ALTB-168, an Immune Checkpoint Enhancer (ICE) targeting P-selectin glycoprotein-1 (PSGL-1), is effective in treating T-cell mediated inflammatory diseases

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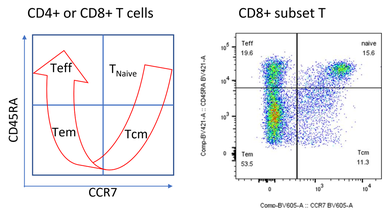
Abstract

P-selectin glycoprotein ligand 1 (PSGL-1), which has historically been known as an adhesion molecule in cell trafficking, was recently discovered to be a novel immune checkpoint regulator that modulates T cell homeostasis. Here we show that PSGL-1 is upregulated in activated T cells compared to naïve T cells. Cross-linking of ALTB-168 (Neihulizumab, an anti-PSGL-1 agonistic antibody) down regulates early T-cell receptor mediated signaling, T cell proliferation, and cytokine secretion. This inhibitory function is independent of the migration function of PSGL-1 as ALTB-168 does not interfere with the binding of P-selectin to PSGL-1. In a pre-clinical GvHD model, ALTB-168 treatment resulted in an increased population of apoptotic CD3 T cells, reduced severity of GvHD and prolonged survival of the mice. In a *trans-vivo* delayed type hypersensitivity (DTH) study where human PBMC, previously sensitized with tetanus toxoid (TT), was implanted in the mouse footpad together with TT, the induration of the mouse footpad was reduced by ALTB-168 in a dose-dependent manner. DTH studies performed in non-human primate (NHP) also showed that ALTB-168 inhibited skin induration as well as the inflammation of the lesion site. Intriguingly, ALTB-168 treatment does not impact the development of T-cell dependent antibody production, suggesting a marginal impact in primary T cells - a significant safety advantage in clinical development. Indeed, in studies across four indication, treating more than 200 subjects, not a single significant adverse events has been reported for ALTB-168. These data support the continued development of ALTB-168, the first anti-PSGL-1 agonistic antibody that down regulates T cell effector function, for the treatment of human inflammatory diseases.

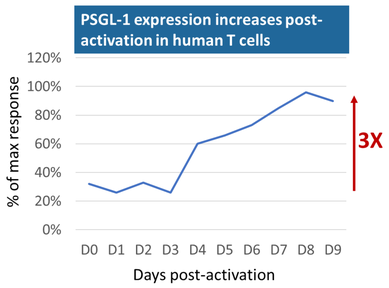
Introduction

- P-selectin glycoprotein ligand-1 (PSGL-1) is a type I transmembrane protein expressed on all leukocytes.
- While historically known for its role as an adhesion molecule to recruit T cells into inflamed tissue, recent findings indicated that PSGL-1 acts as an inhibitory checkpoint regulator of T cell immune responses.
- PSGL-1 is upregulated following T-cell activation. Elevated PSGL-1 expression was reported for T cells with chronic antigen stimulation, and elevated soluble PSGL-1 was reported for inflammatory diseases such as ulcerative colitis.
- ALTB-168, an anti-PSGL-1 IgG4 monoclonal antibody, is an immune checkpoint enhancer that down-regulates T cell functions and can be applied for treating T-cell mediated autoimmune diseases.

