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Detection of Tumor Cells in Leukapheresis Products from Patients with Breast Cancer Using Immunocytochemical Staining Method

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We have used a combination of four monoclonal antibodies (BM7, BM8 against MUC1, 5D3 aganist CK8, 18, 19 and HEA125 against human epithelial antigen) in a sensitive immunocytochemical assay to identify breast tumor cells in PBPC. In model experiments in which small numbers of MCF7 human breast tumor cells were added to PBMN cells, we estimate that there is a 99,7% probability of detecting tumor cells at a prevalence of one tumor cell per million cells by exassessing 4 slides with $1x10^6$ MNC. The probability to detect tumor cells at the same concentration assessing only one slide is 77 %. For evaluation of specificity, we applied the APAAP staining using cocktail as first antibody to PB from healthy donors and the LP products from patients with haematological malignancy. In some cases, cross-staining of non-epithelial cells could be detected. The cross-reacting cells could be distinguished from tumor cells by the combination of staining pattern and cytological details of the cells in question.

In clinical specimens, the proportion of patients with tumor cell positive LP products in this study was 14,3% (6/42) in the adjuvant treatment group, 18,2% (2/11), in the neoadjuvant treatment group and 20,1% (9/43) in the group of patients with metastatic disease.

We analyzed the relationship between the number of PBPC collections and contamination of tumor cells. By statistical analysis, a great number of leukapheresis products performed and tested was significantly correlated to the probability to find a tumor positive LP ($P<0,05$). The

number of leukaphereses depends on the yield of $CD34^+$ cells per leukapheresis**.** To support one cycle high-dose therapy, 2.5 x 10^6 $CD34^+$ cells / kg body weight are needed to gurantee a rapid haematological reconstitution . Our protocols include 2-3 cycle of high-dose therapy, at least 5-7.5 x 10^6 cells/kg body weight should be harvested. On the other hand, the heterogeneity in the patient population and pretreatment of chemotherapeutic agents might result in difference in the mobilization capacity. Therefore, different number of LP are needed for each patient.

Using cytospin preparations, the tumor cells in LP products can be quantified. Tumor cell counts ranged from 0,25 to 5 cell(s) per 10^6 cells per LP products. Only in patients with metastatic disease tumor cell concentrations greater than 1,25 cells/10^6 cells could be detected. In the patients with stage II / III disease, tumor cell counts ranged from 0,25 to 1,25 cells per 1x10^6 cells. As a consequence, the median tumor cell concentration was higher in specimens from patients with metastatic disease (median=0,96) than in specimens from patients in the adjuvant and neoadjuvant treament groups (median = 0,5 and 0,75).

We found no significant difference between epithelial cell positive group and epithelial cell negative group with respect to tumor size, LN involvement, tumor grade, histological type and receptor.

In this study, the effectiveness of the Isolex 300 SA used for selection of CD34+ haemopoietic propenitor and stem cells from blood-derived autografts of patients with high-risk breast cancer. The Isolex 300 was found to be efficient in reducing the amount of contaminating tumor cells. Prior to selection, five LP products contained malignant cells in a concentration between one and two positive cells per 1x10^6 MNCs. All selected products obtained were free of tumor cells. Since the selection procedure leads to a median 100-fold reduction in the number of mononuclear cells and as the median purity of the selected fraction was >90%, a purging efficienty of approximately 3 logs can be achieved.

We conclude that properly performed and controlled immunocytochemical staining of PB cytospins is a sensitive and simple way to detect and quantitate breast cancer cells in PBPC for autotransplantation. Isolex 300 SA for selection of CD34+ haemopoietic propenitor and stem cells from blood-derived autografts resulted in reduction of tumor cells in PBPC. This technique is a potent and efficient tool for the selection of CD34+ haemopoietic propenitor and stem cells of patients with high-risk breast cancer.