Targeting the CD6-ALCAM Pathway to Prevent and Treat Graft vs Host Disease

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Background

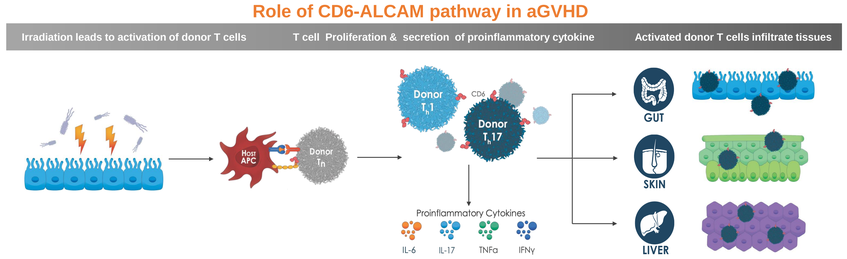
Clinical need:

- Acute graft versus host disease (aGVHD) causes significant morbidity and non-relapse mortality (NRM), occurring in ~50% of patients receiving allogeneic hematopoietic stem cell transplant (HSCT) with standard GVHD prophylaxis regimens.
- aGVHD commonly affects the skin, liver and gastrointestinal tract (GI). Of these, the GI manifestation is the most difficult to treat, with up to 60% incidence, and is a major cause of GVHD-related mortality.
- There are currently no FDA approved therapies for the initial treatment of newly diagnosed aGVHD.

T cells are a major driver of aGVHD:

- T effector cells, particularly CD4+ T helper cells, play a central role in the pathogenesis of aGVHD.
- **CD6** is a co-stimulatory receptor that is highly expressed on CD4+ T helper cells and binds activated leukocyte cell adhesion molecule (ALCAM/CD166), a ligand expressed on antigen presenting cells and various epithelial and endothelial tissues.
- The CD6-ALCAM pathway plays an integral role in modulating T cell activation and trafficking and is central to immune mediated inflammation.

Role of CD6-ALCAM pathway in aGVHD



Targeting the CD6-ALCAM pathway:

- Itolizumab is a humanized IgG1 mAb that binds to CD6 and blocks interaction with ALCAM, thereby inhibiting both the activity and trafficking of T cells without causing T cell depletion.
- Itolizumab has previously been developed and is approved for the treatment of Psoriasis in India, suggesting that it may be used to treat T-cell driven skin diseases. However, it has not been tested previously in gastrointestinal inflammation.

Objective:

We set out to test whether blockade of the CD6-ALCAM pathway was a relevant target to ameliorate aGVHD and if this approach could specifically be used to treat the GI manifestation of aGVHD.

Methods

CD6 Blockade with Itolizumab in humanized model of aGVHD

- Disease initiation: On Day 0, female NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ mice (NSG; Jackson Labs) were irradiated with 100 cGy/rad, followed by IV injection of 1x10^7 human PBMC four hours later. This initiates aggressive acute, xenogeneic GVHD.
- To evaluate the efficacy of itolizumab to prevent the onset of GVHD, mice were administered itolizumab (60 µg or 300 µg), abatacept (150 µg), belatacept (75 µg) or vehicle control (PBS) every other day via IP injection for 35 days beginning on study Day -1 (n=10/group; **Table 1**). Mice were evaluated every other day for body weight, clinical signs and survival. To evaluate the extent of human T cell proliferation, peripheral lymphocytes were immunophenotyped by flow cytometry at Day 10 and Day 35. Study was repeated to examine tissue histology and infiltration at Day 18.
- To evaluate the efficacy of itolizumab (EQ001) in both prevention and therapeutic treatment settings, itolizumab was administered every other day starting on Day -1 (prevention) or Day+5 (treatment) of disease initiation (**Table 2**). The Day +5 time point was chosen for treatment as this was the half-way point between disease initiation and median survival time; moreover, significant expansion of human CD45+ and CD3+ cells is observed at this time point.

Table 1. Groups to evaluate treatment of aGHVD

Group	Treatment	N	Dose	Dose Start
1.	Vehicle	10	N/A	D-1
2.	Itolizumab	10	60 µg	D-1
3.	Itolizumab	10	300 µg	D-1
4.	Abatacept	10	150 µg	D-1
5.	Belatacept	10	75 µg	D-1

Table 2. Groups to evaluate therapeutic treatment

Group	Treatment	N	Dose	Dose Start
1.	Vehicle	10	N/A	D5
2.	Itolizumab (prophylactic)	10	60 µg	D-1
3.	Itolizumab (therapeutic)	10	150 µg	D5