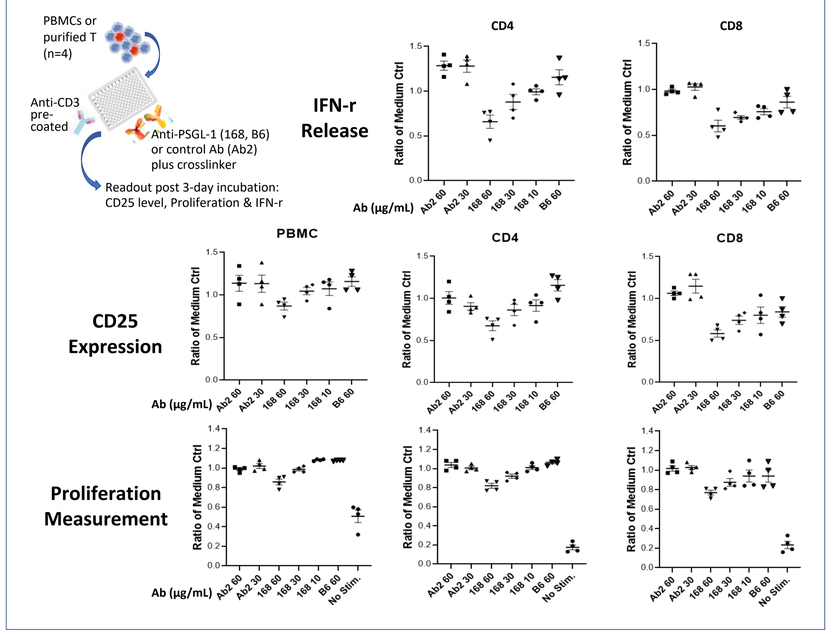


MFI of PSGL-1 staining in different CD8+ subsets from 4 donors

	CD8+ subset			
	Naïve	Tcm	Tem	Teff
Donor 1	15624	18663	24236	28787
Donor 2	15056	15840	21143	23897
Donor 3	21517	24470	30629	32583
Donor 4	20224	22415	25568	29177

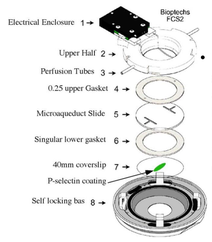


ALTB-168 Is an Agonist Ab That Inhibits Human T cell Proliferation and Activation in vitro



ALTB-168's Agonistic Function Is Independent of PSGL-1's Role in Cell Migration

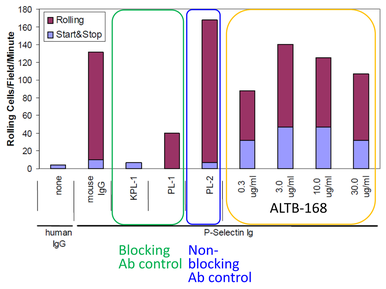
Coating of P-selectin and assembly of Bioptechs flow cell for cell rolling study



- The P-selectin coating was generated by first coating an anti-Fc antibody in the green highlighted area, assembling the flow cell, and then allowing the P-selectin-Ig construct to be captured from solution injected into the flow cells

- The last minute of 10 mins recording was analyzed to quantitate the number of rolling cells per field.
- Start & stop (Blue): the cell paused for > 1 sec between steps

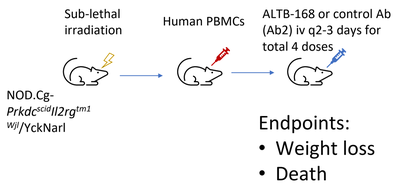
Effect of ALTB-168 on activated T cell rolling on P-selectin under experimental flow conditions



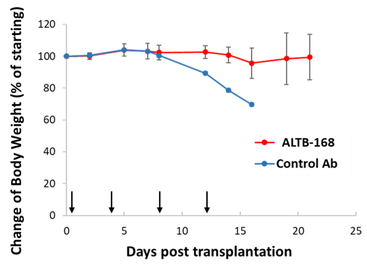
1. KPL-1 and PL-1 are blocking antibodies that inhibit rolling.
2. PL-2 is a binding, but non-blocking antibody that slightly increased the number of rolling cells but had no effect on rolling speed.
3. ALTB-168 appears to decelerate rolling by increasing the duration or number of interactions between bound PSGL-1 and P-selectin.

ALTB-168 Reduced Severity of xGvHD and Prolonged Survival of the Mice

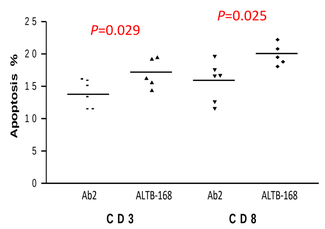
Humanized murine Xenogenic Graft vs. Host Disease (xGvHD) model establishment

