The tetravalent structure of FS118, a bispecific antibody targeting LAG-3 and PD-L1, is required for its novel mechanism of LAG-3 shedding

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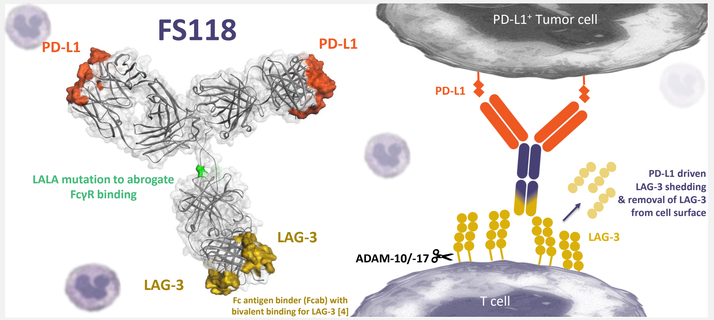
Background

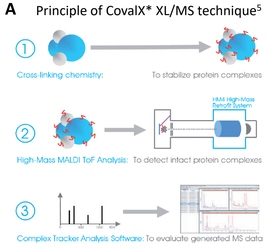
FS118 is a tetravalent bispecific antibody targeting LAG-3 and PD-L1 that can overcome immune suppressive signals with greater preclinical activity than a combination of monoclonal antibodies[1]. *In vivo*, FS118 surrogate downregulates LAG-3 on tumor-infiltrating lymphocytes (TILs) and increases soluble LAG-3 (sLAG-3) in the serum of mice[1]. In a Phase 1 trial, FS118 demonstrated a dose-dependent increase in sLAG-3 in the serum of patients with advanced malignancies[2]. Recent studies have suggested that LAG-3 shedding is essential to overcome resistance to anti-PD-1 immunotherapy[3]. Here, we demonstrate that the tetravalent structure of FS118 is required to mediate this novel LAG-3 shedding mechanism.

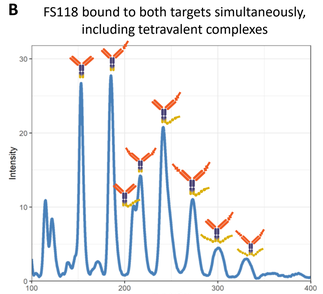
Methods

The binding valency of FS118 was determined using chemically-crosslinked mass spectrometry mapping (XL/MS). Variants of FS118 and FS118 surrogate molecules were generated with differing valency for LAG-3 or PD-L1. Human *ex vivo* assays were performed by co-culturing expanded CD4⁺ T cells with immature dendritic cells (iDCs) in the presence of *Staphylococcal enterotoxin B* and FS118, or controls. sLAG-3 was measured by ELISA. MC38 tumor-bearing C57BL/6 mice were dosed once intraperitoneally with FS118 surrogate or valency variants. TILs were analysed by flow cytometry.



1. Tetravalent binding by FS118 was demonstrated using chemically-crosslinking mass spectrometry mapping (XL/MS)



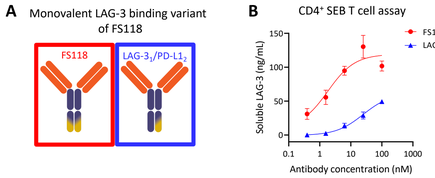


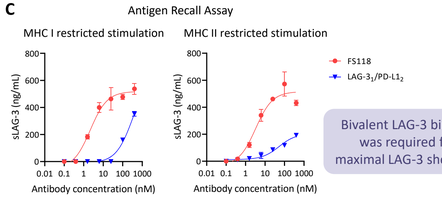
All four binding sites of FS118 bound to LAG-3 and PD-L1 in an *in vitro* system, and demonstrated tetravalency

Data shows binding of FS118 to LAG-3 and PD-L1 antigen in excess of PD-L1 (1:2:10; FS118:LAG-3:PD-L1)

2. Bivalent LAG-3 binding by FS118 was required for maximal LAG-3 shedding

To understand how the structure of FS118 mediates LAG-3 shedding, a valency variant of the tetravalent bispecific FS118, with monovalent LAG-3 binding, was generated and shedding of LAG-3 from T cell subsets explored.

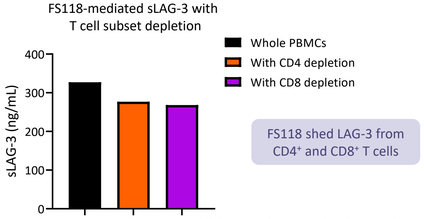




Bivalent LAG-3 binding was required for maximal LAG-3 shedding

(A) The monovalent LAG-3-targeting region comprised a human LAG-3 Fcab paired with a wild-type human IgG CH3 domain using KiH mutations[6].
(B) Expanded human CD4⁺ T cells were activated and cultured with iDCs and SEB plus antibody. Soluble LAG-3 was quantified by ELISA on Day 4.
(C) Human PBMCs were stimulated with CEF MHC I restricted or CEFT MHC II restricted peptide pools, respectively. Soluble LAG-3 quantified by ELISA.

3. FS118 sheds LAG-3 from both CD4⁺ and CD8⁺ T cells

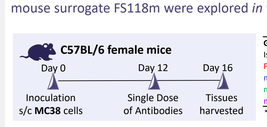


FS118 shed LAG-3 from CD4⁺ and CD8⁺ T cells

CD4⁺ T cells and CD8⁺ T cells were depleted from human PBMCs by magnetic cell separation. Cells were stimulated by pre-coated anti-CD3 and soluble anti-CD28 antibodies for 3 days, in the presence of 100nM FS118. Soluble LAG-3 quantified by ELISA. Data shown from one donor.