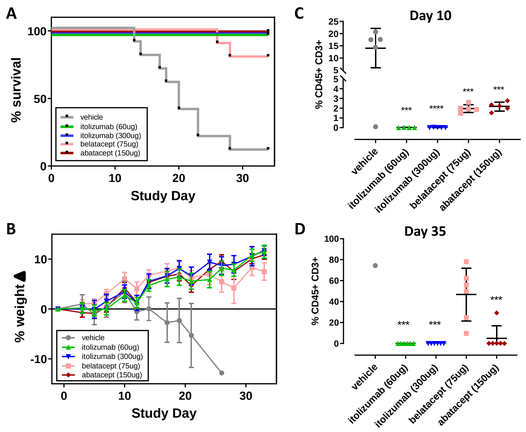
CD6 blockade in TNBS colitis model of gut inflammation

- Disease initiation: At Day 0, female BALB/c mice were administered 2,4,6-trinitrobenzenesulfonic acid (TNBS) intrarectally. TNBS induces colonic inflammation with infiltration by innate cells and Th1 and Th17 cells, important subsets in gut GVHD.
- To evaluate the efficacy of CD6 blockade on inflammation in this tissue, mice were treated with α-mCD6D1 monoclonal antibody (10D12), α-IL-12p40 monoclonal antibody, vehicle or dexamethasone (**Table 3**).

Table 3. Design of Study to Evaluate Gut Inflammation

Group	Treatment	N	Dose
1.	Naive	3	-
2.	Vehicle	12	PBS (EOD)
3.	α-mCD6D1 (10D12)	12	300ug (EOD)
4.	α-IL-12p40	12	1mg (D-1), 200ug (D1, 3, 5)
5.	Dexamethasone	12	1 mg/kg (QD, PO)

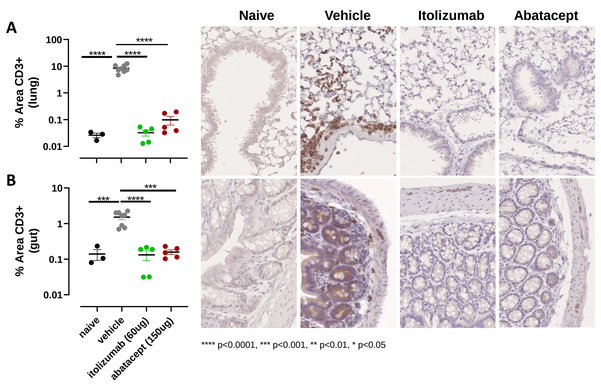
Figure 1. Itolizumab inhibits aGVHD



(A) Kaplan-Meier curve depicting survival by treatment group. Mice treated with itolizumab did not experience mortality compared to 90% mortality in the vehicle-treated mice. (B) Mice treated with itolizumab were protected from disease-related weight loss. Examination of human T cells (human CD45+CD3+) in peripheral blood revealed significant decreases in their prevalence at Day 10 (C) and these levels were maintained at Day 35 (D).

**** p<0.0001, *** p<0.001, ** p<0.01, * p<0.05

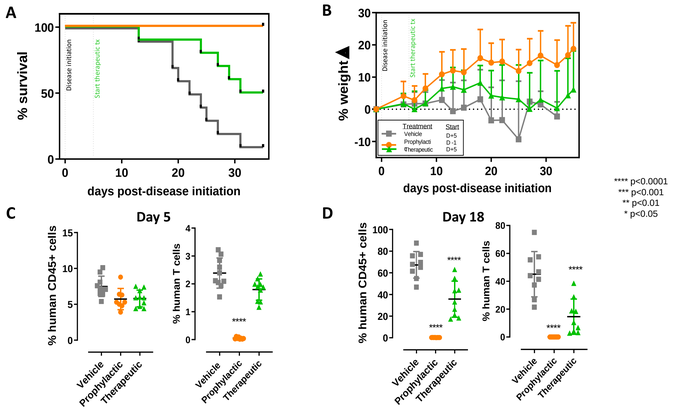
Figure 2. Itolizumab decreases T cell infiltration into tissues



**** p<0.0001, *** p<0.001, ** p<0.01, * p<0.05

Staining for human CD3 in the lung (A) and gut (B) . IHC staining revealed fewer infiltrating T cells in both tissues. Quantification of CD3 staining showed significant decrease in CD3+ T cell infiltration in itolizumab treated mice in comparison to vehicle control.

