

## **Challenge, Innovation and Impact Statement**

The clinical introduction of belatacept, a second-generation blocker of the CD28 costimulation cascade, has seen therapeutic benefits of improved renal function and reduction in donor-specific antibodies in renal transplantation. Furthermore, belatacept spares patients from the cardiovascular and renal fibrosis side effects of calcineurin inhibitors. However, belatacept is associated with higher rates of allograft rejection. An understanding of the pathways contributing to costimulation blockade resistant rejection (CoBRR) is essential. In murine and non-human primate studies, combined blockage of CD28 and CD40 pathways effectively promotes allograft survival. New reagents that selectively target CD40 and CD28 are available for investigation. Beyond this, infection and immune compromise represent the second leading cause of death in transplantation. There is a need to develop strategies to promote immune tolerance that may eliminate the need for long-term immunosuppression. The information gathered from this study will help to simplify immunosuppressive regimens, improve allograft rejection rates, and eliminate infection as a cause of patient morbidity and mortality.

## **Rationale**

T cell activation depends on interaction between TCR-MHC complexes and costimulatory pathways, CD28-CD80/CD86 and CD154-CD40. The Larsen lab and others have demonstrated that disruption of these costimulatory pathways is highly effective at inducing allograft tolerance. Belatacept blocks the binding of CD80/86 to CD28 and CTLA4, an inhibitory pathway that blocks T cell activation but can also activate regulatory T cells. Selective blockade of CD28 could inhibit the activation of effector T cells while allowing the CTLA4-CD80/86 interaction to continue.

Blockade of the CD154-CD40 interaction using anti-CD154 mAb was successful at extending allograft survival in murine and non-human primate models, but clinical investigation was halted secondary to increased risk of thromboembolism. In vitro and in vivo models demonstrated that thromboembolism was the result of Fc region interactions leading to platelet aggregation. Attention was then turned to selective targeting of CD40, however some studies suggest that anti-CD154 ligands are more successful at prolonging graft survival. A new reagent exists, BMS-CD154dAb, that selectively targets CD154 but has an Fc-inert tail thus eliminating the risk of thromboembolic events.

Analysis of autoimmune syndromes has led to an understanding of three important principles of self-tolerance that can be applied to the development of immune tolerance in transplantation: 1) decrease levels of donor-reactive T cells, 2) maintain donor-specific regulatory T cells, and 3) eradicate donor-reactive CD8 T cells. The first and second aims can be achieved by costimulatory pathway blockade as described above. Investigation in the lab of Dr. Rafi Ahmed has demonstrated that high antigen loads can eliminate the memory T cell's ability to proliferate and secrete cytokines. Repeated antigen challenges, using donor bone marrow, may help control donor-specific memory T cells.

## **Specific Aims**

We investigate two hypotheses in our research. **Hypothesis 1** is that selective blockage of CD28 and preservation of the CTLA4 signal using CD28dAb will result in superior renal allograft survival compared to belatacept. Blockade of CD154 with an Fc-silent CD154dAb will synergistically inhibit effector responses and prolong allograft survival. **Hypothesis 2** is that repeated exposure to donor antigen while receiving optimal costimulation blockade will allow for indefinite allograft survival. Testing these hypotheses involves two specific aims:

**Specific Aim 1:** To evaluate third-generation costimulation blockers of the CD40 and CD28 pathways in vitro and in vivo.

**Objective 1:** Assess the effects of third generation CD28 and CD40 pathway blockers on T cell responses in vitro.

**Objective 2:** Use the rhesus renal allograft model to investigate and compare third generation CD28 and CD40 pathway blockers in vivo.

**Specific Aim 2:** To assess the effects of a pulse tolerance induction regimen consisting of repeated antigen exposure during optimized costimulation blockade on renal allograft survival, anti-donor and protective immunity.

**Objective 1:** Test a tolerance pulse protocol using repeated donor bone marrow infusions and optimal costimulation blockade to induce hemopoietic chimerism in non-human primate models.

**Objective 2:** Develop renewable source of donor cells for tolerance maintenance.

**Objective 3:** Use a chemotherapeutic agent, busulfan, to decrease the need for supraphysiologic doses of bone marrow in non-human primate models.