Itolizumab, a Novel Targeted Anti-CD6 Therapy, Induces Cleavage of Cell Surface CD6 and Rapid Onset of Efficacy in Subjects with Newly Diagnosed Acute Graft-Versus-Host Disease

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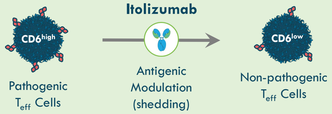
Introduction

- **CD6 is a co-stimulatory receptor** expressed on CD4 and CD8 T effector (T_{eff}) cells that modulates **T cell activity and trafficking**[1]
- The CD6 ligand, activated leukocyte cell adhesion molecule (ALCAM), is **expressed on antigen presenting cells and tissues,** including the skin and gastrointestinal (GI) tract
- CD6 and ALCAM are both **overexpressed during GI inflammation**[2]
- Prior clinical studies validated CD6 as a target relevant to acute graft-versus-host disease (aGVHD) pathogenesis[3]
- **Itolizumab** is a first-in-class antibody therapeutic targeting the CD6-ALCAM pathway by binding to domain-1 of CD6, causing CD6 shedding, and producing $CD6^{low}$ T cells (**FIGURE 1**)
- $CD6^{low}$ T cells are hyporesponsive to stimulation, exhibiting decreased activation, proliferation and cytokine production[4]
- Itolizumab is being evaluated as a treatment for immuno-inflammatory disease in which T_{eff} cells play a central role, including aGVHD

FIGURE 1. ITOLIZUMAB INDUCES CD6 SHEDDING AND PRODUCES $CD6^{LOW}$ NON-PATHOGENIC EFFECTOR T CELLS

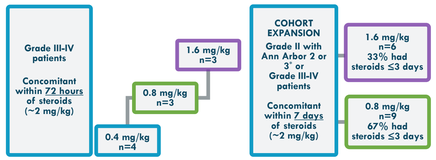


Methods

Study Design

EQUATE

Phase 1b open-label, 3+3, dose escalation study (NCT03763318)
5 itolizumab doses intravenously (IV) bi-weekly (Days 1, 15, 29, 43 & 57)
Subjects followed through Day 337



*Ann Arbor Score: 1, MAGIC Algorithm Probability (MAP) <0.141; 2, MAP 0.141-0.290; 3, MAP >0.290

- EQUATE is a Phase 1b study evaluating itolizumab treatment in subjects with aGVHD
- Primary objectives:
  - Assess the safety and tolerability of IV dosing of itolizumab
  - Determine the optimal IV dose level(s) of itolizumab
- Secondary objectives:
  - Characterize the pharmacokinetics and pharmacodynamics of itolizumab
  - Assess the clinical activity of itolizumab: aGVHD overall response rate (ORR), non-relapse mortality, chronic GVHD, and durability

Pharmacokinetic Methods

- Fresh whole blood was collected at clinical sites for serum collection
- A quantitative enzyme linked immunosorbent assay (ELISA) platform was used to detect and measure the levels of itolizumab in human serum samples

Pharmacodynamic and Immunophenotyping Methods

- Fresh whole blood was collected at clinical sites for peripheral blood mononuclear cell isolation
- Cell surface CD6 expression levels on T cells were determined using flow cytometry on fresh whole blood (performed by Precision For Medicine)
- Soluble CD6 was quantified in serum using a custom electrochemiluminescent assay (performed by Precision For Medicine)
- Flow cytometry staining panels were developed to assess T cell, B cell, innate and myeloid cell populations
- ST2 and REG3α were quantified by ELISA and the Mount Sinai Acute GVHD International Consortium (MAGIC) algorithm[5] was used to calculate the MAGIC algorithm probability (MAP) score (performed by Eurofins Viracor)

Results

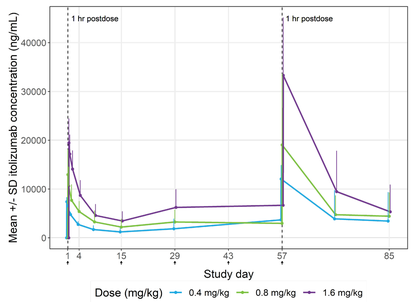
Study Population

- Here we present results from 23 adult subjects with Grade III-IV aGVHD who were enrolled in EQUATE and had PK and PD data available as of Sept 2021
- Subjects initiated IV steroid treatment within 7 days prior to the first dose of study drug
- Itolizumab was administered at dose levels of 0.4 mg/kg (n=4), 0.8 mg/kg (n=10), or 1.6 mg/kg (n=9) IV every 2 weeks
- All subjects received at least one dose and 16 (70%) received at least two doses of itolizumab

Pharmacokinetics

- Itolizumab serum concentrations peaked after the end of the IV infusion
- Thereafter, concentrations gradually decreased with a half-life of approximately 18 days (**FIGURE 2**)
- The increase in itolizumab exposures was approximately proportional to the increase in dose
- There was no evidence of target-mediated effects on itolizumab PK

FIGURE 2. SERUM CONCENTRATION-TIME PROFILE OF ITOLIZUMAB FOLLOWING IV INFUSION TO SUBJECTS WITH ACUTE GVHD

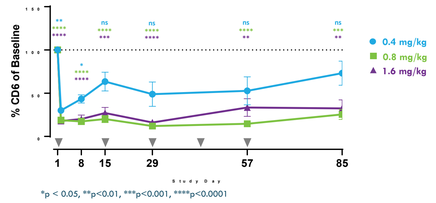


Arrows indicate study visits at which itolizumab doses were administered.

