transplantation. In 16 of the 34 patients the fungal infection was suspected at the beginning. According to the EORTC diagnostic criteria for IFI, 12 patients (75%), had a possible IFI and 4 cases (25%) presented a probable IFI. There were no cases with a proven IFI before death. *Results.* The autopsy demonstrated the presence of fungal infection in 10 patients: in 7 cases there was a clinical suspicion of fungal infection while in three cases it was an unexpected discovery in the autopsy. The organs shown up by the autopsy to be affected by the fungal infection were: lung (9 cases), digestive (6 cases), heart (2 cases), kidney (2 cases), CNS (2 cases) liver (2 cases) spleen (1 case), mediastinic mass (1 case), and pancreas (1 case). It is relevant that in most patients, the organic involvement other that lung was not suspected before their death, and it was responsible for very outstanding clinical manifestations during the end stage of the illness: superior vena cava syndrome (1 case), serious heart arrythmias (1 case), profuse diarrhea (1 case), renal failure (1 case), and hepatic failure (1 case). *Conclusion*: Our study shows high incidence of clinical suspected IFI at the end-stage disease not confirmed with the autopsy, and the complexity of the clinical manifestations associated to this type of infections.

## 1253

### CD40 LIGAND AND CALCIUM IONOPHORE TREATMENT OF DENDRITIC CELLS FROM HEALTHY DONORS AND PATIENTS WITH MONOCLONAL GAMMOPATHY OF UNKNOWN SIGNIFICANCE AND MULTIPLE MYELOMA

L. Kovarova, J. Michalek, M. Penka, R. Hajek

*University Hospital, BRNO, Czech Republic*

*Backgrounds.* Dendritic cells (DC) are the most potent antigen-presenting cells that can initiate adaptive immune response. They can differentiate from peripheral blood precursors and as an immature dendritic cells react to wide range of stimuli. Upon the activation/maturation process they change their phenotypic, morfologic and functional characteristics. The ability to acquire and activate blood DCs makes them a valuable source for future immunotherapy trials, but there are inconsistent reports about the functional state of dendritic cells from patiens with multiple myeloma (MM). *Aims.* Comparison of 48h treatment of immature dendritic cells with different stimuli as CD40 ligand (CD40L) and calcium ionophore (CI). Searching for differences in phenotype of DCs from healthy donors and patiens with MGUS and MM after stimulation. *Methods.* Ficoll-Hypaque-separated peripheral blood mononuclear cells (PBMC) from 10 healthy donors and 12 patients (7 MM and 5 MGUS) were used. Adherent precursors of DCs were cultured with GM-CSF and IL-4. CD40L and/or CI were added in day 1 or 4 to generate mature DCs. Multicolor flowcytometric analysis was done in day 0 and after harvest of DCs in day 3 or 6. Following monoclonal antibodies were used: CD11c, CD80, CD83, CD86, lineage mixture, CCR2, CCR5, CCR7, IL-12, MIP-1a, HLA-DR. *Results.* The highest percentage of CD83, characteristic marker of mature DCs, was found in 3rd day of culture after stimulation CD40L and also CI. In the 6th day was the average percentage of CD83 decreased to the half of 3rd day. There was found no differences between donors and patients. Expression of HLA-DR was relatively constant, independent on the time of the harvest or type of the stimulation and again without differences between groups of patients and donors. Expression of costimulation molecule CD80 slowly increased in 6th day of culture after CD40L stimulation, but CD86 was higher after CI stimulation. Chemokines receptors CCR2 and CCR5, markers of immature DCs, were expressed in low density as well as CCR7, marker of mature DCs. There was some evidence, that CCR7 was increased in healthy donors. Production of cytokine IL-12 and chemokine MIP-1a were also low. *Summary/conclusion*. Addition of CD40L and/or CI to an immature DCs obviously didn´t evoke their maturation, because there were found no strong expression of CCR7, IL-12 and MIP-1a. We didn´t found significant diferences between DCs generated from healthy volunteers and patients.

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## 1254

### THE PREPARATION OF MYELOMA-SPECIFIC CYTOTOXIC T CELLS BASED ON INTERFERON γ PRODUCTION

D. Ocadlikova,[1] L. Zahradova,[1] L. Kovarova,[1] M. Penka,[2] R. Hajek,[1] J. Michalek[3]

[1]*LEHABI, BRNO, Czech Republic;* [2]*Department of Clinical Hematology, BRNO, Czech Republic;* [3]*Cancer Immunobiology Center, DALLAS, USA*

