Abstract 6550

In Vitro Characterization of Fully Human Antagonistic anti-BTLA Monoclonal Antibodies

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Abstract

Introduction: B and T lymphocyte attenuator (BTLA) is an immune checkpoint receptor essential for immune homeostasis and tolerance. Unlike PD-1 or CTLA-4, BTLA is broadly expressed on lymphoid and some myeloid cells, including T cells, B cells, dendritic cells, and macrophages. Engagement with its ligand HVEM (herpesvirus entry mediator) transmits inhibitory signals that suppress immune activation. Within tumors, BTLA contributes to immune evasion by dampening anti-tumor responses. Given its unique expression profile and regulatory function, we developed monoclonal antibodies (mAbs) targeting BTLA as an immunotherapeutic strategy to enhance anti-tumor immunity.

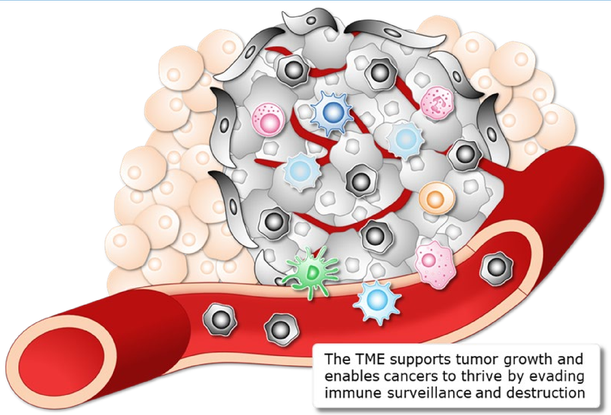
Methods: Fully human anti-BTLA mAbs identified by hybridoma screening were reformatted as huIgG4 (S228P/L235A) and transiently expressed using Curia's TunaCHO® platform. The S228P mutation prevents Fab-arm exchange, stabilizing mAb structure, while L235A minimizes FcγR binding and effector functions. Binding potency was determined by ELISA; kinetics by Octet® biolayer interferometry (BLI) against human and cynomolgus BTLA. BLI also assessed FcγR, FcRn (at pH 6.0 vs 7.2), and C1q binding to evaluate recycling potential and complement activation. Neutralization of HVEM binding to BTLA was quantified by modified ELISA and cell-based reporter assays.

Results: Six fully human anti-BTLA mAbs and clinical candidate comparator JS004 displayed similar ~1 nM binding potencies (EC50) values to human and cyno BTLA by ELISA. Clone 13-F7-A exhibited the highest affinity (KD < 1 nM) for both human and cyno BTLA among all 7 mAbs tested. Four mAbs and the comparator JS004 were high affinity binders to human and cyno BTLA (KD 1-32 nM). All six fully human mAbs exhibited reduced FcγR binding relative to JS004. Each bound FcRn at pH 6.0 but not at 7.2, consistent with normal endosomal recycling. None bound C1q, whereas a positive-control IgG1 did bind to C1q as expected. In an ELISA-based biofunctional assay, all mAbs blocked HVEM binding to BTLA with similar IC50 values (~1 nM). In a T cell reporter assay, four antibodies and the comparator JS004 reversed HVEM:BTLA-dependent suppression of TCR→NFAT→luciferase signaling (IC50: 9-31 nM).

Conclusions: We generated potent, high affinity, human/cyno cross-reactive, fully human antagonistic anti-BTLA mAbs. Targeting BTLA offers promising opportunities for cancer immunotherapy and may demonstrate strong synergy when combined with other checkpoint antagonists, potentially overcoming resistance mechanisms and improving clinical outcomes.

Introduction

Tumors Create a Toxic, Immunosuppressive Microenvironment (TME)



Solid Tumors with HVEM Upregulation

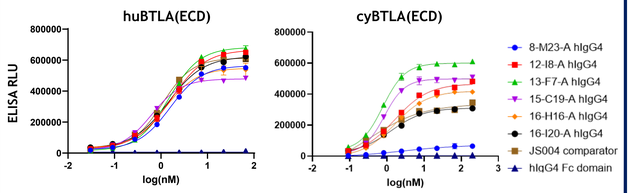
- Melanoma: HVEM is overexpressed in both metastatic melanoma samples and cultured cell lines. It is often found physically adjacent to BTLA+ tumor-infiltrating lymphocytes (TILs), making it a prime candidate for BTLA blockade[1].
- Non-Small Cell Lung Cancer (NSCLC): HVEM is upregulated in approximately 18-20% of NSCLC biopsies. Its expression is often independent of PD-L1, suggesting that BTLA-targeted therapy could benefit patients who do not respond to traditional PD-1 inhibitors[2].
- Colorectal Cancer (CRC): High HVEM levels in malignant lesions are linked to advanced pathological stages and poor prognosis. Preclinical studies show that blocking the HVEM-BTLA interaction can significantly reduce tumor growth in CRC models[3].
- Gastric & Digestive Cancers: Upregulation of both HVEM and BTLA in gastric cancer tissues correlates with lymph node metastasis and decreased overall survival[1].
- Hepatocellular Carcinoma (HCC): HVEM expression is associated with postoperative recurrence and poor survival outcomes in liver cancer[1].
- Glioblastoma & Glioma: Increased HVEM expression is found in aggressive glioma subtypes and is linked to poorer prognosis[3].
- Breast Cancer: High HVEM expression is linked to aggressive tumor features and poor prognosis in invasive breast cancer patients[3].
- Prostate Cancer: Recent studies indicate that HVEM negatively regulates the anti-tumor response in prostate cancer, and its blockade has shown promise in humanized mouse models[1].

