preliminary results do not show an increase in the early or late apoptosis of CD34+ cells, following cryopreservation. We hope to standardise this method for routine evaluation of CD34 + cell viability of the grafts to be used in haematopoietic transplantation. This work was supported by a grant from CFICS n°266/99 - Ministério da Saúde

360 POSTER

### Relationship between immune abnormalities post-high dose chemotherapy with stem cell support in patients with solid tumors and tumor type and stage

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**Background:** High-dose chemotherapy (HDC) with stem cell rescue induces profound immunosupression. Recovery of cell-mediated and antibody-mediated immunity takes 1-2 years and inversion of CD4/CD8 ratio persists for at least 1 year. Infusion of peripheral blood derived hematopoietic stem cells(PBSC) results in faster recovery of blood counts than bone marrow infusion. Is immunological recovery also faster?

**Aims:** We have evaluated immunological recovery after HDC+ PBSC and factors influencing immune recovery.

**Patients and methods:** Lymphoid subpopulations in peripheral blood were quantified by flow-citometry using surface markers CD3, CD4, CD8, CD19 and CD56. IgG, IgA, IgM and IgE concentrations were also measured. These parameters were measured 1, 2, 3, 6, 9, 12, 145, 18, 21 and 24 months (mo) after PBSC infusion in 41 consecutive patients (p) (9 males ans 32 females) treated with HDC+PBSC at our institution for metastatic breast cancer (20 pts), non-metastatic high-risk breast cancer (>10 axillary nodes or stage III) (10 pts), non-Hodgkin's lymphoma (7 pts) or other solid tumors (4 pts).

**Results:** The duration of cellular and humoral immune recovery was markedly different according to tumor type and stage but not to the number of CD34+ cells infused. As for cell-mediated immunity, median time to CD4/CD8 >0.8 was 3 mo (range 2-6) for pts with non-metastatic breast cancer versus 9 mo (2-24+) for metastatic breast cancer ($p<.05$), 6 mo (1-9+) for non-Hodgkin's lymphoma and 6 mo (1-8+) for other tumors.

**Conclusions:** Cell-mediated immune recovery after HDC+PBSC is faster than that reported for bone marrow infusion and differs according to tumor type and stage.

## Growth factors/cytokines

361 POSTER

### High incidence of thrombosis using G-CSF in the treatment of chemotherapy-induced neutropenia

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**Background:** G-CSFs are widely used as potent myelopoietic stimulators. However, its activity is not restricted to the myelopoietic system and several observations suggest that G-CSF could interfere with the hemostatic balance. The prevalence of thrombosis in cancer patients has been estimated as up to 15%. In order to assess the interaction between G-CSF and hemostasis, a retrospective analysis, was performed on 409 patients, treated with G-CSF and chemotherapy from 1996 to 2000.

**Patients and Methods:** 287 were females and 122 were males, the mean age was 63,4 years, (range 43-76). No significant risk factors were detected; All pts had normal renal, hepatic and hematologic function and were divided in three groups according to the number of total treatments with subcutaneous G-CSF at the standard dosage of 5 mg/kg. In the first cohort (A) (n=188, 46%) pts were treated from 1 to 5 administrations of G-CSF, while the second (B) (n=135, 33%) and third cohorts (C) (n=86, 21%) respectively received from 6 to 10 and over 10 administrations. Thrombosis events occurred in 154 patients (37,65%) distributed as follows: Cohort (A): 18,18%, Cohort (B): 25,32%, Cohort (C): 54,49%.

**Results:** Thromboembolic complications of central venous catheter were observed in 57,8% of patients, while thrombosis of intra-arterial catheter and deep vein thrombosis were respectively 3,25% and 31,8% of cases. Seven patients (4,55%) developed subclavian vein thrombosis, pulmonary embolization in 1,95% of cases and in only one patient (0,65%) autopsy revealed acute multifocal cerebral venous thrombosis.

**Conclusions:** These observations indicate that G-CSF administration may induce a higher risk of thrombosis and a careful monitoring of the venous circolation should be done.

362 POSTER

### IL-2 effect on NK cell phentoype of PBPC from healthy donors and patients

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Clinical studies have demonstrated that culturing PBPC in IL-2 enhances the generation of killer cells capable of lytic activity against malignant cells. The aim of this study was to evaluate the expression of NK associated markers, adhesion molecules and killer and activation-related receptors on CD56bright and CD56dim NK cells, before and after incubation of PBPC with IL2.

PBPC from 6 healthy donors (HD) and 4 patients were cultured with IL2 for 24h and studied by flow cytometry using 4-colour staining with anti-CD3 and anti-CD56, which allows the selection of NK cells defined as CD3- and CD56+, and two additional markers.

In HD, the majority of NK cells presented, before and after incubation, a CD56dim phenotype, whereas only 5% and 7%, respectively, was CD56bright. Although these two NK populations share several surface markers, statistically significant differences ($p<0,05$) were observed between them: pre-incubation -CD11c, CD16, CD57, CD94, CD158a, Granzyme B (GB) and HLA-DR; post- incubation - CD2, CD11c, CD16, CD57, CD94, CD158a, GB and HLA-DR. Following IL2 incubation there were differences within each CD56+ population: CD56brihgt cells showed an increased % of CD2+ and GB+ cells and a decreased % of CD161+ cells, whereas CD56dim cells demonstrated an increased % of CD69+ and NKb1+ cells and a decreased % of CD16+ cells.

Similar to HD cells, patient NK cells present a different phenotypic pattern for CD56bright and CD56dim cells. Comparing CD56+ populations of HD and patients we were able to detect various differences: pre-incubation - patient CD56dim cells demonstrated an increased % of CD16+ and CD94+ cells while CD56bright cells showed a decreased % of HLA-DR+ cells; post-incubation - whereas no discrepancies were encountered in CD56bright cells, the CD56dim population had an increased % of CD16+ and CD94+ cells and a decreased % of CD158a+ and GB+ cells. In what concerns pre-post incubation, a significant increase of CD69+ cells was observed in both CD56+ populations of patient NK cells.

Our preliminary results indicate that a 24 h incubation with IL-2 induces an increased % of CD69+ cells, not only in HD but also in patient NK cells. These results seem to agree with the function of CD69, one of the earliest activation markers acquired during NK cell activation. While comparing results of HD with those of patients, we were able to observe a differentiated pattern of some surface antigens specific for NK cells. Determining to what extent these cell surface receptors are functionally significant in NK cells will depend on further investigation, namely cytotoxic essays.

## Head and neck cancer

363 POSTER

### Limited dose external beam irradiation and interstitial iridium192 implant in definitive treatment of carcinoma of the oropharynx. Long term results

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**Purpose:** To evaluate long term treatment results of definitive radiation therapy in the treatment of carcinoma of the oropharynx.

**Materials And Methods:** 215 patients with biopsy-proven carcinoma of the oropharynx were treated during January, 1979 to October, 1995 at Long Beach Memorial Medical Center, California. There were 132 males and 80 female patients with median age of 60 (range 24 to 82 years). Forty-two patients had stage II disease and 173 patients had stage III/IV (AJCC) tumors. The external beam irradiation included the primary site as well as