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to the DNA replicative, recombination and repair and nucleic acid metabolism pathways. Hierarchical clustering analysis of 347 differentially expressed cell cycle and apoptosis genes again separated the early and late passage cells into two groups and a node of genes up-regulated in late passages cells included two genes involved with replication senescence, cyclin-dependent kinase inhibitor 1A (p21, Cip1) (CDKN1A) and cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4) (CDKN2A) and one that inhibits TRAIL mediated apoptosis, tumor necrosis factor receptor superfamily, member 10d (TNFRSF10D, DCR2, CD264). Flow cytometry analysis confirmed greater expression of TNFRS10D on late passage cells. BMSC senescence is associated with distinct molecular changes. Early passage BMSCs may have more immune modulatory properties.

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THERAPEUTIC POTENTIAL OF GAMMADELTA T-CELLS IN CONTROLLING CMV AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Introduction: Allogeneic stem cell transplantation (allo-SCT) is hampered by GvHD, infections like CMV and relapse of disease. Gammadelta T-cells (gdT-cells) seem to be important in virus control but also in malignancy control. CMV infections are associated with an increased expansion of Vd1+T cells. Therefore, we investigated frequency and function of gdT-cells after allo-SCT in order to assess their therapeutic potential.
Methods: PBMCs at time points within 3 months after allo-SCT of 17 patients were sampled. CMV viral load was monitored by PCR. Phenotype and frequency of gdT-cells and alpha beta T-cells (abT-cells) were analyzed by flow cytometry. GdT-cells of 2 patients with CMV reactivation were isolated and expanded. Frequency and clonality of Vd1+, Vd2+ and Vd3+T cells were measured with spectratyping. The reactivity against CMV infected fibroblasts of Vd2- and Vd2+T-cells was tested using an IFN-g ELISPOT.
Results: We observed an increased expansion of Vd1+T-cells after allo-SCT in patients during CMV reactivation as assessed by flow cytometry and spectratyping. Furthermore Vd1+ but not Vd2+T-cells from these patients reacted against CMV infected fibroblasts. Moreover, following pp65-reactive abT-cells in HLA-A2+ patients indicated that Vd1+T cells precede an abT-cell response. Finally Vd1+T cells were able to induce antigen independent maturation of dendritic cells (DC) via CD1c.
Conclusion: GdT-cells are present in patients after allo-SCT, have the potential to eradicate CMV infected fibroblasts and possibly the potential to spread an immune response to abT-cells via DC maturation. This strongly supports the idea to explore gdT-cells as cell population for immune interventions after allo-SCT.

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DEVELOPMENT OF CD19-SPECIFIC CENTRAL MEMORY DERIVED T CELL PRODUCTS FOR THE TREATMENT OF CD19$^+$ HEMATOLOGIC MALIGNANCIES

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A key parameter affecting the therapeutic potency of adoptive T cell transfer is the extent to which infused cells persist and expand in vivo. Ex vivo expanded CD8$^+$ effector T cells derived from CD45RO$^+$CD62L$^+$ central memory (T_{CM}) precursors are epigenetically programmed to persist and revert to memory T cells following adoptive transfer. T_{CM} are a rare subpopulation of lymphocytes in peripheral blood, therefore we sought to develop a clinical-scale immunomagnetic purification method to isolate CD8$^+$ T_{CM} for subsequent genetic modification to express CD19-specific chimeric antigen receptors (CAR). Using a two step process by which monocytes, naïve and CD4$^+$ T cells are first depleted from PBMC, followed by positive selection of CD62L$^+$ T_{CM}, we have found that CD8$^+$ T_{CM} can be enriched from 2-8% to $>$ 80% purity. Following isolation, CD8$^+$ T_{CM} can be activated with CD3/CD28 Dynal beads, transduced two days later with cGMP grade self-inactivating lentivirus (MOI 1.5), then expanded with 50U/ml IL-2 and 0.5ng/ml IL-15. This process results in the procurement of cell numbers for clinical use within 3-6 weeks without further ex vivo stimulation (i.e., 1-3x10^9 final product from 7-15x10^6 CD8$^+$ T_{CM}). These T_{CM} -derived effector cell products ($T_{E(CM)}$) are 80-90% CAR$^+$ and exhibit CD19-specific cytolytic function and IFN-g production. Additionally, these products consist of a broad repertoire of $T_{E(CM)}$ based on TCR Vβ usage, retain expression of central memory markers such as CD62L/CD28, and, upon adoptive transfer into immunodeficient NOG mice, exhibit in vivo engraftment fitness in a huIL-15 dependent manner. This process has undergone a series of full scale qualification runs under cGMP and our group will be applying CD19-specific CD8$^+$ $T_{E(CM)}$ for post autologous HSCT adoptive therapy of high risk lymphoma as an innovative strategy to deliver engineered GVL effectors for eradication of minimal residual disease.

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BIOTHERAPY ORDER ENTRY (BOE) – ELECTRONIC PHYSICIAN ORDERS FOR CELLULAR THERAPY PRODUCTS

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Electronic systems for physician orders have largely focused on medications but orders for cellular products are also a potential source of communication errors that may affect patient safety. As part of a quality improvement effort, we developed and implemented an electronic order entry system for therapeutic cellular products of various types. Biotherapy Order Entry (BOE) was built to include three types of physician orders: 1) cell collection, 2) cell processing and 3) product release for administration. For each order type, electronic templates are created based on requirements of specific treatment plans or IRB-approved clinical research protocols. Each order set imports patient demographics from the hospital registration system and patient registration information from the Quality Assurance for Clinical Trials (QACT) information system. Individual templates for each treatment plan or research protocol are reviewed and approved before activation and orders can only be placed after consent to a treatment plan or protocol has been confirmed. For example, template orders for collection of hematopoietic stem cells specify cellular product type and the target number (or range) of cells requested. Processing orders for hematopoietic stem cells specify manufacturing steps such as CD34 selection, cryopreservation or thawing and desired cell dose. Release orders specify the cell type and cell dose needed for administration. When cryopreserved products are available for administration, physicians can select individual products for release. Since templates are specific for individual treatment plans or protocols, physician order choices are restricted by the requirements of the protocol each patient has been registered to. This system was implemented in June 2010 after extensive in-house planning, development, validation and end-user training. This quality improvement process gave us the opportunity to review and improve the workflow processes in all areas (clinical, collections, registration, processing, information management) related to cellular therapy.