*Backgrounds.* Autologous hematopoietic stem cell transplantation has been considered recently as part of a standard treatment strategy in patients with multiple myeloma (MM). Here we attempted to enhance the immunotherapeutic potential of autologous T cells based on selection of myeloma-reactive lymphocytes *in vitro*. *Aims.* The aim of this study was to identify and characterize autologous myeloma-reactive T cells *in vitro* and to evaluate their cytotoxic effect. *Methods.* Irradiated myeloma cell line ARH 77 or patient's myeloma cells were used as tumor antigen for dendritic cells loading. Peripheral blood mononuclear cells of 8 healthy volunteers and 10 MM patients were used for repeated stimulation of T lymphocytes. Activated T cells producing interferon γ were isolated using immunomagnetic separation (MACS) (Miltenyi Biotech) and expanded *in vitro* by phytohemaglutinin and high concentrations of interleukin 2. A specific cytotoxicity against myeloma cells was tested after the expansion with propidium iodide or 7-amino actinomycin D. Activated T cells were labeled with CFSE. Allogeneic T cells and interferon γ negative fraction of T cells served as controls. *Results.* In an allogeneic setting with ARH 77 cells the enrichment of interferon γ positive T cells by magnetic beads in healthy donors started from a median of 2.83% (1.97-4.58%) to 48.57% (15.14- 82.98%) after MACS and from 1.91% (1.14-3.4%) to 73.14% (3.9-88.75%) after MACS in $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells, respectively. Interferon γ positive T cells were further expanded *in vitro* from $0.5\times10^6$ to a median of $160\times10^6$ ($150\times10^6$-$420\times10^6$) T cells within 4 weeks and the test of cytotoxicity has demonstrated a high degree of specific killing of ARH 77 myeloma cells 69.17% (38.04-78.23%). Cytotoxicity of expanded interferon γ negative T cells was negligible. In an autologous setting with autologous myeloma cells used as an antigen, the enrichment of interferon γ positive T cells from MM patients started from 1.12% (0.27-6.2%) to 7.85% (0.42-12.6%) after MACS and from 1.9% (0.37-14.4%) to 14.7% (1.28-71.4%) after MACS in $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells, respectively. Interferon γ positive T cells were expanded in vitro from $0.12\times10^6$ ($0.05\times10^6$-$0.4\times10^6$) to $88.5\times10^6$ ($35\times10^6$-$226\times10^6$) within 8-12 weeks and the test of cytotoxicity has demonstrated only a modest specific killing of autologous multiple myeloma cells (18.88%) and allogeneic ARH 77 cells (18,21%). *Conclusions.* These data demonstrate a promising tumor-specific effect of allogeneic myeloma-reactive T cells but only a modest effect in an autologous setting in patients with MM. Whether that is due to a low MACS enrichment or low immunogenicity of autologous myeloma cell needs to be further clarified.

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## 1255

### AMINO ACID SEQUENCES OF T CELL RECEPTOR REACTING AGAINST MULTIPLE MYELOMA

S. Dudova,[1] L.K. Kovarova,[2] R.H. Horak,[2] R.H. Horvath,[3] M.P. Penka,[4] R.H. Hajek,[5] D. Ostroveanu[6]

[1]*FN Brno, BRNO, Czech Republic;* [2]*LEHABI, FN Brno, BRNO, Czech Republic;* [3]*Genex CZ, BRNO, Czech Republic;* [4]*OKH, FN Brno, BRNO, Czech Republic;* [5]*IHOK, FN Brno, BRNO, Czech Republic;;* [6]*Fundeni Clinical Institute, BUCHAREST, Romania*

*Backgrounds.* Multiple myeloma (MM) is a disease caused by malignant proliferation of B lymphocytes in the bone marrow. Recently, high-dose chemotherapy with autologous hematopoietic transplantation has been considered a standard treatment for patients with advanced stages of MM. Such treatment delays relapse but it is not curative and almost all patients ultimately develop recurrent disease. Based on preclinical and clinical studies it is evident that myeloma-reactive T lymphocytes play an important role in immunologic response to this malignant disease. Myeloma-reactive T lymphocytes have been shown to be a promising approach in adoptive cellular immunotherapy aside autologous transplantation of bone marrow graft. *Aims.* Our aim was to analyse T cell receptor (TCR) sequences reacting against multiple myeloma. Experimental study was performed in 10 patients to provide information on the specificity and spectrum of recognized antigens. *Methods.* Dendritic cells loaded with apoptotic bodies from magnetically isolated myeloma cells have been used to stimulate autologous T lymphocytes. Activated myeloma-specific T cells were identified and expanded. After mRNA isolation the anchored reverse transcription using modified version of SMART method was done. PCR product was cloned into plasmid vector, transformed in bacterial cells and individual clonotypes were sequenced. *Results.* Oligoclonality of TCR receptor was demonstrated in myeloma specific *in vitro* expanded T lymphocytes, in one case monoclonal population of tumor specific T cells was found. These findings support the assumption of myeloma specific antigens stimulating only certain autologous T lymphocytes. *Conclusions.* Structural characterization of TCR receptor of myeloma specific clones provides further evidence for the role of these T lymphocytes in immunotherapy. Receptor

sequence determination can be used as a marker for evaluation of the vaccine strategy.

