NFAT reporter assay (from Bioscience). (B, C) SED superantigen induced NFAT reporter gene transcription in the presence of the indicated antibodies (B) and the summary of IC_{50} and top activity fold change (C).

ES005 effectively reverses LAG3 driven inhibition of T cell activation



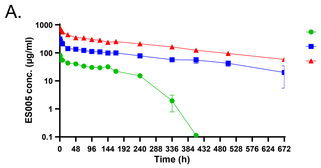
(A) Schematic diagram of the antigen specific T cell activation assay. (B) Antigen pulsed IL-2 secretion of T cells in the presence of the indicated antibodies.

ES005 monotherapy potently inhibits tumor growth in EMT6 syngeneic tumor model



EMT6 (murine mammary carcinoma cell line) cells inoculated human LAG3 knock-in Balb/c mice were treated with the indicated antibodies (n=7). (A) Average tumor growth curves for each group. (B) Individual tumor growth curves for each mouse. CR: complete response; ns.: not significant; **** p<0.0001.

ES005 was well tolerated in cynomolgus monkeys with favorable PK Profile



B.

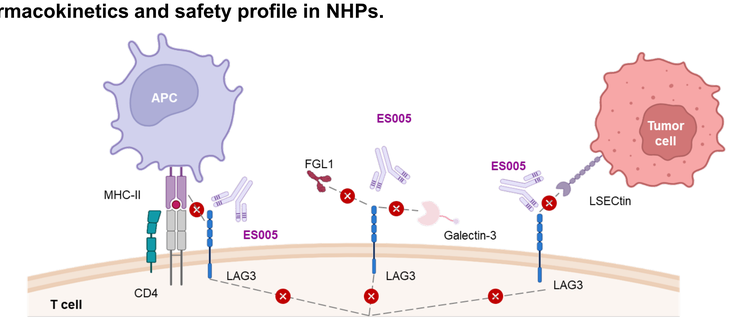
Parameter	Unit	3 mpk	10 mpk	30 mpk
$T_{1/2}$	hour	24.0	210	254
C_{max}	μg/ml	83.7	309.0	816.5

(A, B) A single dose of ES005 was administered to cynomolgus monkeys via intravenous infusion at 3, 10, and 30 mg/kg. (A) Serum concentration-time profiles of ES005 by dose group. (B) PK parameters summary. $T_{1/2}$: half-life in the terminal phase; C_{max}: maximum plasma concentration.

In the dose range finding (DRF) study, ES005 was administered to cynomolgus monkeys once weekly (QW) via intravenous infusion at 25, 75 and 150 mg/kg for 5 times. No test article-related adverse effects were observed. No-observed-adverse-effect level (NOAEL) is 150 mg/kg.

SUMMARY

ES005 specifically recognizes human LAG3 with high affinity. It binds to a unique epitope on LAG3 D1 domain that is distinct from benchmark antibodies. ES005 potently blocks LAG3 binding to multiple ligands (MHC-II, LSECtin, FGL1). ES005 can reverse LAG3-driven inhibition of NFAT reporter gene transcription and T cell activation in a dose-dependent manner. In a syngeneic mouse breast tumor model, ES005 significantly inhibited tumor growth *in vivo*. ES005 has excellent pharmacokinetics and safety profile in NHPs.



By blocking inhibitory LAG3, ES005 effectively activates T cells functionalities