Advantages of BTLA-targeted Cancer Immunotherapy

- PD-1/PD-L1 Independence...: HVEM expression is often not linked to PD-L1 status, offering an alternative pathway for "cold" tumors[4].
- ...as well as Synergistic Potential: Combining anti-BTLA mAbs with anti-PD-1 has shown enhanced T cell restoration in early clinical trials[5].
- Exhaustion Reversal: BTLA is typically expressed on terminally exhausted T cells that are the most dysfunctional and resistant to standard PD-1 therapy[6]; blocking BTLA can "un-brake" these specific cells to restore their killing capacity.
- Bidirectional Control: Unlike many checkpoints, BTLA:HVEM signaling is bidirectional. While BTLA sends "stop" signals to T cells, HVEM can send survival signals to tumor cells. Blocking this axis potentially hits both sides: it reactivates the immune system and stops the tumor from receiving survival cues[7].
- Broad Immune Impact: Beyond T cells, BTLA is expressed on B cells, NK cells, and macrophages. BTLA antagonism can improve NK cell activity and promote anti-tumor macrophage activity[7].

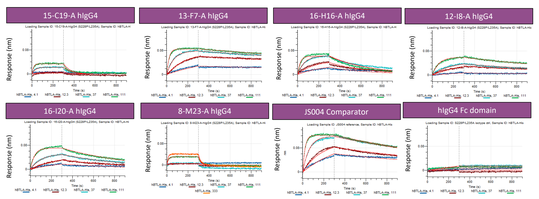
Results

Fig 1: ELISA Binding Potency



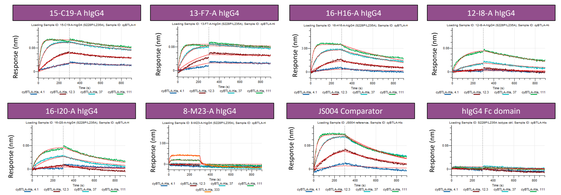
Dose dependent mAb binding curves for human and cyno BTLA

Fig 2: huBTLA Binding Affinity by BLI



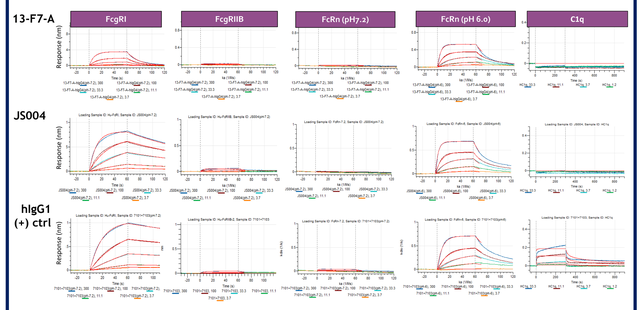
5 min association, 15 min dissociation, and 4 concentrations of huBTLA tested for binding to mAbs loaded on AHC biosensors

Fig 3: cyBTLA Binding Affinity by BLI



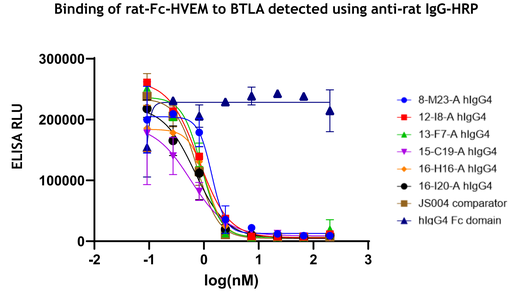
5 min association, 15 min dissociation, and 4 concentrations of cyBTLA tested for binding to mAbs loaded on AHC biosensors

Fig 4: FcγR, FcRn and C1q Binding by BLI



1 min association, 1 min dissociation, and 5 concentrations of mAbs tested for binding to FcgR and FcRn loaded on biosensors. mAbs were loaded on biosensors for C1q kinetics.

