The Development and Characterization of AT-1501, an Anti-CD40LG Antibody Lacking Fc Effector Function

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Introduction

CD40LG is a costimulatory type II transmembrane receptor for CD40. T helper cell CD40LG binding to CD40 on antigen-presenting cells induces multiple downstream immune and inflammatory responses. These include B and T cell clonal expansion; antibody production, class-switching, and maturation; and pro-inflammatory cytokine and chemokine production. Hu5c8 is a humanized Ig1 antibody that binds with high affinity to CD40LG and showed promising therapeutic potential for organ transplant and autoimmune diseases. However, unpredicted on-target thromboembolic events caused by Fc-effector function and platelet activation occurred in human clinical trials, limiting its therapeutic potential (Kawai 2000; Boumpas 2003). AT-1501 is a humanized IgG1, kappa monoclonal antibody that blocks CD40LG (CD154, gp39) binding to CD40. AT-1501's ligand binding region is based upon Hu5c8, the humanized IgG1, kappa antibody with high affinity binding to CD40LG. AT-1501 was designed to mitigate the risk of thrombotic events by introducing-substitutions in the hu5c8 heavy chain hinge and hinge-proximal CH2 constant domain sequence (C220S/C226S/C229S/P238S, Kabat EU numbering). We analyzed AT-1501 binding affinity to CD40LG and verified blockade of CD40LG function on activated cells. We verified successful ablation of FC effector function compared to Hu5c8 by assessing binding to FCγRs and to C1q. Lastly, we verified lack of in vitro platelet activation in the presence of AT-1501-CD40 immune complexes.

Critical Objectives

(1) Assess binding affinity of AT-1501 clones to CD40LG compared to historical Biogen anti-CD40LG antibody Hu5c8 to ensure high affinity binding (Figure 1).

(2) Assess FC effector function of AT-1501 clones compared to Hu5c8. Based on historical data abrogation of FC effector is a critical component for clinical development (Figure 2).

(3) Assess ability of AT-1501 to block CD40LG signaling on human peripheral blood lymphocytes (Figure 3).

(4) Demonstrate that AT-1501 does not activate human platelets in vitro compared to Hu5c8 (Figure 4).

Methodology

Figure 1 CD40LG Binding: CD40LG binding potency was determined using an ELISA assay with recombinant human CD40LG (R&D or BioLegend) as the capture protein. Four clones of AT-1501 were compared to anti CD40LG antibody Hu5c8. Abatacept was used as a negative control. The binding curves were analyzed fitting each curve with a four parameter logistic function and determining the IC50s and confidence intervals for relative binding potency

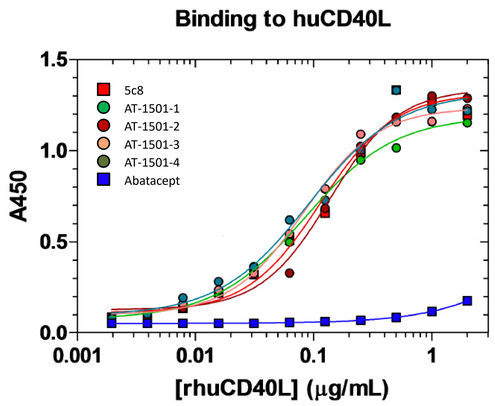


Figure 2 Binding to Fc Receptors: Fc receptor binding was determined using a sandwich ELISA assay with AT-1501, Hu5c8 parent antibody (Hu5c8 is also referred to as 5c8), or Abatacept (negative control antibody) as capture antibodies. Binding curves of recombinant human FcγRI, FcγRIIa, FcγRIIIa and FcγRIIIb (R&D Systems) were generated. The FcR binding curves were fitted using a four parameter logistic function.

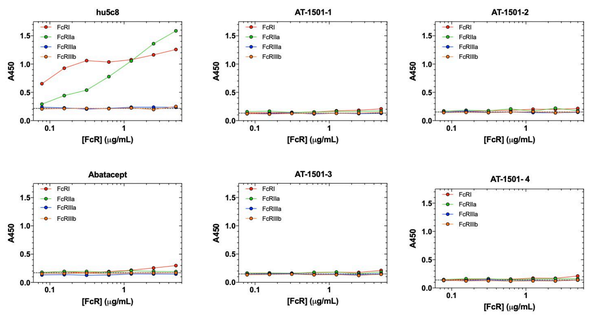


Figure 3: Binding to Human PBMCs: Human peripheral blood lymphocytes were isolated from heparinized whole blood and diluted in PBS. Prepared cells were activated by overnight exposure to a pre-mixed cocktail with optimized concentrations of PMA (phorbol 12-myristate-13-acetate) and ionomycin. Activation of cells by PMA and ionomycin leads to an increase in cell surface expression of CD154 (CD40LG), which can be detected by FACS after staining the cells with AF488-5c8-F(Ab')2. Competition for binding cell surface CD40LG was assessed by incubation with Abatacept (negative control), Hu5c8 or AT-1501.