Pharmacodynamics

- Itolizumab rapidly decreased surface CD6 levels on T cells ($p<0.001$; all dose groups) in a dose-dependent manner (**FIGURE 3**)
- Surface CD6 levels remained below baseline throughout treatment
- At 0.4 mg/kg, CD6 trended toward baseline by Day 15; at higher doses, CD6 remained significantly reduced ($p<0.0001$)
- Similar results were observed in CD8+ T cells

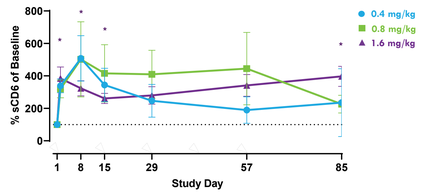
FIGURE 3. SURFACE LEVELS OF CD6 ON CD4+ T CELLS



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

- As cell surface CD6 decreased, there was a corresponding elevation in soluble CD6 (sCD6) in serum, indicating that itolizumab induces release of cell surface CD6 (**FIGURE 4**)
- Assessment of serum proinflammatory cytokines revealed a decrease from baseline in IL-2 receptor (data not shown)

FIGURE 4. SERUM SOLUBLE LEVELS OF CD6



Immunophenotyping

- By Day 15 following the first dose, itolizumab 0.8 and 1.6 mg/kg increased the ratio of T regulatory (T_{reg}) to T_{eff} cells (**FIGURE 5**)
- In addition, at Day 15, a decrease in the T cell activation marker programmed cell death protein 1 (PD-1) on T_{eff} cells was observed at all 3 dose levels, suggesting that itolizumab reduces the activation state of T cells (**FIGURE 6**)

FIGURE 5. DAY 15 T_{REG} : T_{EFF} CELL RATIO†

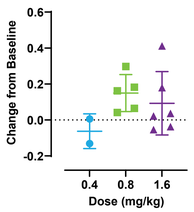
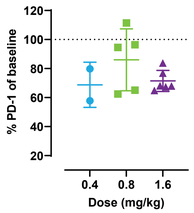


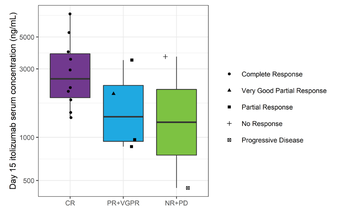
FIGURE 6. PD-1 ON T_{EFF} AT DAY 15†



Exposure-Response Analysis

- Since the majority (94%) of subjects who achieved a clinical response did so after the first dose, the relationship between itolizumab concentrations and efficacy was evaluated at Day 15
- Higher itolizumab concentrations correlated with a higher rate of complete response (CR) at Day 15 (mean concentration for CR, 3199 ng/mL; partial response [PR] or very good PR, 1823 ng/mL; no response or disease progression, 2048 ng/mL) (**FIGURE 7**)

FIGURE 7. EXPLORATORY EXPOSURE-RESPONSE RELATIONSHIP AT DAY 15



Shapes represent subject-level data as denoted in the legend. In the boxplots, the bold line represents the median, the lower and upper hinges correspond to the first and third quartiles, and the lower and upper whiskers extend to the smallest and largest values, respectively, within 1.5 times the interquartile range. Interim data; subject to change.
Note: The subject categorized as NR at Day 15 (with Grade IV skin aGVHD at Day 1) in the right panel was assessed as such due to organ staging criteria but was showing clinically significant improvement in skin symptoms by the Investigator and attending dermatologists.

MAGIC Biomarkers and Clinical Response

- At baseline (Day 1), 8 subjects had low MAP scores (≤0.290) and 10 subjects had high MAP scores (>0.290)
- Subjects with low baseline MAP scores had a higher CR rate at Day 15 (MAP low: 67%, MAP high:50% and a lower NRM rate at Day 169 (MAP low: 12.5%, MAP high: 50%; **FIGURE 8**); these numerical trends did not reach statistical significance, possibly due to small sample size
- The overall survival probability was higher in subjects with low MAP scores on Day 1 relative to those with high MAP scores on Day 1 (**FIGURE 9**)

FIGURE 8. CR & NRM RATES IN MAP LOW vs. MAP HIGH

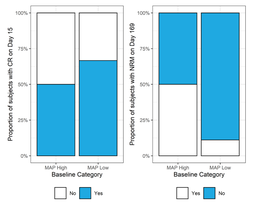
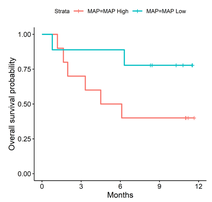


FIGURE 9. OVERALL SURVIVAL IN MAP LOW vs. MAP HIGH



Conclusion

- Itolizumab is associated with rapid and durable decreases in cell surface CD6 and increases in serum sCD6 in subjects with aGVHD
- The pharmacodynamic effects associated with the 0.4 mg/kg dose are likely insufficient for maximal efficacy
- The observed association between itolizumab serum concentrations and clinical response highlights the importance of achieving high concentrations early in the treatment period to maximize pharmacodynamic effects and clinical efficacy
- These results provide evidence of itolizumab activity at the doses evaluated in the EQUATE study and were used to optimize dose selection for the ongoing Phase 3 EQUATOR study (NCT05263999)

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Disclosures

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