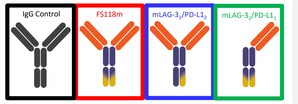
4. Tetravalent FS118m surrogate demonstrated effective surface LAG-3 reduction on TILs and limited compensatory up-regulation in the periphery

As tetravalent binding by FS118 was required to drive optimal LAG-3 shedding *in vitro*, and our previous studies[1] demonstrated that an FS118 surrogate drove a decrease in LAG-3 expression by TILs, valency variants of the mouse surrogate FS118m were explored *in vivo* in tumor-bearing mice.

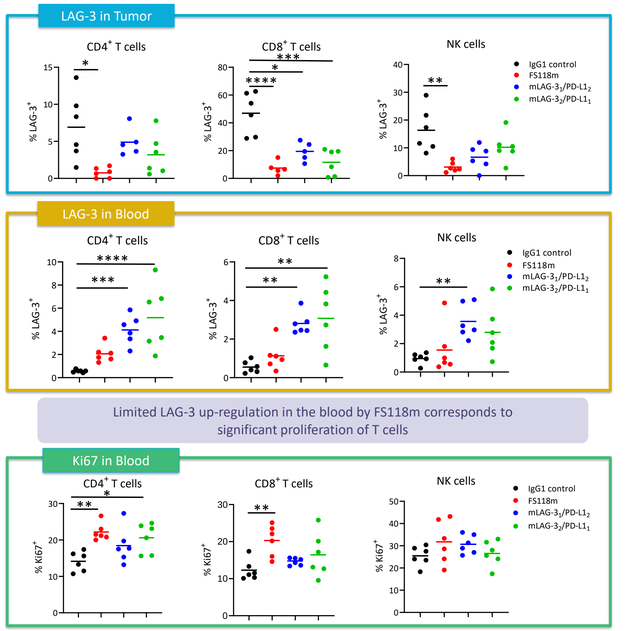


GROUP	DOSE (mg/kg)
Isotype Control	10
FS118m	10
mLAG-3₁/PD-L1₂	10
mLAG-3₁/PD-L1₁	6.66*
mLAG-3/PD-1	10

*Reduced dose based on MW of protein



Tetravalent FS118m significantly reduced cell surface LAG-3 on TILs but not in the periphery, suggesting tetravalency is key to limiting compensatory up-regulation of LAG-3

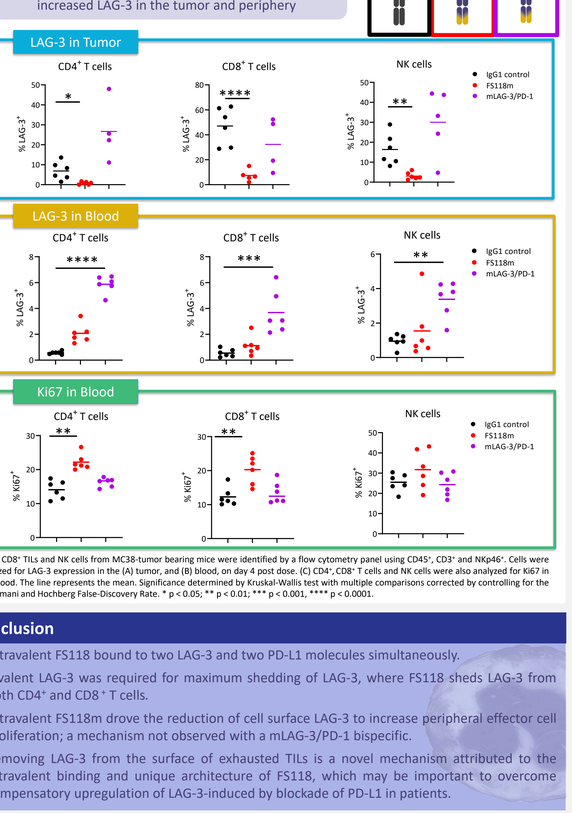


Limited LAG-3 up-regulation in the blood by FS118m corresponds to significant proliferation of T cells

CD4⁺, CD8⁺ TILs and NK cells from MC38-tumor bearing mice were identified by a flow cytometry panel using CD45⁺, CD3⁺ and NKp46⁺. Cells were analyzed for LAG-3 expression in the (A) tumor, and (B) blood, on day 4 post dose. (C) CD4⁺, CD8⁺ T cells and NK cells were also analyzed for Ki67 in the blood. The line represents the mean. Significance determined by Kruskal-Wallis test with multiple comparisons corrected by controlling for the Benjamani and Hochberg False-Discovery Rate.
* p < 0.05; ** p < 0.01; *** p < 0.001, **** p < 0.0001.

5. Reduction of cell surface LAG-3, and increased proliferation of T cells in the blood, was driven by binding to PD-L1 but not PD-1

Whereas FS118m reduced LAG-3, which corresponded to increased proliferating T cells in the blood, mLAG-3/PD-1 increased LAG-3 in the tumor and periphery



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Conclusion

- Tetravalent FS118 bound to two LAG-3 and two PD-L1 molecules simultaneously.
- Bivalent LAG-3 was required for maximum shedding of LAG-3, where FS118 sheds LAG-3 from both CD4⁺ and CD8⁺ T cells.
- Tetravalent FS118m drove the reduction of cell surface LAG-3 to increase peripheral effector cell proliferation; a mechanism not observed with a mLAG-3/PD-1 bispecific.
- Removing LAG-3 from the surface of exhausted TILs is a novel mechanism attributed to the tetravalent binding and unique architecture of FS118, which may be important to overcome compensatory upregulation of LAG-3-induced by blockade of PD-L1 in patients.

Acknowledgements
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