iNKT Cell Depletion – Reappearance study in Cynomolgus Macaques

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Invariant Natural killer T (iNKT) cells are a small subset of T lymphocytes (ranging from 0.01 – 0.1% of CD3+ T cells) that share surface markers and functional characteristics with both conventional T cells and natural killer (NK) cells. Unlike T cells, they recognize glycolipid antigens presented by the MHC class-I-like protein CD1d rather than peptide antigens. In contrast to most T cell subpopulations, which have diverse sequences for their T Cell Receptors (TCRs), iNKT cells express a uniquely rearranged, highly conserved, semi-invariant TCR-α chain (Vα24-Jα18 in humans), which preferentially pairs with specific TCR-β chains (Vβ11 in humans). iNKT cells are rapid-onset cells with a universal receptor, like innate cells. They are also like the adaptive system because they share properties of T cells including required thymic positive selection and recognition of antigen presented on the MHC-I like molecule CD1d. As such, they serve as a bridge between the two systems where they can play both pro-inflammatory or immuno-regulatory roles either to enhance or attenuate developing immune responses, respectively. iNKT cells have been shown to be involved in mediating tissue injury and inflammation following ischemia reperfusion injury (IRI) in multiple organ systems. There is a growing understanding that chronic IRI is associated with the pathophysiology of Sickle cell disease (SCD). Our studies and that of others (Wallace et al. Blood 114:667-676, 2009) have found an increased ratio of activated iNKT cells in peripheral blood of patients with SCD at baseline compared to normal volunteers.

Figure 1: iNKT cell activation status

Healthy Volunteers: ~9% CD69+ iNKT Cells

SCD Patients: ~50% CD69+ iNKT Cells

Whole Blood samples were stained for iNKT cells using monoclonal antibodies against CD3, Vα24 as well as the anti Vα24-Jα18 invariant TCR loop mAb 6B11.

Activation was assessed by CD69 expression levels. Cells were gated on conventional T cells expressing the Vα24 variable alpha chain (Pink) as well as on iNKT (Blue) cells that express the Vα24 variable alpha chain but also the unique Vα24-Jα18 rearrangement

Our profile of blood iNKT cells of SCD patients vs. African American volunteers found that CD69+ iNKT cells of the SCD patients averaged 50.5% of all iNKT cells, while healthy control averaged 8.9% (Figure 1)
NKT Therapeutics has developed a fully humanized monoclonal Antibody (NKTT120) that specifically binds the CDR3 loop of the human and non-human primate (NHP) invariant T cell receptor. **Figure 2** shows the distribution of iNKT cell numbers in peripheral blood of humans and NHP (Cynomolgus Macaque). NKTT120 can deplete iNKT cells in human iTCR transgenic mice and NHPs. In the course of our IND enabling studies we conducted a depletion reappearance study in NHPs to learn more about the specificity of our antibody for iNKT cells and the ability of iNKT cells to recover following depletion. Animals (n=3 per group) were dosed once with 4 different concentrations of NKTT120 (0.01 mg/kg, 0.03 mg/kg, 0.1 mg/kg and 0.3 mg/kg) (Figure 3). iNKT cells and other lymphocytes were monitored daily for the first 7 days and weekly thereafter using flow cytometry. iNKT cell recovery was defined as all 3 animals in a group reaching a ratio of at least 0.03% iNKT cells of CD3+ T cells) for two consecutive weeks. iNKT cells were depleted within 24 hours at all doses. There was no significant change in other cells of the lymphocytic series. To date the 2 lowest dose groups of animals have recovered in week 5 and week 7, respectively.

**Figure 3: iNKT depletion and reappearance in Cynomolgus Macaques**

The kinetics of recovery in the higher dose animals is in accord with slower recovery after a expected full tissue depletion in these doses. While animals to date did not cross the arbitrary 0.03% threshold to call recovery, iNKT cells are measurable in all groups after full depletion. Overall our study showed that we can safely deplete iNKT cells in non-human primates and that iNKT cells can recover after depletion.

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