Itolizumab-induced Modulation of Cell Surface CD6 is a Pharmacodynamic Marker of Drug Activity in SLE Patients

POSTER 1766

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Introduction

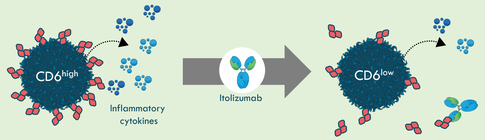
CD6 is a costimulatory receptor that is highly expressed on T cells and plays an important role in their activity and trafficking. CD6 promotes immune synapse formation, T cell activation, and migration into tissues by binding activated leukocyte cell adhesion molecule (ALCAM). Excessive activation through the CD6-ALCAM pathway has been implicated in the pathogenesis of multiple autoimmune and inflammatory diseases, including systemic lupus erythematosus (SLE) and lupus nephritis (LN). Therefore, the ability to modulate the level of T cell activation through the CD6-ALCAM pathway is beneficial to disease resolution.

Pathogenic activity

- Cytokine production
- Proliferation
- CD6-ALCAM pathway activation

Attenuated activity

- Cytokine production
- Proliferation
- CD6-ALCAM pathway activation



Itolizumab is a novel first in-class monoclonal antibody (hIgG1κ) specific for CD6 that is currently being evaluated as treatment for SLE and LN. Mechanistically, itolizumab has been shown to block the optimal engagement between CD6 and ALCAM, thereby decreasing signaling through the CD6-ALCAM pathway. Here, we demonstrate that prolonged exposure to itolizumab induces cleavage of cell surface CD6, leading to decreased T cell activity, and that the levels of surface and soluble CD6 in SLE patients dosed with itolizumab is a pharmacodynamic marker of drug activity. Results of clinical assessments are reported separately (poster 1750).

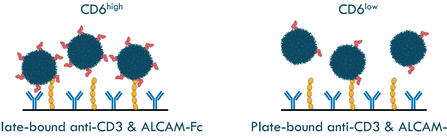
Methods

Evaluation of surface and soluble levels of CD6

Mechanistic studies were carried out ex vivo using PBMCs (peripheral blood mononuclear cells) from healthy volunteers. Samples from patients dosed with itolizumab, including blood, serum and urine, were collected as part of the EQUALISE trial, an open label Phase 1b 2-part study evaluating the safety, tolerability, pharmacokinetics (PK), pharmacodynamics (PD), and clinical activity of subcutaneous doses of itolizumab (0.4 to 3.2 mg/kg on Day 1 and Day 15) in patients with SLE (NCT04128579). Cell surface CD6 was assessed by flow cytometry using an anti-CD6 antibody that does not compete with the binding of itolizumab, while soluble CD6 in cell supernatants, serum and urine was quantified by an electrochemiluminescence assay.

Assessing functionality of CD6^{high} vs. CD6^{low} cells in vitro

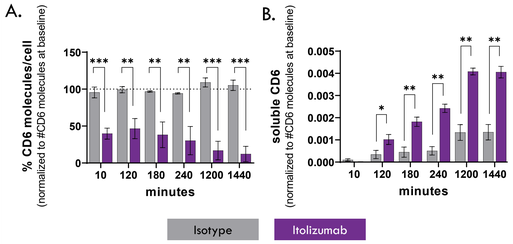
PBMCs are thawed and incubated with itolizumab or isotype at 37°C for 24 hours to generate CD6^{low} and CD6^{high} cells, respectively. Treated cells are harvested and washed with complete media to remove excess antibody treatment. CD6^{low} and CD6^{high} cells are activated via plate-bound anti-CD3 (clone: UCHT1) and ALCAM-Fc for at least 20 hours. Following stimulation, cells are harvested and stained for total surface levels of CD6 and activation markers (CD25, CD69, CD71 and PD-1).



Results

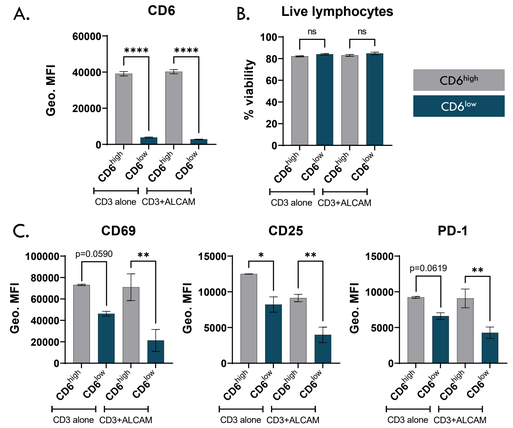
Itolizumab induces a time-dependent loss of CD6 from the T cell surface and a concomitant increase in soluble CD6 in the supernatant (FIGURE 1).