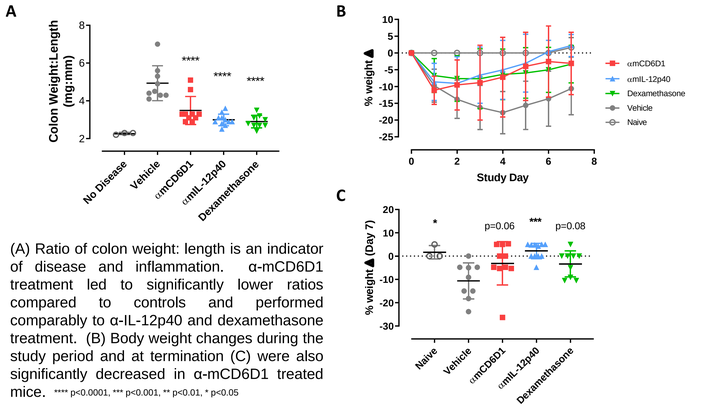
Figure 3. Treatment with itolizumab improves survival



**** p<0.0001
*** p<0.001
** p<0.01
* p<0.05

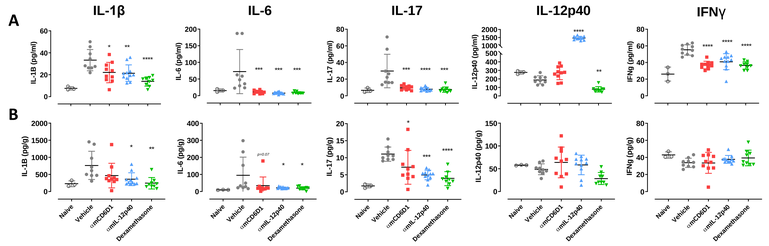
(A) Kaplan-Meier curve depicting survival by start of treatment with itolizumab. Mice treated with itolizumab at Day -1 were fully protected from mortality; mice that were treated at Day 5 experienced significantly less mortality vs. vehicle-treated mice. This same pattern was reflected in the % weight loss (B). (C) Examination of human lymphocytes (left panel) and human T cells (right panel) in peripheral blood show that at Day 5, prophylactic treatment significantly inhibited T cell proliferation. Mice that had not yet begun therapeutic treatment were similar to vehicle control mice. (D) By Day 18, mice that were therapeutically treated with itolizumab (Day 5) exhibited significantly lower of human lymphocytes and T cells vs. vehicle control mice.

Figure 4: CD6 blockade inhibits gut inflammation



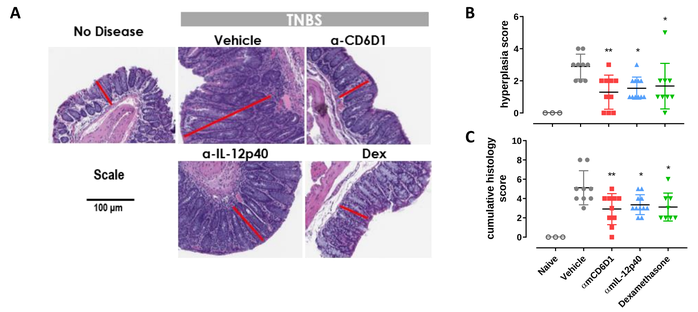
(A) Ratio of colon weight: length is an indicator of disease and inflammation. α-mCD6D1 treatment led to significantly lower ratios compared to controls and performed comparably to α-IL-12p40 and dexamethasone treatment. (B) Body weight changes during the study period and at termination (C) were also significantly decreased in α-mCD6D1 treated mice. **** p<0.0001, *** p<0.001, ** p<0.01, * p<0.05

Figure 5. CD6 blockade reduces inflammatory cytokines



(A) Inflammatory cytokines were quantitated in serum (A) and colon tissue (B). α-mCD6D1 treated animals experienced significant decreases in levels of IL-1β, IL-6, IL-17, and IFNγ in serum and of IL-1β, IL-6, IL-17 in tissue while IFNγ was not elevated in colon tissue of any of the diseased mice. This suggests that CD6 blockade is able to reduce inflammatory cytokines specifically in gut tissue. **** p<0.0001, *** p<0.001, ** p<0.01, * p<0.05

Figure 6. CD6 blockade inhibits gastrointestinal pathology



(A) H&E staining of colon. Red line delineates epithelial layer and illustrates hyperplasia of the tissue. Tissue sections were examined and scored by a trained pathologist to assess hyperplasia (B) and pathology (C). CD6 blockade resulted in improved hyperplasia and cumulative histology scores that were similar to treatment with αIL-12p40 and dexamethasone. **** p<0.0001, *** p<0.001, ** p<0.01, * p<0.05

Conclusions

- **We demonstrate for the first time that *in vivo* blockade of the CD6-ALCAM pathway can reduce incidence and severity of GVHD and associated gut inflammation, highlighting this as a promising therapeutic approach for GVHD and other T cell-driven inflammatory diseases.**
- Currently, a Phase 1b is ongoing to assess itolizumab in the treatment of newly diagnosed aGVHD. The **EQUATE trial** is currently open at US Centers; more information can be found on clinicaltrials.gov (NCT#03763318).