REMOVAL OF MYELOID CELLS FROM AUTOLOGOUS LEUKOCYTES USED FOR CHIMERIC ANTIGEN RECEPTOR (CAR) T CELL MANUFACTURING IMPROVES FINAL PRODUCT CONSISTENCY AND YIELDS

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Early phase clinic trails of T cells genetically engineered to express Chimeric Antigen Receptors (CAR) have been promising. CD19-CAR T cells have been used successfully in a number of clinical trials to treat non-Hodgkin’s lymphoma and acute lymphocytic leukemia (ALL) and clinical trials of GD2-CAR T cells for the treatment of osteosarcoma and neuroblastoma are underway. Most CAR T cell manufacturing protocols make use of autologous peripheral blood mononuclear cell (PBMC) concentrates collected by apheresis, however, the lymphocyte-rich PBMC concentrates are also enriched for monocytes and contain small but variable quantities of red blood cells, platelets and neutrophils and prior to beginning CAR T cell manufacturing the PBMC concentrates are generally enriched for lymphocytes or CD3+ cells.

We initially manufactured CD19- and GD2-CAR T cells using autologous PBMC concentrates enriched for T cells by selection with the anti-CD3/CD28 beads. These same anti-CD3/CD28 beads were used to stimulated T cell expansion. While the method was, in general, effective, we found that the quantities of GD2-CAR T cells produced were less than the quantities of CD19 CAR T cells produced. In addition, T cells from some patients failed to expand at all. Further investigation found that the presence of large quantities of monocytes or granulocytes in some PBSC concentrates which was associated with poor in vitro CAR T cell expansion.

Myeloid derived suppressor cells (MDSCs) that inhibit T cell proliferation are present in sarcoma and ALL patients. These MDSCs may have a monocyte or neutrophil phenotype. We hypothesized that MDSCs in the PBSC concentrates bound non-specifically to the anti-CD3/CD28 beads and more rigorous enrichment of the starting material for lymphocytes would improve CAR T cell yields and reduce the incidence of manufacturing failures. We modified the T cell enrichment method to include a monocyte-depleting plastic adherence step. This change improved T cell expansion, but it was not completely effective at removing contaminating monocytes and granulocytes and did not completely eliminate manufacturing failures.

To provide better depletion of monocytes and granulocytes we subjected PBMC concentrates to counter flow elutriation instrument. We manufactured 8 CD19- and 5 GD2-CAR T cell products from elutriated lymphocytes. All 13 CAR T cell manufacturing procedures yielded sufficient quantities of T cells to meet the dose criteria. The 13 CAR T cell products contained 2,166±1,113 x10^6 CD3+ cells and 1,064±877 x10^6 transduced CD3+ T cells. The CAR T cell products manufactured from elutriated lymphocytes yielded significantly more CD3+ cells and transduced CD3+ cells than that of those manufactured from anti-CD3/CD28 bead selected and plastic adherence selected cells.

These results show CAR T cell manufacturing yields are greater and more consistent when manufacturing is initiated with lymphocytes that have few contaminating myeloid cells.