**­­­­­­­­­­­Development of a multiplex autoantibody assay for the screening of renal transplant recipients**

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**Background and Aim**: Pretransplant donor-specific anti-HLA antibodies are associated with a decreased kidney graft survival, whereas the relevance of autoantibodies or non-HLA antibodies remains uncertain. Depending on their specificity these non-HLA antibodies have been associated with chronic transplant glomerulopathy or a higher number of acute rejection episodes. Moreover, autoantibodies against non-HLA antigens have been found in sera from patients on the waiting list for kidney transplantation. Several small studies suggest that detection of preformed non-HLA antibodies might be useful in identifying patients at increased risk for graft loss. As there are no commercial assays available to determine these antibodies we are developing a multiplex autoantibody assay on a Luminex platform using magnetic beads (MagPlex® Microspheres). In this assay, patient sera will be incubated with magnetic beads coated with human recombinant proteins and the presence of IgG against these non-HLA proteins will be evaluated. Finally, we will determine the clinical relevance of pretransplant non-HLA antibodies on graft survival in a large cohort of renal transplant recipients. **Methods**: Based on a literature review, we selected 14 non-HLA antigens with wide expression in humans and most highly expressed in the kidney. First, 15 proteins (14 non-HLA proteins and transferrin as a negative control) were expressed in HEK293 cells. The vectors were designed in such a way that by using the restriction enzymes BglII and XhoI we can place the various inserts with the different proteins of interest. The inserts starts with the restriction site for BglII, then a signal peptide, HaloTag®, 6-His-tag, the gene of interest and ending with the restriction site XhoI. A signal peptide was included to promote protein secretion into the supernatant for easier protein purification. The 6-His-tag is useful for protein purification and the HaloTag® for the covalent binding of the protein to Luminex beads. **Results**: Using Western blot we confirmed that the correct HaloTag-6-His-Tag proteins were produced. The proteins were further purified with a HisTrap™ High Performance column on an AKTA Protein Purification system using 1M imidazole to elute them from the column. Several fractions were pooled and dialysed in PBS to remove any remaining imidazole that might interfere in binding to the magnetic beads. HaloTag Amine Ligands were conjugated to the beads via the activated carboxylic acid of the COOH radical group. Subsequently, all different HaloTag-6-His-Tag proteins will be coupled to different beads enabling a multiplex non-HLA autoantibody assay. As a final validation step, non-HLA specific antibodies will be used to determine whether the bead-protein complex is functional by measuring the median fluorescence intensity (MFI) on a LABScan 100 flow analyzer. Empty (uncoated) beads and beads coated with only HaloTag Amine Ligands will be used as a negative controls. **Conclusions**: Currently we have purified 9 of the 15 recombinant proteins and 2 of the Luminex assays are validated. In the coming months the remaining assays will be validated. Subsequently patient sera from the PROCARE cohort, comprising of all kidney transplant recipients in the Netherlands between 1995 and 2005, almost 5000 sera in total, will be screened for the presence of these non-HLA antibodies. From these assays the clinical relevance of these antibodies in renal transplant recipients will be determined.