Conditionally active, therapeutic lymphotoxin beta receptor (LTBR) agonist bispecific antibodies for induction of tertiary lymphoid structures in the treatment of solid tumors

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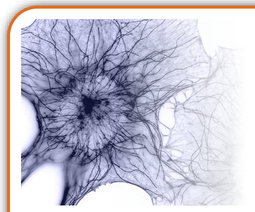
Abstract #LB123

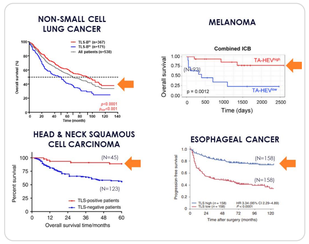


INTRODUCTION

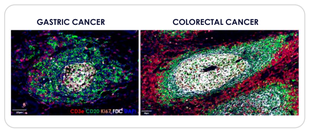
The presence of tertiary lymphoid structures (TLS) and associated high endothelial venules (HEV) in tumors strongly correlates with improved prognosis and treatment outcomes across solid tumors (examples right) [1-5]. These clinical observations highlight the therapeutic potential of inducing TLS and/or boosting TLS functions, which include acting as a point of entry and site of education for immune effector cells locally in the tumor [6].

Although TLSs have previously been observed to occur in tumors (examples right; Mestag), it is only more recently that they have been recognized as sites of T cell priming and activation in situ in the tumor [6]. This critical role in anti-tumor immunity is underscored by the inclusion of TLS in the revised cancer immunity cycle [6].

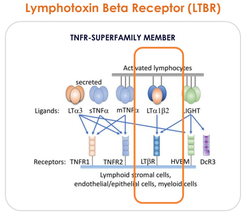
Presence of TLS Correlates with Better Survival
Examples from NSCLC, Melanoma, HNSCC and ESCC



TLS in Human Tumors
Observed by Multiplex Immunofluorescence



Lymphotoxin Beta Receptor (LTBR)



Lymphotoxin beta receptor (LTBR) signalling is essential for lymphoid structure development [7,8]. Ectopic expression of LTBR ligands lymphotoxin αβ and LIGHT is sufficient for TLS formation in vivo but challenging for therapeutic development [9-11]. The activation of LTBR on fibroblast reticular cells (FRCs) is believed to be a critical step in TLS induction [12,13].

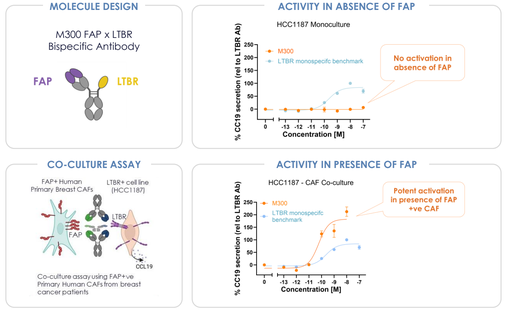
Here we present a human bispecific antibody, M300, which conditionally agonizes LTBR in the tumor on co-engagement with fibroblast activation protein (FAP), a tumor microenvironment-specific marker expressed by cancer associated fibroblasts.

In vitro characterisation of the human therapeutic bispecific antibody is presented, along with in vivo preclinical evaluation of a surrogate M300 bispecific antibody binding to mouse LTBR and mouse FAP in murine tumor models.

M300 FAP X LTBR BISPECIFIC ANTIBODY

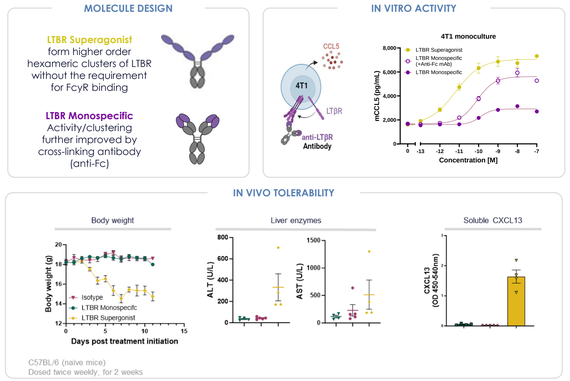
Therapeutic human FAP X LTBR bispecific antibodies were identified following an extensive screening campaign combining LTBR and FAP binding arms on an engineered human Fc with minimal FcγR binding.

M300 Bispecific Antibody: Conditional Activation of LTBR



In vitro assays using primary cancer associated fibroblasts and LTBR positive cells demonstrated that conditionally active bispecific antibody M300 potently activated LTBR uniquely in the presence of FAP expressing cells.