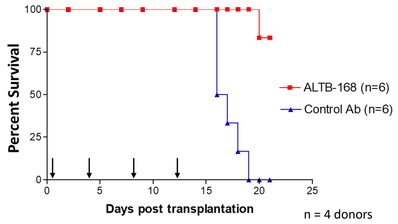


Endpoints:
- Weight loss
- Death



Increased apoptotic T-cells was detected in the Splenocytes of xGvHD mice

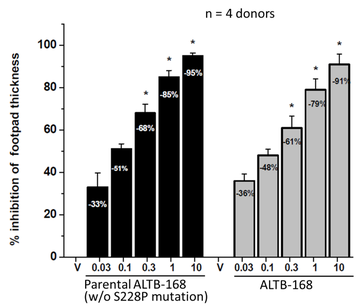




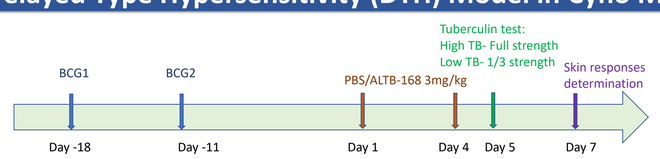
n = 4 donors

Inhibition of Humanized Mouse trans-vivo Delayed Type Hypersensitivity (trans-vivo DTH) Response by ALTB-168

ALTB-168 was dosed intraperitoneally at 0.03, 0.1, 0.3, 1 and 10 mg/kg one hour before paw injections with PBMCs with or without tetanus toxoid. Increase in paw thickness was measured after 24 hours. Pooled percent inhibitions of foot pad thickness in response to AbGn-168 and AbGn-168H treatment in trans-vivo DTH is plotted. (mean ± SEM, n=4 donors) * P < 0.05 versus vehicle control using ANOVA. V: vehicle.

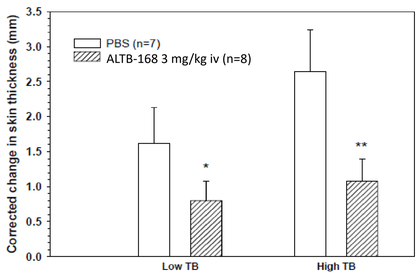


ALTB-168 Reduced Skin Inflammation in a Tuberculin-induced Delayed Type Hypersensitivity (DTH) Model in Cyno Monkey

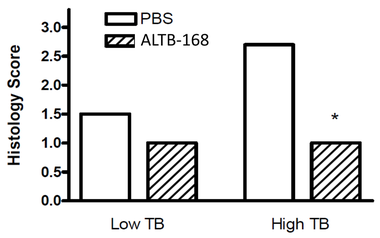


Skin thickness was measured twice before and twice after challenge. Following the final measurements monkeys were euthanized and skin biopsies were taken and evaluated for grades of inflammation severity.

ALTB-168 at 3 mg/kg inhibited DTH Responses in Tuberculin Challenged Cyno Monkeys



Mean ± SEM changes in skin thickness are shown. * p=0.015 compared to the PBS treated group. ** p<0.0001 compared to the PBS treated group (ANCOVA).



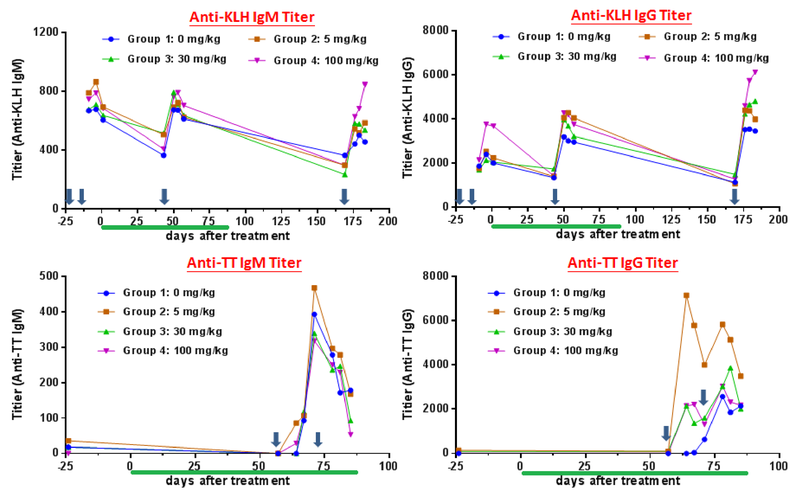
. * p=0.002 (Mann Whitney test)

