ALTB-268, a Tetravalent Anti-PSGL-1 Antibody Derived From ALTB-168, Shows Enhanced Potency in Treating T-cell Mediated Inflammatory Diseases Allowing for Subcutaneous Administration

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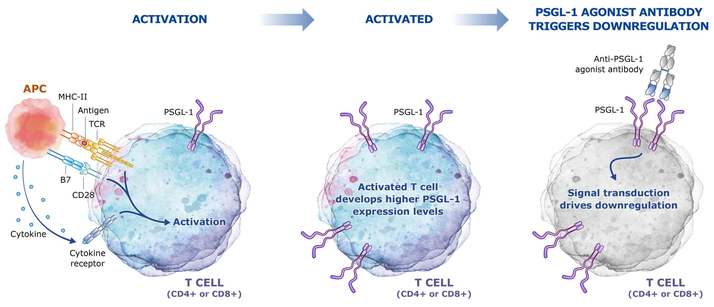
Abstract

We have previously discovered a novel anti-PSGL-1 monoclonal antibody, ALTB-168 (Neihulizumab), that acts as an immune checkpoint enhancer (ICE) by down-regulating T effector function. With this unique mechanism of action, ALTB-168 has been advanced clinically for the treatment of T-cell mediated inflammatory diseases. ALTB-168 was found to induce inhibitory signaling upon binding to PSGL-1, which is enhanced by cross-linking with anti-human antibody *in vitro*.

An Fv engineered tetravalent antibody, with four PSGL-1 binding sites, can potentially facilitate the clustering of cell surface PSGL-1 and the downstream signaling compared to a conventional bivalent antibody. Here we show that a tetravalent version of ALTB-168, named ALTB-268, demonstrated greater than 10-fold higher potency in *in vitro* T cell activation inhibition assays compared to ALTB-168. When compared in a human-mouse *trans-vivo* delayed-type hypersensitivty (DTH) study as well as in a non-human primate (NHP) DTH study, greater than 3-fold higher potency was observed for ALTB-268. The increased potency is likely related to differences in stoichiometry and increased avidity rather than increased affinity, as a single 268 molecule can bind to more PSGL-1 compared to a single 168 molecule, while similar affinity for both ALTB-168 and ALTB-268 was measured by SPR or ELISA. Most importantly, a similar safety profile as ALTB-168 was observed for ALTB-268 in NHP toxicology assessments, with a NOAEL of 120 mg/kg in a definitive 28-day weekly repeat-dose toxicity study, and a bioavailability of 70% by subcutaneous (SC) route. These data support the clinical development of ALTB-268, sc, for the treatment of T-cell mediated inflammatory diseases.

Introduction

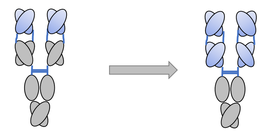
- **P-selectin glycoprotein ligand-1 (PSGL-1)**, is a type I transmembrane protein expressed on all leukocytes and is historically best known for its role in cell trafficking via selectin binding.
- PSGL-1 has been shown to be one of the key immune checkpoint regulators by AltruBio[1, 2] and by independent 3rd party labs[3, 4] .
- AltruBio has discovered anti-PSGL-1 agonistic antibodies, ALTB-168/ALTB-268, that act as **immune checkpoint enhancer (ICE)** by down-regulating T cell function.



- ALTB-168 has been clinically validated for safety, tolerability and efficacy in Psoriasis, Psoriatic Arthritis, Ulcerative Colitis and acute GvHD patients. (see FOCIS Poster # Tu221)
- In the present study, we have demonstrated ALTB-268 shows enhanced potency in treating T-cell mediated inflammatory diseases.

ALTB-268 Has Doubled the Binding Domains Compared to ALTB-168

- ALTB-268 is a tetravalent Fc fusion single chain diabody derived from ALTB-168.



ALTB-168
Humanized anti-PSGL-1 antibody with a hinge stabilized IgG4 backbone

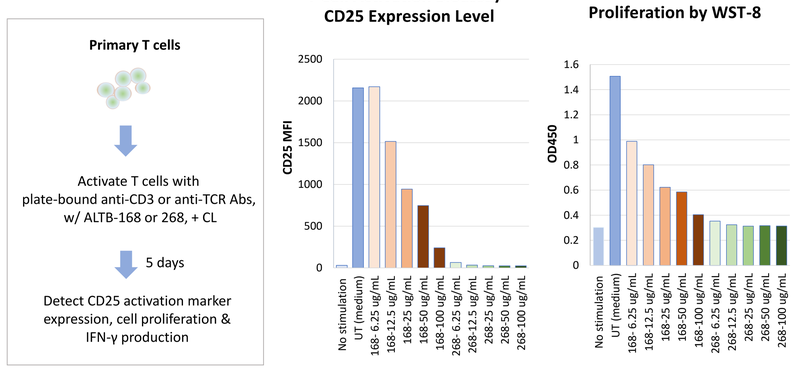
ALTB-268
Fc fusion protein, containing tandem diabodies to create a tetravalent binder to PSGL-1

- ALTB-268 and ALTB-168 showed similar affinity by BIAcore SPR or ELISA.