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## 1256

### CRITERIA FOR CORD BLOOD DONOR SELECTION ON THE BASIS OF ROC CURVE ANALYSIS

M. Solves,[1] A. Perales,[2] V. Mirabet,[1] M. Arnao,[3] A. Soler,[1] R. Roig[1]

[1]*Transfusion Centre, VALENCIA, Spain;* [2]*Hospital Universitario la Fe, VALENCIA, Spain;* [3]*Hospital de la Ribera, ALZIRA, Spain*

The main limitation factor for a wide use of umbilical cord blood (CB) for transplantation is the cell dose. In this sense, many cord blood banks have set a total nucleated cells (TNC) content ranging from 60 to 100 x 107 as minimum required values for storing the units. In order to optimise cord blood banking and reduce the number of UCB units deferred before processing, an effort in donor selection is mandatory. Many authors have showed that placental and neonatal weight influence hematopoietic content of cord blood units. To establish obstetric criteria for selection of cord blood units before cryopreservation. In order to determine the optimal placental and neonatal weight for selecting cord blood donors according to the number of TNC, we have performed Receiver Operating Characteristic (ROC) curve analysis. ROC curve is a graphical technique commonly used to find optimal cut off value of a test using sensitivity and specificity data. We thought it could be useful to determine cut off values of placental and neonatal weight for an optimal selection of UCB units.

**Table 1.**

| *TNC×10⁷* | *Cut-off* | *Area under the* | *95% confiance* |
|---|---|---|---|
| 60×10⁷ | | | |
| Neonatal weight | ≥ 3190 | 0.635±0.013 | 0.616-0.653 |
| Placental weight | ≥ 646 | 0.685±0.013 | 0.666-0.704 |
| 70×10⁷ | | | |
| Neonatal weight | ≥ 3195 | 0.638±0.012 | 0.619-0.656 |
| Placental weight | ≥ 645 | 0.682±0.012 | 0.662-0.701 |
| 80×10⁷ | | | |
| Neonatal weight | ≥ 3195 | 0.632±0.011 | 0.614-0.651 |
| Placental weight | ≥ 635 | 0.676±0.011 | 0.656-0.695 |
| 90×10⁷ | | | |
| Neonatal weight | ≥ 3195 | 0.631±0.011 | 0.612-0.649 |
| Placental weight | ≥ 635 | 0.648±0.011 | 0.629-0.668 |
| 100×10⁷ | | | |
| Neonatal weight | ≥ 3195 | 0.624±0.011 | 0.605-0.642 |
| Placental weight | ≥ 635 | 0.637±0.011 | 0.617-0.657 |

*Results.* We revised 2590 cord blood units collected at Valencia Cord Blood bank for a four-year period. Mean TNC content of UCB before processing was 107.65→54.74×07. Mean neonatal weight and placental weight were 3313.36→430.7 g. and 652.2→122.1 g. ROC curve analysis was performed with MedCalc software for windows v. 7.4.2.0. Variable was considered 0 or 1 if TNC was < or > 60, 70, 80, 90, and 100×107, respectively and classification variables were considered placental weight and neonatal weight. Results are shown on the following Table. We conclude this statistical analysis can be helpful to determine cut off value of placental/neonatal weight according to the required limit of TNC for each bank. This approach would reduce the number of collected units that are refused before processing.

## 1257

### ANALYSIS OF THE CD34+ CELLS CONTENT OF THE CORD BLOOD UNITS STORED IN A REGIONAL CORD BLOOD BANK

M. Solves,[1] V. Mirabet,[1] M. Arnao,[2] F. Carbonell-Uberos[1], D. Planelles,[1] A. Soler,[1] R. Roig[1]

[1]*Transfusion Centre, VALENCIA, Spain;* [2]*Hospital De La Ribera, ALZIRA, Spain*

