subunit of the human telomerase complex (hTERT). This enzyme is involved in immortalisation of cancer cells, and is expressed in high levels in 80-90% of all human tumors. Overexpression is partly due to gene amplification in the tumor cells. The gene is turned off in most normal human tissues, and hTERT is therefore an attractive target for immunotherapy, where hTERT may represent a "universal" tumor associated antigen. Based on these new, tumor specific and tumor associated antigens, candidate cancer vaccines have been tested in clinical trials involving patients with pancreas, colorectal and lung carcinomas and melanomas. The majority of these trials have been small phase I/II protocols primarily focused on safety aspects, protocol optimisation and immunological endpoints. By using and intradermal route of injection and the use of GM-CSF as an adjuvant 50-90% of patients demonstrate immune responses to the vaccines, depending on the antigen and patient group. In some trials a significant association between increased survival and an immune response was observed, indicating that such vaccines may have a clinical potential.

41

## Treatment of melanoma by adoptive transfer of CTL

Brigitte Dreno[1], Francine Jotereau[2]. [1] *Department of Skin Oncology Unit of Cellular and Gene Therapy;* [2] *INSERM U 463, France*

The two different approaches in the treatment of melanoma by immunotherapy are active immunotherapy which is represented by vaccination and passive immunotherapy with cellular therapy and mainly Tumor Infiltrating Lymphocytes (TIL). These ones used in melanoma stage IV (AJCC) induce a response rate of about 35% with a short duration of clinical response. Two main points are related to the efficiency of TIL: firstly the tumor burden and secondly the injection of specific T cytotoxic lymphocytes. Concerning the first point, it appears that the TIL would be more efficiency with a low tumor burden. Concerning specific cytotoxic T lymphocytes of melanoma antigens, they are considered as crucial in the anti-tumor response. However, until now a direct correlation between specific T cytotoxic activity and clinical response has never been demonstrated. In this context, the aim of our study was to demonstrate the efficiency of TIL used as adjuvant therapy in melanoma stage III after lymph node resection and to determine the percentage of specific TIL injected and the correlation with the clinical response.

88 eight patients have been included in an adjuvant randomized open study after positive lymph node resection. One arm (n = 44) received IL2 alone during 2 months, 6 weeks after the surgery and the other arm (n = 44) TIL and IL2 in the same condition. Moreover, among the TIL arm, the percentage of specific cytotoxic T lymphocytes has been determined in 26 patients by studying the intra-cytoplasmic production of gamma interferon by TIL incubated with autologous melanoma cell line. With a median follow up of 3 years, this study shows a significant decrease of relapse (73% without relapse in TIL arm versus 44% in IL2 arm $p = 0.019$) and an increase of survival in patients treated with TIL with only one invaded lymph node ($p = 0.026$). The percentage of specific TIL injected varied between 0, 3% and 10%. The injection of specific TIL was significantly associated with a decrease of relapse and an increase of survival ($p = 0.02$).

In conclusion, this study demonstrates for the first time, a clinical benefit of TIL used as adjuvant therapy in melanoma stage III with a correlation between relapse and overall survival and the injection of specific cytotoxic T cells.

42

## Genetic alterations in intraductal and invasive breast cancer

M. Van de Vijver. *Antoni van Leeuwenhoekhuis, Amsterdam, The Netherlands*

Breast cancer develops by transformation of normal epithelial cells into carcinoma in situ, followed by progression to invasive breast cancer, which can than acquire the potential to metastasize. There are several types of breast cancer, and consequently several distinct genetic pathways can be identified. Lobular carcinoma in situ and invasive lobular carcinoma are characterized by inactivation of the E-cadherin gene; well differentiated ductal carcinoma in in situ (DCIS) is characterized by frequent inactivation of an unidentified tumor suppressor gene on chromosome 16q; p53 mutation and HER2 gene amplification are involved in the development of poorly differentiated DCIS and invasive carcinoma.