ALTB-168 Shown to be Well Tolerated in Pre-clinical NHP Study

- **Cyno monkey established as appropriate model for efficacy and safety demonstration**
  - ➢ ALTB-168 binds to monkey T cells and negatively regulates TCR signal, which in turn inhibits cell proliferation and cytokine secretion in response to CD3 signaling
- ***In vivo* general toxicity findings in cynomolgus monkeys**
  - ➢ 4-weeks twice weekly *iv* and *sc* administration toxicity studies
    - There were no macroscopic or microscopic changes attributable to ALTB-168.
    - No evidence of bone marrow inhibition was observed.
    - Samples of plasma showed no evidence of an effect of treatment on circulating cytokines (IL-2, IL-4, IL-5, IL-6, TNF-α and IFN-γ).
    - There were no changes in the immune cells measured in peripheral blood or in lymphoid tissues attributable to the administration of ALTB-168.
    - NOAEL determined at 100 mg/kg twice per week
  - ➢ 13-week twice weekly *iv* toxicity study with 39-week recovery phase
    - Well tolerated at all dose levels and no general toxicity observed. NOAEL also determined at 100 mg/kg twice per week
    - T cell dependent antibody response (TDAR) assay demonstrates no immunotoxic potential resulted from ALTB-168 administration.

Monkey TDAR study with 2 immunogenic antigens design,

- There were no ALTB-168-related changes of anti-KLH or anti-TT specific IgM and IgG levels following immunization with KLH and TT



— ALTB-168 dosing phase. First day of dosing as d1 (n = 14 each dose group) . Arrows indicated the time of Ag immunization.

ALTB-168 Is Clinically Validated for Safety, Tolerability and Efficacy in Multiple Indications

ALTB-168 (iv administration) is well tolerated, with dose dependent reductions in disease activity in Psoriasis, Psoriatic Arthritis, Ulcerative Colitis, and acute GvHD.

Indication	Pt Count	Outcome
Healthy Volunteer	48 (12 placebo)	Safety, tolerability
Psoriasis	54 (17 placebo)	Safety, tolerability, clinical effect vs. placebo
	50 (17 placebo)	Safety, tolerability, clinical effect vs. placebo
Psoriatic arthritis	20	Safety, tolerability, durability of response
Ulcerative colitis	10 (Pilot study)	Safety, tolerability, dose finding
	14 (Main study)	Clinical effect competitive vs. SoC
SR-aGVHD	IST: 4	Dose finding
	(1a): 13 (1b): 24	Clinical effect highly competitive in combination with Ruxolitinib
Front-line aGVHD	IST: 10	3 of 7 treated patients showed CR
Total Subjects	**247 participants (201 treated) to date**	

Conclusions

- **Selectively inhibiting or deleting activated T lymphocytes represents a promising therapeutic approach for the treatment of autoimmunity.**
- **AltruBio discovered that the anti-PSGL-1 agonistic antibody, ALTB-168, can down-regulate activated T-cells and restore the immune system to a state of balance.**
- **ALTB-168's function is independent of PSGL-1's role in cell migration.**
- **NHP studies demonstrated excellent safety profile of ALTB-168. Efficacy of ALTB-168 is demonstrated in DTH studies and GvHD disease models.**
- **ALTB-168 is clinically validated for safety, tolerability and efficacy in treating human inflammatory diseases including Psoriasis, Psoriatic Arthritis, Ulcerative Colitis, and acute GvHD.**

References & Acknowledgement

1. Huang CC, et al, Eur J Immunol. 2005; 35: 2239-49
2. Chen SC, et al, Blood 2004; 104: 3233-42
3. Tinoco et al., 2016, Immunity 44, 1190–120
4. Hope et al., 2023, Cell Reports 42, 112436

5. Thanks to Department of Immunology and Inflammation, Boehringer Ingelheim Pharmaceuticals, Inc. for scientific input and the works in cell rolling, *tv*-DTH, NHP DTH and NHP Toxicity study .