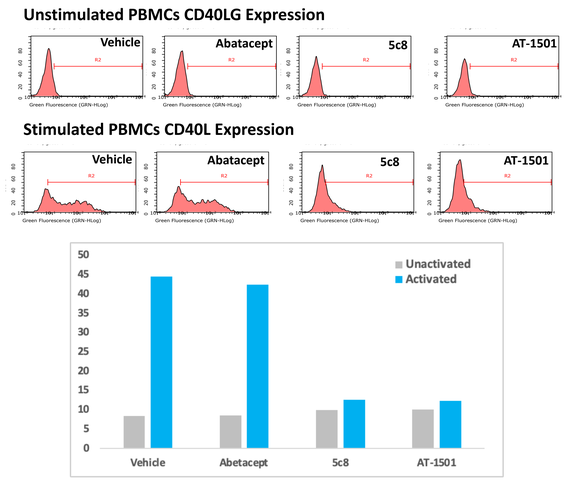
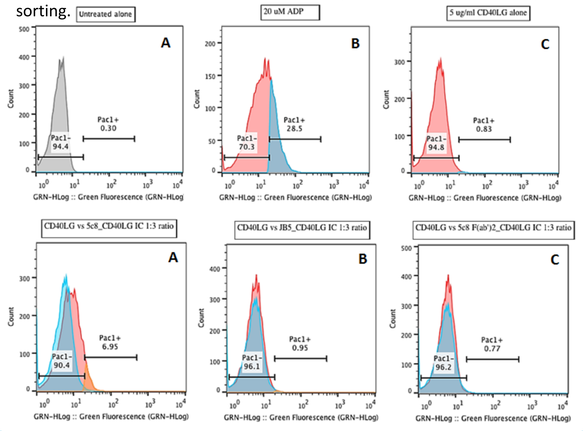


Figure 4: In Vitro Platelet Activation: Platelet activation was measured by flow cytometric detection of the well-established platelet activation marker PAC-1 (activated GPIIb/IIIa), which is not detectable unless platelets have been activated. Blood from three healthy volunteer donors was incubated with either ADP, a direct platelet agonist, or soluble CD40LG (sCD40LG) complexed to anti-CD40LG antibodies Hu5c8, AT-1501 (JB5), or to Hu5c8 F(Ab')2 lacking an Fc region. Complexes were incubated for 30 minutes at room temperature, then with PAC-1-FITC. Detection antibody was added followed by FACS sorting.



Results

AT-1501 and 5c8 have similar binding affinity to CD40LG, with an EC50 of 100 ng/mL. Abatacept, a CTLA4-Fc fusion protein that does not bind to CD40LG, was used as a negative control. Unlike Hu5c8, the amino acid modifications in AT-1501 ablate Fc-effector function leaving no detectable binding to FCγRs or C1q.

AT-1501 and Hu5c8 inhibited the binding of AF488-5c8-F(Ab')2 to activated human peripheral blood lymphocyte cells, whereas the negative control antibody Abatacept did not block AF488-5c8-F(AB')2 binding to CD40LG on activated PBMCs.

The incubation of immune complexes of intact Hu5c8:sCD40LG with platelets from healthy controls caused clear induction of PAC-1 expression on platelet surfaces when examined by FACS. Immune complexes of AT-1501 (JB5-D2):CD40LG produced no increase in detectable PAC-1. These results are consistent with the AT-1501's lack of Fc effector function and lack of ability to activate platelets.

PAC-1 staining was not apparent in untreated platelets, platelets that had been exposed to AT-1501-D2:sCD40LG IC, or hu5c8 F(Ab')2 (lacks Fc region) in the presence of sCD40LG. The positive control activator ADP and intact 5c8:sCD40LG IC caused platelet activation leading to surface PAC-1 expression, right-shifting PAC-1 fluorescence by FACS analysis.

Conclusion

- AT-1501 is a humanized IgG1 monoclonal antibody designed to lack Fc effector function.
- AT-1501 binds CD40LG with affinity similar to that of the antecedent anti-CD40LG antibody Hu5c8.
- AT-1501 does not bind FcRγ receptors and does not bind Complement.
- In vitro studies demonstrated AT-1501's ability to block CD40LG signaling on activated human peripheral blood mononuclear cells.
- Unlike Hu5c8, AT-1501 does not activate human platelets in the presence of immune complexes.
- Based on these data, AT-1501 is an inviting candidate for further IND-enabling studies and a potential candidate for clinical development.