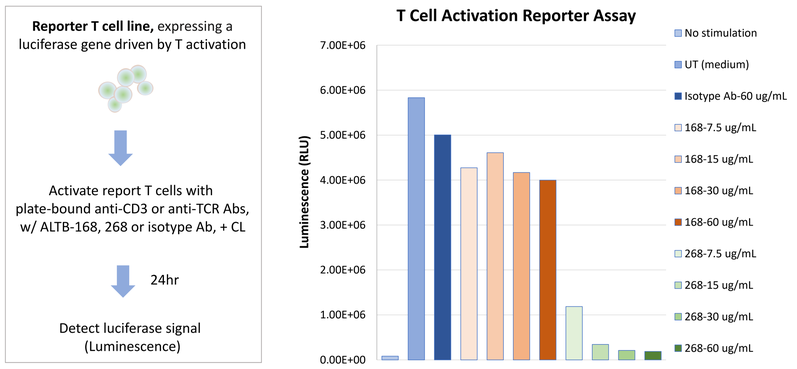
			ALTB-168	ALTB-268	Fold
BIAcore	PSGL-1 band 1	Kd (M)	2.17 x 10^{-9}	1.62 x 10^{-9}	1.34
BIAcore	PSGL-1 band 2	Kd (M)	11.6 x 10^{-9}	4.74 x 10^{-9}	2.45
ELISA	PSGL-1	EC_{50}(ng/mL)	7.06	5.06	1.40

ALTB-268 Shows Enhanced T Cell Inhibition *in Vitro*

- **T Cell Activation Bioassay-1**



- **T Cell Activation Bioassay-2**

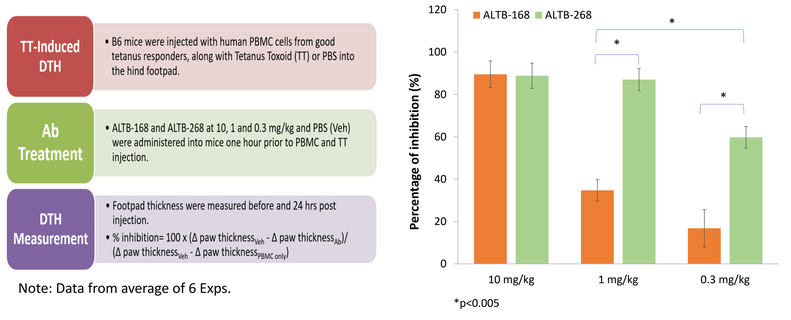


- **Summary**
  - ALTB-268 shows higher potency than ALTB-168 in down-regulating T cell receptor mediated signaling and T cell effector function, including activation, proliferation and also cytokine secretion (data not shown).

ALTB-268 Shows Greater Potency Than ALTB-16 *in Vivo*

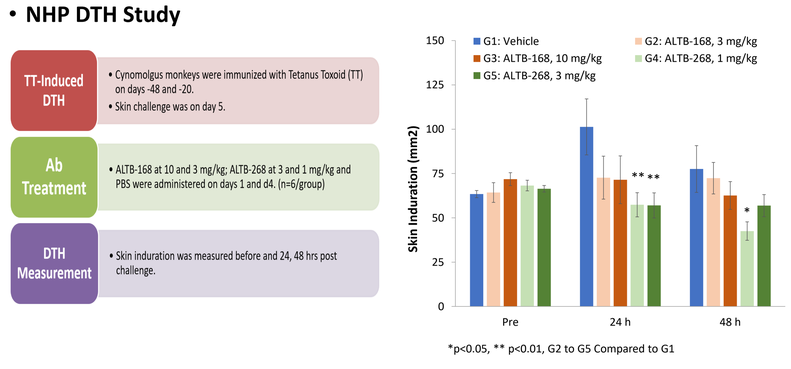
- Inhibition of delayed-type hypersensitivity (DTH) response, a response mainly mediated by antigen-specific memory T cells, is used for ALTB-168/268 efficacy assessment.