Fig 5: ELISA Biofunction IC50 Binding Inhibition



Dose dependent inhibition of BTLA binding to HVEM by anti-BTLA mAbs

References

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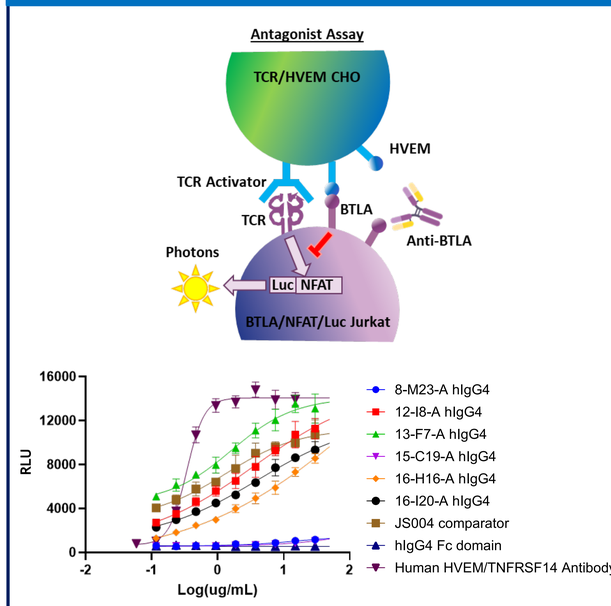
Fig 6: T cell Reporter Assay



Four anti-BTLA mAbs, JS004 comparator, and anti-HVEM (+) control Ab reverse HVEM:BTLA-dependent suppression of TCR signaling

Binding Affinities and Functional Characterization of Fully Human BTLA mAbs

	BTLA:HVEM Inhibition IC50 (nM)	BTLA Binding Affinity BLI KD (M)		BTLA Binding Potency ELISA EC50 (nM)		Human FcgR and C1q Binding Affinity BLI KD (M)							
Ab ID	T cell Reporter Assay	ELISA	huBTLA	cyBTLA	huBTLA	cyBTLA	FcgRI	FcgRIIA	FcgRIIB	FcgRIIIA-F	FcgRIIIA-V	FcgRIIIB	FcRn pH 7.2	FcRn pH 6.0	C1q
15-C19-A	n.d.	0.57	3.15E-08	1.05E-09	0.63	0.85	1.54E-07	3.07E-08	1.78E-04	n.d.	n.d.	n.d.	n.d.	4.64E-08	n.d.
13-F7-A	10.9	0.91	8.57E-10	4.26E-10	1.45	0.77	2.18E-07	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	6.80E-08	n.d.
16-H16-A	30.9	1.12	7.52E-09	3.25E-09	1.22	1.65	2.42E-07	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	8.99E-08	n.d.
12-I8-A	22.5	0.8	5.29E-09	1.26E-08	1.65	1.63	1.76E-07	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	5.10E-08	n.d.
16-I20-A	25.9	0.69	6.84E-09	1.94E-08	1.42	1.29	1.10E-07	n.d.	6.21E-08	n.d.	n.d.	n.d.	n.d.	4.67E-08	n.d.
8-M23-A	n.d.	1.4	3.29E-07	2.14E-06	1.58	2.55	2.65E-07	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	8.88E-08	n.d.
JS004 Reference Ab	9.0	0.76	1.59E-09	5.78E-09	1.07	0.33	2.11E-08	n.d.	2.06E-07	n.d.	n.d.	n.d.	n.d.	6.30E-08	n.d.
hIgG4 Fc (-) ctrl	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.89E-07	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.94E-08	n.d.
Curia hIgG1 (+) ctrl	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	1.28E-08	5.19E-07	6.03E-08	9.42E-08	5.97E-08	n.d.	n.d.	6.55E-08	7.15E-10

Favorable (green) or problematic (red) features

n.d. not detected
n.t. not tested

Conclusions

- We generated four potent, high affinity, human/cyno cross-reactive, fully human antagonistic anti-BTLA mAbs. Three antagonists, 13-F7-A, 16-H16-A, and 12-I8-A, had reduced FcgRI binding and no binding to FcgRIIB compared to clinical candidate JS004, and thus may have improved pharmacokinetics and reduced chance of FcR-dependent adverse events (e.g. cytokine release syndrome or other immune-mediated toxicities).
- Targeting BTLA is ideal since its ligand HVEM is expressed and/or upregulated in the TME of many cancers including melanoma, NSCLC, CRC, gastric cancer, glioblastoma, and prostate cancer and generally corelates with reduced overall survival[1,3].
- Targeting BTLA offers promising opportunities for cancer immunotherapy and may demonstrate strong synergy when combined with other checkpoint antagonists, potentially overcoming resistance mechanisms and improving clinical outcomes.