FIGURE 1. ITOLIZUMAB ALTERS SURFACE AND SOLUBLE LEVELS OF CD6



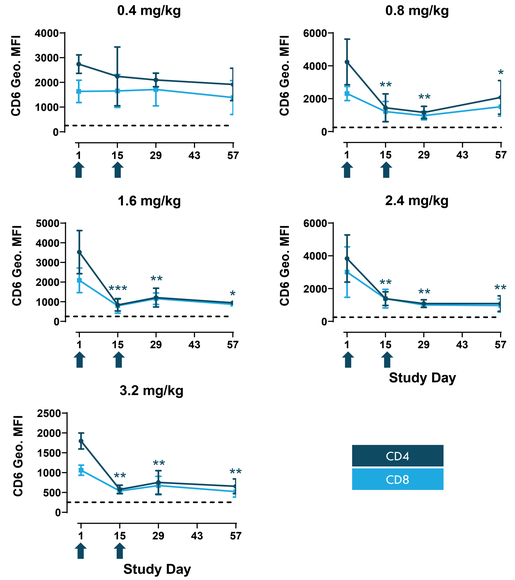
PBMCs from 3 different donors were incubated with 10 ug/mL of itolizumab and (A) cell surface expression of CD6 was assessed on CD4 T cells and (B) soluble CD6 was quantified in the PBMC supernatant at the indicated timepoints. The calculated number of CD6 receptors on CD4 T cells at baseline as assessed by flow cytometry was used to normalize values across the 3 donors. *** p<0.001, ** p<0.01, * p<0.05

FIGURE 2. CD6^{LOW} CELLS ARE HYPORESPONSIVE TO STIMULATION COMPARED TO CD6^{HIGH} CELLS IN THE PRESENCE OF ALCAM



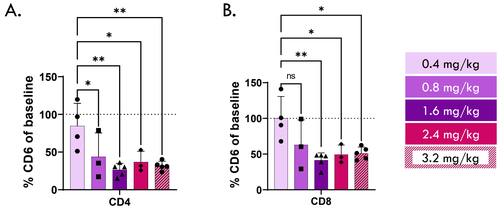
(A) Levels of CD6 on CD6^{low} cells remain significantly lower than CD6^{high} cells following activation even in the absence of itolizumab. (B) Differences observed between CD6^{low} and CD6^{high} are not due to differences in cell viability. (C) Surface expression of activation markers including CD69, CD25 and PD-1 is lower on CD6^{low} cells following stimulation in the presence of ALCAM. Similar trend observed with CD71 (data not shown). Data representative across three PBMC donors. Activation markers expressed as geometric mean fluorescence intensity (Geo. MFI), or average expression, on CD4+ cells. ****p<0.0001, **p<0.01, *p<0.05

FIGURE 3. LOW LEVELS OF SURFACE CD6 IS MAINTAINED WITH ITOLIZUMAB



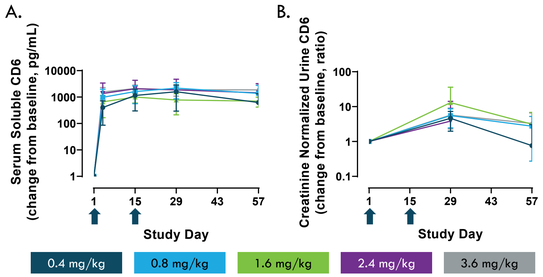
Surface levels of CD6 on CD4 and CD8 T cells decreased in SLE subjects from the EQUALISE trial following itolizumab treatment as expressed by average fluorescence of CD6. While baseline (pre-drug) levels of CD6 is variable across subjects, loss of surface CD6 is observed across all doses. Surface levels of CD6 throughout the course of the study is more variable on subjects treated with a lower dose of itolizumab (0.4 mg/kg). Subjects treated with a higher dose of itolizumab (0.8 – 3.2 mg/kg) maintain low levels of CD6 weeks after the last dose (FIGURE 3). Arrows indicate when subjects received itolizumab dose. Dashed line indicates no surface CD6 as determined by the fluorescence minus one (FMO) control. Statistics shown for CD6 on CD4 T cells compared to baseline. Total N=26 subjects included in analysis across the five dose cohorts.

