Development and Characterization of SL-325, a DR3-targeted, Antagonistic Antibody for the Treatment of IBD

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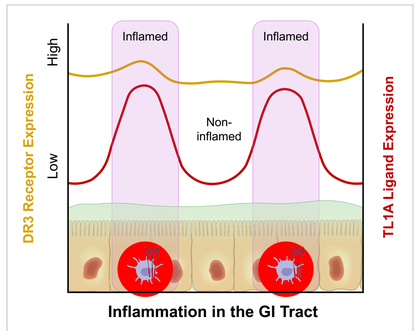
1. Rationale for Targeting DR3 in IBD

- Death Receptor 3 (DR3, TNFRSF25) is the sole signaling receptor for TL1A.
- Dysregulation of the TL1A/DR3 axis has been implicated in multiple inflammatory diseases, including inflammatory bowel disease (IBD).
- TL1A is found selectively in actively inflamed tissue, where it is primarily expressed by antigen presenting cells in an inducible and transient manner.
- Neutralization and blockade of soluble and membrane forms of TL1A, respectively, by monoclonal antibodies (mAb) has shown significant clinical responses in IBD patients.
- In contrast, DR3 is constitutively expressed by lymphocytes, and is found in greater abundance than TL1A in both actively inflamed and adjacent uninflamed tissue.



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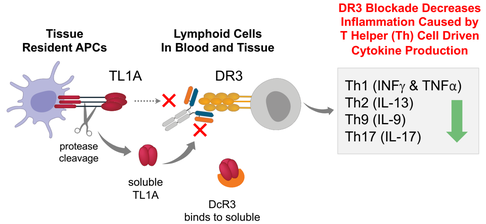
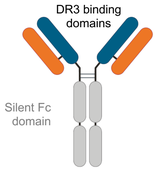
2. SL-325 Mechanism of Action



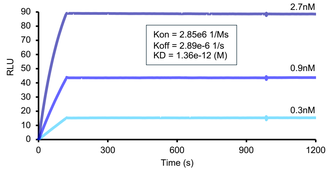
- TL1A is expressed primarily on tissue-resident antigen presenting cells (APCs), and binds to the DR3 receptor and the soluble decoy receptor (DcR3)
- DR3 is expressed by circulating and tissue-resident lymphoid cells. SL-325 binds DR3 with high affinity and blocks cell surface and soluble TL1A-induced activation of lymphoid cells
- SL-325 does not bind to DcR3 and thereby preserves its function

3. High Affinity DR3 Binding, Specificity and TL1A Blocking Activity

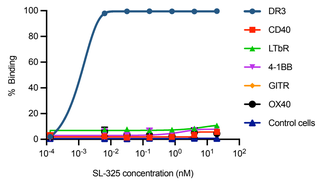
A. **SL-325 is a fully Fc-silenced mAb**



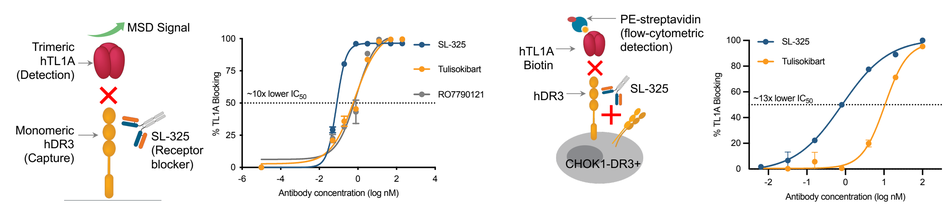
B. **SL-325 Binds DR3 with low pM affinity (SPR)**



C. **SL-325 only binds to human DR3 on cells with high specificity** *(no binding to other TNFRs)*

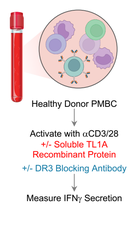


D. **SL-325 Blocks TL1A Binding to DR3 and Trimerization of DR3**

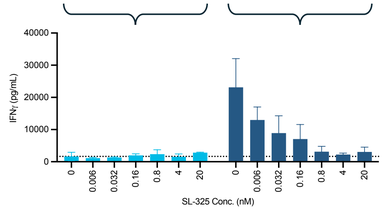


A) Structure of SL-325 mAb. B) Binding kinetics of SL-325 to DR3 by SPR (Biacore). C) Cell surface binding of SL-325 to DR3 and other TNF receptors by flow cytometry D) MSD-based TL1A competition assay comparing SL-325 against Tulisokibart and RO7790121(left panel); Flow cytometry based TL1A competition assay comparing SL-325 and Tulisokibart (right panel). Sl-325 binding to DR3 does not trigger receptor internalization (data nor shown). Studies were performed using a sequence equivalent of Tulisokibart and RO7790121.

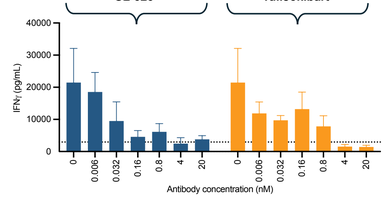
4. SL-325 Blocks Soluble and Membrane TL1A-Induced Immune Cell Activation with No Evidence of Agonistic Properties



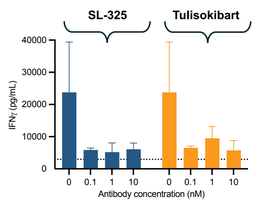
A. **Agonist Format αCD3/28** **Antagonist Format αCD3/28+TL1A**