- **Trans-vivo DTH Study**



Note: Data from average of 6 Exps.

- **NHP DTH Study**



- **Summary**
  - ALTB-268 shows greater than 3-fold higher potency than ALTB-168 in both DTH models.

ALTB-268 Demonstrates Subcutaneous Administration Potential in NHP Bioavailability Study

- **Study Design**

Group No.	Test Material	Dosing Regimen	Dose Level (mg/kg/dose)	Route	No. of Animals
1	ALTB-268	Single dose	6	SC	3
2	ALTB-268	Single dose	6	IV	3
3	ALTB-268	Weekly x 4	20	IV	3
4	ALTB-268	Weekly x 4	80	IV	3

- **Summary**
  - The bioavailability of ALTB-268 via SC route was approximately **70%** (AUC_{last} 595 *vs* 852 day•µg/mL) at 6 mg/kg.

Summary of PK Parameters Following a Single Dose of ALTB-268 in Cyno Monkey

PK Parameters	Units	Dose Group (RoA)	
		6 mg/kg (SC)	6 mg/kg (IV Infusion)
T_{max}	Day	3.33	1.34
C_{max}	µg/mL	98.2	183
CL	mL/kg/day	NA	7.36
V_{ss}	mL/kg	NA	31.6
$T_{1/2}$	Day	1.54	1.97
AUC_{last}	Day•µg/mL	595	852

AUC = area under the plasma concentration-time curve; AUClast = AUC from time 0 to the last quantifiable concentration ; CL = total clearance; Cmax = maximum plasma concentration; IV = intravenous; ; NA = not available; PK = pharmacokinetic; RoA = route of administration; SC = subcutaneous; T1/2 = terminal half-life; Tmax = time to reach maximum plasma concentration; Vss = volume of distribution at steady state

- No abnormal clinical symptoms, food consumption nor body weight loss was observed during in-life period at all dose levels.
- No abnormality were observed in hematology, serum chemistry, urine and coagulation-related parameters.
- No ALTB-268-related changes in cytokine parameters (IFN-γ, IL-2, IL-6, IL-8, TNF-α) evaluated.

ALTB-268 Demonstrates Excellent Safety & Tolerability in Cynomolgus Monkeys

- **Study Design**

Group No.	Test Material	Dosing Regimen	Dose Level (mg/kg)	Route	No. of Animals[a]			
					Main Study		Recovery Study	
					M	F	M	F
1	Control Article	Weekly x 4	0	SC	3	3	2	2
2	ALTB-268	Weekly x 4	6	SC	3	3	-	-
3	ALTB-268	Weekly x 4	30	SC	3	3	2	2
4	ALTB-268	Weekly x 4	120	SC	3	3	2	2

[a] Main Study animals were euthanized on Day 29. Recovery animals were euthanized on Day 85.

- **Summary**

4 weekly-repeated SC administration of ALTB-268 was well-tolerated in cynomolgus monkeys at dose levels up to and including 120 mg/kg/dose. There were no clinical observations, ophthalmic findings, alterations in food consumption, inject site reactions, electrocardiology abnormalities, changes in body weight, neurological/vital signs, cytokines, immunophenotyping parameters, and clinical pathology parameters, or macroscopic gross findings that were considered ALTB-268 related. Based on the results under the conditions of this study, the **no-observed-adverse-effect level (NOAEL)** was considered to be **120 mg/kg/week**. At the NOAEL, the combined gender mean Cmax and AUC (0-7 days) values following the last dosing occasion were 948 µg/mL and 5460 µg•days/mL, respectively.

Conclusions

- AltruBio discovered that anti-PSGL-1 agonistic antibodies, ALTB-168/ALTB-268, serve as immune checkpoint enhancers that down regulate TCR signaling and T cell effector function, restoring the immune system to a state of balance.
- ALTB-268 is tetravalent with higher potency compared to ALTB-168.
- Enhanced potency, excellent bioavailability, safety and tolerability enables ALTB-268 to be administered by subcutaneous delivery.
- These data support the clinical development of ALTB-268 for the treatment of T-cell mediated inflammatory diseases.

References

1. Huang CC, et al, Eur J Immunol. 2005; 35: 2239-49
2. Chen SC, et al, Blood 2004; 104: 3233-42
3. Tinoco R et al, Immunity, 2016; 44: 1190-1203
4. Hope et al, Cell Reports, 2023; 42: 112436