Some clinical studies have shown that graft selection should be based principally on CD34⁺ cell dose and grafts should contain at least 1.7 x $10^5$ CD34⁺ cells per kilogram of recipient´s body weight. However, criteria for selecting collections suitable for freezing and storage are not standardized. Although most banks have set a total nucleated cells (TNC) content ranging from 60 to 100x$10^7$ as initial minimum required values for storing the units, only a few banks selecting cord blood units on the basis of their CD34⁺ cell content. Aims. To analyse the CD34⁺ cell content of the cord blood units stored at the Valencia cord blood bank and the characteristics of the units according to their CD34⁺ cell content. We reviewed the data of 2149 cord blood units stored at Valencia cord blood bank and selected on the basis of their TNC content (more or equal than 100×$10^7$). CD34⁺ cells were quantified by flow cytometry. CB sample was taken directly from the bag and after volume reduction-before cryopreservation and 5×10× cells were incubated using monoclonal antibodies conjugated CD45 fluorescein and CD34 phycoerythrin (Becton Dickinson) and 7 amino-actomicin D as marker of DNA staining. Flow cytometric analysis was performed using Cell Quest software. ProCount progenitor cell enumeration kit was used in comparison with our standard protocol, giving similar results. Total CD34+ cells content was calculated by multiplying the CD34 percentage per TNC. A total of 2149 cord blood units were stored for a 5 years-period. Mean TNC, CD34⁺ cell percentages and total CD34⁺ cells were112.37→37.17×$10^7$, 0.36 → 0.25% and 41.79→34.74×$10^5$, respectively. From these units, 489 (22%) had a total CD34⁺ cell content less than 20×$10^5$. Characteristics of the units according to their CD34 cell content are shown in the table. Conclusions. In order to increase the quality of cord blood units stored, the CD34 cell content should be considered as a selection criteria of cord blood units for cryopreservation and storing.

**Table 1.**

| CD34+ Content x 10e5 | N (% of stored units) | CD34+ x 10e6 | CD34+ (%) |
|---|---|---|---|
| < 20 | 489 (22.5%) | 12.18 ± 5.49 | 0.13 ± 0.06 |
| ≥ 20 | 1660 (77.5%) | 50.51 ± 34.92 | 0.43 ± 0.24 |
| ≥ 30 | 1218 (56.6%) | 59.80 ± 36.53 | 0.49 ± 0.25 |
| ≥ 40 | 865 (40.2%) | 69.98 ± 38.96 | 0.55 ± 0.27 |
| ≥ 50 | 610 (28.4%) | 80.53 ±4 2.1 | 0.62 ± 0.29 |
| ≥ 60 | 434 (20.2%) | 91.15 ± 45.8 | 0.68 ±0.32 |
| ≥ 70 | 292 (13.6%) | 104.14 ± 50.99 | 0.74 ± 0.36 |
| ≥ 80 | 223 (10.3%) | 113.14 ± 55.33 | 0.79 ± 0.39 |
| ≥ 90 | 160 (7.4 %) | 124.40 ± 61.81 | 0.84 ± 0.44 |
| ≥ 100 | 106 (4.9%) | 139.60 ± 71.34 | 0.88 ± 0.52 |

## 1258

### MESENCHYMAL STEM CELLS CONTRIBUTE TO THE HEALING PROCESS AND FUNCTIONAL IMPROVEMENT OF ISCHEMIC INJURED KIDNEY IN RAT MODEL

S.K. Park, S.B. Bae, C.K. Kim, N.S. Lee, K.T. Lee, J.H. Won, D.S. Hong, H.S. Park, J.K. Kim

*Soonchunhyang University Hospital, BUCHEON-SI, GEYONGGI-DO, South-Korea*

*Objective*. Renal failure is a common disease with high morbidity and mortality. Ischemic injury is one of the most common cause of renal failure. Recent studies have reported that adult bone marrow-derived cells can contribute to renal remodeling and a dramatic repopulation of the mesangium. Moreover, there was a report that the role of bone marrow-derived hematopoietic stem cells in the regeneration of the renal tubular epithelium after ischemic injury in mice. When ischemic injury is inflicted on targeted organ, MSCs may migrate to the site of damage, undergo differentiation, and promote structural and functional repair. We evaluated whether bone marrow-derived MSCs contribute to the healing process and improve renal function in injured kidney of rat by ischemia. *Materials and Methods.* Right nephrectomy was performed in six-week-old SD rat. And the left renal artery and vein were clamped for 45 min followed by 2/3 nephrectomy was done and then clamp releases to allow perfusion. MSCs prelabeled with green fluorescent protein (GFP) injected via tail vein. Peripheral blood was collected serially for evaluation of blood urea nitrogen and creatinine and functional evaluation was done with radioisotope renal scan. Histologic study and confocal microscopic evaluation were performed at 4 days, 1 week, and 4 weeks after MSCs injection. *Results.* We demonstrated that GFP positive cells were detected in damaged kidney by confocal microscopy and engrafted MSCs promoted healing process by ischemic injury. Also engrafted MSCs differentiated into tubular epithelial cells, thereby restoring renal structure. In the group with MSCs injection, the levels of blood urea nitrogen and creatinine were lower than control group without MSCs injection (BUN Day 4, control group; 65.0±8.1, MSC infusion group; 31.1±5.1). And MSCs injected rats demonstrated that renal func-