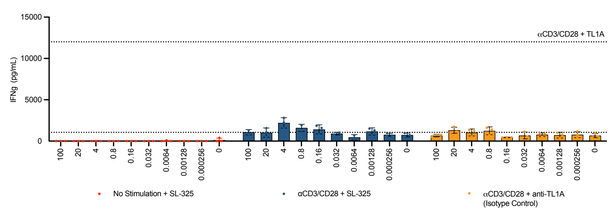
B. **Soluble TL1A (antagonist format)**



C. **Membrane TL1A (antagonist format)**



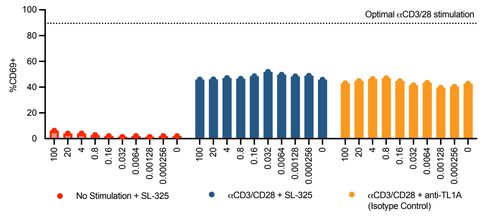
D. **Plate-bound SL-325 (agonist format)**



A) Healthy donor PBMCs were activated using a sub-optimal quantity of αCD3/CD28 antibody mix in the absence (agonist format) or presence (antagonist format) of soluble TL1A protein (100ng/ml). SL-325 was titrated in both conditions and the amount of IFNγ production was measured after 72 hours in culture. B) The ability of SL-325 to block IFNγ secretion in the 'antagonist format' conditions was compared to a benchmark anti-TL1A antibody (Tulisokibart). C) Human donor PBMCs were co-cultured with HEK293-TL1A* cells to confirm that SL-325 was capable of blocking cell surface expressed TL1A protein induced IFNγ secretion. The anti-TL1A antibody, Tulisokibart was used as a benchmark control. D) SL-325 was immobilized on to a 96-well microtiter plate ('plate-bound') at increasing concentrations to mimic antibody cross-linking and cultured with sub-optimally activated healthy donor PBMCs to assess potential agonistic properties of SL-325. Soluble TL1A was used as a positive control.

5. SL-325 Binding to DR3 Does Not Trigger T cell Activation or Proliferation

A. **No CD4 T cell Activation**



B. **No Treg Expansion**

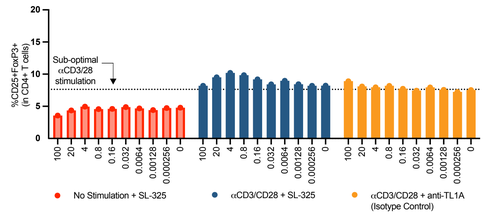
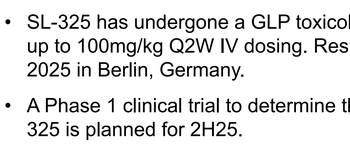


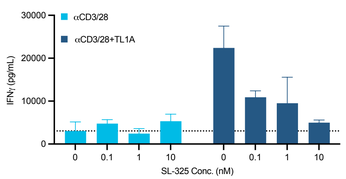
Plate bound SL-325 cultured with sub-optimally activated human donor PBMCs was assessed for its potential to activate CD4+ T cells (A) and proliferate regulatory T cells (Treg; B) by binding to DR3 over a 72-hour period. An optimal dose of αCD3/CD28 antibody mix was used as a positive control in A (top dashed line).

6. SL-325 Blocks TL1A-Induced Activation of IBD Patient PBMCs and Protects Epithelial Barrier Disruption Caused by Immune Cell Activation

A. **Ulcerative Colitis Patient**



B. **Crohn's Disease Patient**



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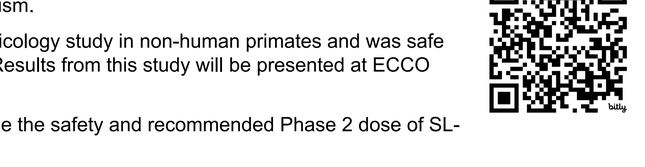
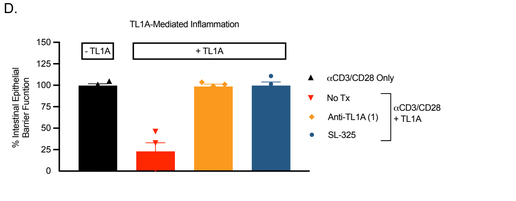


Source: www.AltisBiosystems.com

D.



Ulcerative Colitis (A) and Crohn's disease (B) patient PBMCs were sub-optimally activated with αCD3/CD28 antibody mixture in the absence (agonist format) or presence (antagonist format) of soluble TL1A protein. SL-325 was titrated in both conditions and the amount of IFNγ production was measured after 72 hours in culture. C) An *ex vivo* 2-D human primary intestinal epithelial cell model system (RepliGut® system; AltisBiosystems) was used to study the protective effects of blocking DR3 on activated T cells and epithelial cells during inflammatory cytokine-driven epithelial barrier disruption. D) Human PBMCs were pre-activated with aCD3/CD28 antibody mixture and added to the basal reservoir in the transwell system once the intestinal epithelial monolayer barrier was established. Soluble TL1A (100ng/ml) was added together with either SL-325 or a comparator anti-TL1A antibody (each at 10ug/ml) and the TEER was measured after 72 hours. Note that aCD3/CD28 +TL1A condition triggers TNFα production that leads to epithelial barrier disruption in this model.

7. Conclusions

- SL-325 is a fully Fc-silenced humanized IgG monoclonal antibody that has demonstrated high affinity binding to human DR3 and potent antagonistic properties with no evidence of residual agonism.
- SL-325 has undergone a GLP toxicology study in non-human primates and was safe up to 100mg/kg Q2W IV dosing. Results from this study will be presented at ECCO 2025 in Berlin, Germany.
- A Phase 1 clinical trial to determine the safety and recommended Phase 2 dose of SL-325 is planned for 2H25.