FIGURE 4. DOSE-DEPENDENT REDUCTION IN SURFACE CD6 IN SLE PATIENTS FOLLOWING THE FIRST DOSE OF ITOLIZUMAB



Cell surface CD6 on (A) CD4 and (B) CD8 T cells decreases following the first dose of itolizumab and greater loss of surface CD6 is observed with higher doses of itolizumab. Data is expressed as % of baseline (pre-drug) and shown for Day 15 (14 days post the first dose, pre the second). **p<0.01, *p<0.05

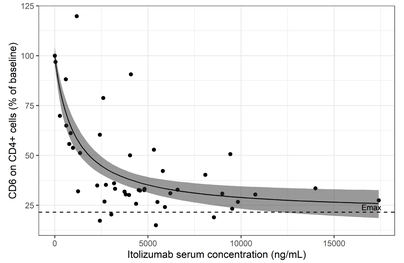
FIGURE 5. ITOLIZUMAB INCREASES SOLUBLE CD6 IN SERUM AND URINE



(A) Levels of soluble CD6 in serum and (B) urine increased following the first dose of itolizumab across all five cohorts of subjects enrolled in the EQUALISE trial. Data shown as change from baseline (pre-drug). Arrows indicate when subjects received itolizumab dose.

Levels of CD6 on CD4 T cells decreased with increasing itolizumab serum trough concentrations, with a maximum decrease of approximately 80% from baseline following two subcutaneous doses to patients with SLE (FIGURE 6).

FIGURE 6. ITOLIZUMAB REDUCES SURFACE CD6 IN A CONCENTRATION-DEPENDENT MANNER IN PATIENTS WITH SLE



Circles denote observed data, black line indicates model fit (Emax=-78.5%, EC_{50}=11009 ng/mL), gray shading represents 95% confidence intervals. Maximal loss occurs at 1.6 mg/kg, suggesting that higher doses are not necessary to achieve the greatest inhibition of T cell activity.

Conclusions

Cell surface CD6 regulates T cell activity and CD6^{high} cells are highly pathogenic with associations in numerous autoimmune and inflammatory diseases. Itolizumab induces loss of cell surface CD6 without T cell depletion thereby modulating hyperactive T cell activity and converts a pathogenic CD6^{high} cell into a less pathogenic CD6^{low} cell. Furthermore, CD6^{low} cells are hyporesponsive to stimulation even in the absence of itolizumab, suggesting that treatment with itolizumab results in prolonged modulation of T cell activity.

The same mechanism is observed in subjects dosed with itolizumab. Both cell surface and soluble CD6 can be used to monitor PD activity of itolizumab in serum and urine of itolizumab-dosed subjects. Both the decrease in surface CD6 and increase in soluble CD6 could have downstream effects on the resulting T cell phenotype as well as structural cells that express ALCAM, both of which are currently being investigated. In conclusion, our results show that the novel mechanism of CD6 modulation by itolizumab is efficacious in fine-tuning T cell activity. This may contribute to the resolution of diseases where the CD6-ALCAM pathway is highly active.

Acknowledgments

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Disclosures

The study was funded by Equillium, Inc. The presenting author (**DC**) declares the following conflicts of interest during the past 24 months in relationship to this presentation: Current employee, stockholder and owns intellectual property/patents of Equillium. **LC** is an employee and stockholder of Equillium and was an employee of Genentech/Roche and Principia. **JA** is an employee and stockholder of Equillium. **KP** has had consultancy roles and was an employee and stockholder of Equillium JR is an employee, officer, and stockholder of Equillium. **MF** is an employee and stockholder of Equillium and was an employee of Arena. **DT** is an employee, officer, and stockholder of Equillium and was an employee of Principia and current consults with Chinook. **CP** has had consultancy roles and received grant/research support with Equillium and has consultancy roles with Kidneycure and Progentec. **CN** is an employee and stockholder of Equillium. **SC** is an employee, officer, and stockholder of Equillium.

Additional Information

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