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 was an obvious fungal infection. They were considered of undetermined origin. The pathology report described the image of candidiasis in the lungs of 8 patients. The organs 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 phytohemagglutinin and high concentrations of interleukin 2. A specific cytotoxicity against myeloma cells was tested after the expansion and induction of interferon 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 cells by magnetic beads in healthy donors started from a median of 2.85% (1.97-4.58%) to 48.57% (15.14-82.96%) after MACS and from 1.91% (1.14-3.34%) to 73.14% (9.89-85.75%) after MACS in CD3+CD4+ and CD3+CD8+ T cells, respectively. Interferon γ positive T cells were further expanded in vitro from 0.5×10^6 to a median of 160×10^6 (150×10^6-420×10^6) 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% (54.94-72.35%). Cytotoxicity against allogeneic MM 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% (0.57-14.4%) to 14.7% (1.28-71.4%) after MACS. The highest percentage of CD83, 2.83% (1.97-4.58%) to 48.57% (15.14-82.98%) after MACS and from 0.5 ×10^6 to 88.5×10^6 (35×10^6-226×10^6) 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 autologous myeloma-reactive T cells but only a modest effect in an autologous setting in patients with MM. Whether that is due to low MACS enrichment or low immunogenicity of autologous myeloma cells need to be further clarified.

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1253
CD40 LIGAND AND CALCIUM IONOPHORE TREATMENT OF DENDRITIC CELLS FROM HEALTHY DONORS AND PATIENTS WITH MONOCLONAL GAMMOPATHY OF UNKNOWN SIGNIFICANCE AND MULTIPLE MYELOMA
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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 cell present antigens to T lymphocytes. 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), mediastinum mass (1 case), and pancreas (1 case). It is relevant that in most patients, the organic involvement other than 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 arrhythmias (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.

1254
THE PREPARATION OF MYELOMA-SPECIFIC CYTOTOXIC T CELLS BASED ON INTERFERON γ PRODUCTION
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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. Irradiated autologous 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 phytohemagglutinin and high concentrations of interleukin 2. A specific cytotoxicity against myeloma cells was tested after the expansion and induction of interferon D. The highest percentage of CD83, 2.83% (1.97-4.58%) to 48.57% (15.14-82.98%) after MACS and from 0.5 ×10^6 to 88.5×10^6 (35×10^6-226×10^6) within 8-12 weeks and the test of cytotoxicity has demonstrated a high degree of specific killing of ARH 77 myeloma cells 69.17% (54.94-72.35%). Cytotoxicity against allogeneic MM 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% (0.57-14.4%) to 14.7% (1.28-71.4%) after MACS. The highest percentage of CD83, 2.83% (1.97-4.58%) to 48.57% (15.14-82.98%) after MACS and from 0.5 ×10^6 to 88.5×10^6 (35×10^6-226×10^6) 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 autologous myeloma-reactive T cells but only a modest effect in an autologous setting in patients with MM. Whether that is due to low MACS enrichment or low immunogenicity of autologous myeloma cells need to be further clarified.

Funding: Supported by the grant: IGA MZCR 1A/8907-5.

1255
AMINO ACID SEQUENCES OF T CELL RECEPTOR REACTING AGAINST MULTIPLE MYELOMA
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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 5 patients in 10 patients to provide the evidence 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, TCR-reacting reverse transcription using modified version of SMART method was done. PCR product was cloned into plasmid vector, transformed in bacterial cells and individual clones 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 supported 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**

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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 10^7 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 values of 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

<table>
<thead>
<tr>
<th>TNC x 10^7</th>
<th>Cut-off</th>
<th>Area under the ROC curve</th>
<th>95% confidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>60 x 10^7</td>
<td>Neatant weight</td>
<td>≥3190</td>
<td>0.635±0.013</td>
</tr>
<tr>
<td>60 x 10^7</td>
<td>Placental weight</td>
<td>≥646</td>
<td>0.685±0.013</td>
</tr>
<tr>
<td>70 x 10^7</td>
<td>Neatant weight</td>
<td>≥3195</td>
<td>0.638±0.012</td>
</tr>
<tr>
<td>70 x 10^7</td>
<td>Placental weight</td>
<td>≥645</td>
<td>0.682±0.012</td>
</tr>
<tr>
<td>80 x 10^7</td>
<td>Neatant weight</td>
<td>≥3195</td>
<td>0.632±0.011</td>
</tr>
<tr>
<td>80 x 10^7</td>
<td>Placental weight</td>
<td>≥635</td>
<td>0.676±0.011</td>
</tr>
<tr>
<td>90 x 10^7</td>
<td>Neatant weight</td>
<td>≥3195</td>
<td>0.631±0.011</td>
</tr>
<tr>
<td>90 x 10^7</td>
<td>Placental weight</td>
<td>≥635</td>
<td>0.648±0.011</td>
</tr>
<tr>
<td>100 x 10^7</td>
<td>Neatant weight</td>
<td>≥3195</td>
<td>0.624±0.011</td>
</tr>
<tr>
<td>100 x 10^7</td>
<td>Placental weight</td>
<td>≥635</td>
<td>0.673±0.017</td>
</tr>
</tbody>
</table>

**Results:** We revised 2590 cord blood units collected at Valencia Cord Bank for a four-year period. Mean TNC content of UCB before processing was 107.65±54.74 x 10^7. 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 < 60, 70, 80, 90, and 100 x 10^7, 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.

### 1258

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


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**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 radiouisotope renal scan. Histologic study and confoocal 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 confoocal 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±3.1, MSC infusion group; 31±5.1). And MSCs injected rats demonstrated that renal func-

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