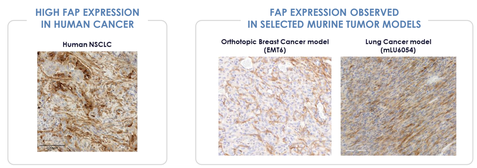
Tolerability of Unconditional LTBR Agonists



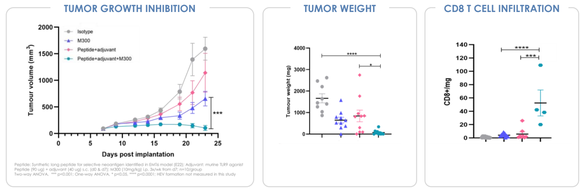
An unconditional LTBR superagonist was generated as a means of exploring untargeted LTBR agonism and associated tolerability. The LTBR superagonist demonstrated enhanced activity in vitro compared to an LTBR monoclonal antibody, however treatment of non-tumor bearing mice with the LTBR superagonist showed increased serum CXCL13 associated with increased liver enzymes and loss of body weight compared to the LTBR monoclonal antibody.

IN VIVO PROOF OF MECHANISM

A mouse surrogate M300 FAP x LTBR bispecific antibody was used to study mechanism of action in vivo. Effects on tumor growth inhibition, HEV formation and changes in the tumor immune microenvironment were determined using high-FAP expressing, low-immunogenic tumor models representative of human disease.

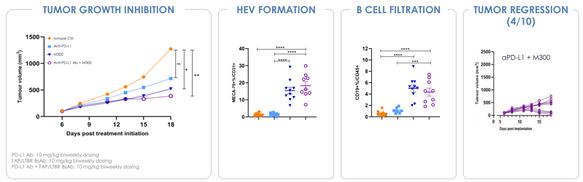


Tumor Regressions in EMT6 Model Upon Treatment with M300 + Peptide Antigen



M300 surrogate bispecific antibody showed significant anti-tumor efficacy in the high-bar, low antigen EMT6 orthotopic model; including potent anti tumor responses in combination with tumor peptide antigen (together with adjuvant used to represent tumor antigen in the tumor microenvironment) leading to tumor regression. Combination treated tumors also demonstrated significantly increased T cell infiltration, consistent with potent anti-tumor effect.

Tumor Regressions in EMT6 Model Upon Treatment with M300 + Anti-PD-L1

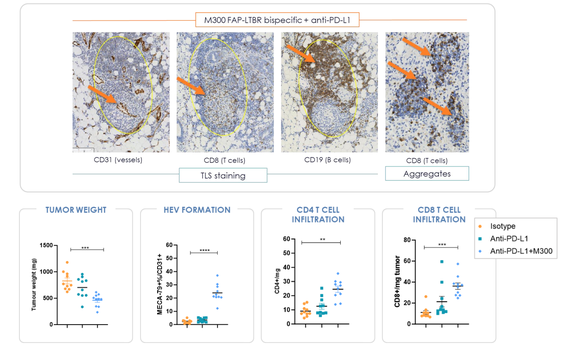


Treatment of the orthotopic EMT6 tumor model with mouse surrogate M300 monotherapy and in combination with anti-PD-L1 resulted in significantly decreased tumor growth, increased HEV formation and increased B cell infiltration. Tumor regression was observed in 4/10 mice in the combination group.

TLS FORMATION IN VIVO

The ability of M300 to induce the formation of TLS and inhibit tumor growth in vivo was determined using a mouse lung cancer model driven by RAS mutation.

Formation of TLS and Tumor Growth Inhibition in Lung Cancer Model with M300 + PD-L1 Combination



Histology data demonstrated that treatment of the mLU6054 lung tumor model with a combination of surrogate FAP x LTBR M300 and anti-PD-L1, but not anti-PD-L1 alone, led to the formation of TLS structures containing organized lymphocyte aggregates with the appearance of germinal centres and accumulation of T and B cells. The combination of anti-PD-L1 and M300 surrogate also inhibited tumor growth and resulted in increased HEV formation and T cell infiltration.

CONCLUSIONS

- Conditionally active M300 FAP x LTBR bispecifics activate LTBR in the tumor microenvironment and induce HEV and TLS formation, leading to potent monotherapy activity in vivo, and to tumor regression in combination with CPI or tumor peptide antigen.
- These data support the development of the M300 FAP x LTBR bispecific for the treatment of solid tumors as monotherapy and in combination with standard of care.

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