Several studies have revealed that the spectrum of genetic alterations in the in situ tumors is comparable to that of the invasive carcinomas. An important question remains which genetic alterations are involved in the progression from carcinoma in situ to invasive breast cancer. We have compared the genetic alterations in both the in situ and invasive component of the same tumor. Of 12 invasive breast carcinomas with a relatively large in situ component, we have microdissected both components, isolated DNA and performed comparative genomic hybridization (CGH). In some tumors, we have found a few distinct differences between otherwise identical genome profiles of both components, suggesting that the number of genetic alterations involved in breast cancer progression is limited. In one tumor, we found high-level amplification of a region on 8q in the invasive component only. This region was further analyzed by fluorescence in situ hybridization (FISH). We believe that amplification of a region on chromosome 8q is involved in the progression of carcinoma in situ to invasive breast carcinoma.

43

## Genetic alterations in putative precursor lesions to breast cancer

S.R. Lakhani. *Breakthrough Breast Cancer Centre, Molecular Pathology, London, UK*

The multistep model of carcinogenesis in the breast suggests a transition from normal epithelium to invasive carcinoma via non-atypical and atypical hyperplasia and in situ carcinoma. The introduction of mammographic screening has led to the increased detection of 'borderline lesions' and this has highlighted deficiencies in our understanding of these lesions.

**Atypical Ductal Hyperplasia (ADH):** ADH is a controversial lesion, which shares some but not all features of DCIS. Despite clear diagnostic criteria agreement even amongst experienced breast pathologists has been low. Lakhani (1995) demonstrated that LOH identified at loci on 16q and 17p in invasive carcinoma is also present in ADH with a similar frequency. This indicates that ADH is a neoplastic proliferation. There is support for this view in the literature. The studies demonstrate that within the limits of current investigations, there is no significant difference between ADH and DCIS. ADH as currently defined may represent a small focus of DCIS rather than a separate entity.

**Hyperplasia of Usual Type (HUT):** Retrospective studies indicate that this lesion has a relative risk of 2 for the subsequent development of invasive carcinoma. O'Connell et. al. have demonstrated LOH at many different loci in HUT with frequencies ranging from 0-15%. These figures are similar to those of Lakhani et. al. (1996) - range 0% at 13q to 13% at 17q. These frequencies are lower than in DCIS and ADH (range 25-55%). At least a proportion of non-atypical hyperplasias are also clonal, neoplastic proliferations.

**Apocrine Hyperplasia:** Apocrine papillary hyperplasia is considered to be a benign lesion despite a similar architecture to low grade DCIS. Jones et al have investigated genetic alterations in 10 benign apocrine hyperplasia and compared these to apocrine ductal carcinoma in-situ (DCIS) and invasive apocrine carcinomas of the breast using comparative genomic hybridisation (CGH). All lesions exhibited DNA copy number changes. The average number of alterations in apocrine hyperplasia was 4.1 compared to 10.2 in apocrine DCIS and 14.8 in invasive carcinoma. The changes show considerable overlap with those identified in in-situ and invasive apocrine carcinoma. The data suggest that apocrine hyperplasia may be a precursor of apocrine carcinoma.

**Normal tissues:** LOH identified in invasive carcinoma is also present in morphological normal lobules. The frequency and significance remains unknown

44

## Molecular biology of malignant lymphoma: implications for diagnosis

P.M. Kluin. *Academisch Ziekenhuis Groningen, Dept of Pathology, Groningen, The Netherlands*

Malignant lymphomas consist of Hodgkin lymphomas (HL) and Non-Hodgkin lymphomas (NHL; 90% of B- and 10% of T-cell origin). Normal and neoplastic B- and T-cells express unique B-cell receptors (BCR) and T-cell receptors (TCR), respectively. These receptors are shaped after gene rearrangements in precursor B- and T-cells. Thereafter BCR genes undergo additional modifications, hypermutation and class switching. Recent studies suggested that all gene alterations are mediated by DNA breaks and repair; therefore they also can lead to chromosomal translocation. B-cells undergo these alterations on the functional and non-functional allele. Many investigators focused on the functional BCR allele. Apart from providing useful clonality assays, these studies also led to the concept that B-NHL can be distinguished in pregerminal center, germinal center and postgerminal center lymphomas. A clinically relevant implication is that chronic lymphocytic leukemia (CLL), formerly thought to be one disease, consists