805 POSTER

Tumor growth retardation mediated by T-cells following a hybrid-based vaccination/adoptive cellular combination therapy

R. Savai[1], R.T. Schermuly[2], S.S. Pullamsetti[2], M. Schneider[1], M. Obert[3], H. Traupe[3], F. Grimminger[2], G.A. Banat[1]. [1]*Medical Center University of Giessen, Department of Hematology and Oncology, Giessen, Germany;* [2]*Medical Center University of Giessen, Department of Internal Medicine, Giessen, Germany;* [3]*Medical Center University of Giessen, Department of Neuroradiology, Giessen, Germany*

Cancer immunotherapy with dendritic cell–tumor cell hybrids induces polyclonal stimulation against a variety of tumor antigens including unknown antigens. Hybrids can prime cytotoxic T cells, which subsequently develop anti-tumor responses.
The aim of this study was to enhance the known anti-tumor effect of hybrid vaccination (HC-Vacc) and hybrid-primed adoptive T cell therapy (HC-ACT) using the poorly immunogenic Lewis lung carcinoma (LLC1) model. The strategy used was a combination of a double hybrid vaccination alternating with HC-ACT (HC-Vacc/ACT).
Using flat-panel volumetric computer tomography and immunohistochemistry, we demonstrated a significant retardation of tumor growth (85%). In addition, a significant delay in tumor development, a reduction in the number of pulmonary metastases and increased survival times were observed. Furthermore, the tumors displayed significant morphological changes and increased apoptosis, as shown by upregulation of gene expression of the pro-apoptotic markers FAS, caspase 8 and caspase 3. The residual tumor masses seen in the HC-Vacc/ACT-treated mice were infiltrated with CD4+ and CD8+ lymphocytes and showed elevated interferon gamma (IFNG) expression. Moreover, splenic enlargement observed in HC-Vacc/ACT-treated mice reflected the increased functionality of T cells, as also indicated by increased expression of markers for CTL activation, differentiation and proliferation (CD28, ICOSL, TNFRSF13 and TNFSF14).
Our findings indicate that the combination therapy of dendritic cell–tumor cell hybrid vaccination with adoptive T cell therapy is a very effective and a promising immunotherapeutic regimen against poorly immunogenic carcinomas.

Radiotherapy

Oral presentations (Thu, 27 Sep, 09.00−11.00)
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Are TGF-beta 1 polymorphisms potential predictors of fibrosis risk after radiotherapy? – a subset analysis from the DAHANCA 6 and 7 protocols

C.N. Andreassen[1], C. Grau[2], J. Alsner[1], J. Overgaard[1], on behalf of the Danish Head and Neck Study Group. [1]*Aarhus University Hospital, Dept. of Experimental Clinical Oncology, Aarhus, Denmark;* [2]*Aarhus University Hospital, Dept. of Oncology, Aarhus, Denmark*

Background: Several studies have investigated the impact of TGFB1 SNPs upon risk of late toxicity after radiotherapy. In four studies, the TGFB1 position −509 T and codon 10 Pro alleles were significantly associated with enhanced complication risk (Quarmby 2003, Andreassen 2003 and 2004, Giotopoulos 2007). One study demonstrated a similar but non-significant association (De Ruyck 2006). Nevertheless, in a recent study of 120 breast cancer patients, the association could not be reproduced (Andreassen 2006).
Materials and Methods: 99 patients given definitive radiotherapy for head and neck cancer as part of the DAHANCA 6 and 7 protocols were genotyped for the TGFBI position −509 C/T and codon 10 Leu/Pro SNPs. During routine follow-up the patients were prospectively scored for soft tissue fibrosis. Median length of follow-up was 59 months. 44 patients were given 6 fractions a week, 55 were given 5 fractions a week. 62 patients were treated for localised laryngeal cancer. Using actuarial analysis the 'fibrosis free survival' (grade 2+) was calculated for each polymorphic genotype.
Results: The genotype distributions were; position −509; C/C 51%, C/T 43%, T/T 6% and codon 10; Leu/Leu 33%, Leu/Pro 56%, Pro/Pro 11%. The fraction of patients with grade 2+ fibrosis for each genotype was; position −509; C/C 19/50, C/T 17/43, T/T 2/6, and codon 10; Leu/Leu 12/33, Leu/Pro 23/55, Pro/Pro 3/11 No significant differences in 'fibrosis free survival' were found between the genotypes at position −509 or codon 10.
Conclusion: In this series of patients, the previously observed associations could not be detected. The predictive value of TGFB1 SNPs remains to be clarified and large well powered studies are highly warranted. The present study will be extended to a larger subset of patients from the DAHANCA protocols. This work was carried out as part of the ESTRO GENEPI project.

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Impact of SNP's in risk genes on fibrosis after radiotherapy

K. Borgmann[1], I. Boeckelmann[1], S. Borstelmann[1], A. Raabe[1], O. Zschenker[2], U. Hoeller[3], D. Rades[4], E. Dikomey[5]. [1]*University Hospital Hamburg-Eppendorf, Oncology, Hamburg, Germany;* [2]*Dep. of Radiation Oncology, University of California, San Francisco, USA;* [3]*Dep. of Radiotherapy, Vivantes Medical Center, Berlin, Germany;* [4]*Dep. of Radiation Oncology, University Hospital Schleswig-Holstein, Luebeck, Germany;* [5]*Oncology, University Hospital Hamburg-Eppendorf, Hamburg, Germany*

Background: Individual radiosensitivity is discussed to be mediated by single nucleotide polymorphisms (SNPs) in so called risk genes. This study was performed to identify risk genes causing an increased susceptibility to radiation-induced damage, in terms of chromosomal aberration determined in vitro as well as radiation-induced fibrosis following radiotherapy.
Materials and Methods: Blood samples were collected from 69 patients with breast cancer Stage I/II who had undergone breast conserving surgery and adjuvant radiotherapy applying 1.8 to 2.5 Gy per fraction with a median reference dose of 55 Gy. Fibrosis was evaluated using the LENT/SOMA score. The median follow up time was 12 years. Blood samples were analysed for SNP's in TGFbeta1 (C-509T), XPD (A → C, exon 23), SOD2 (C1183T), XRCC1 (G-A, exon 10), and ATM (G557A) applying the RFLP method or MassArray™ technology, respectively. In 47 out of 69 blood samples chromosomal damage was determined using the metaphase technique.
Results: In total 15/69 (22%) of the patients developed a fibrosis of grade 2 or 3, respectively. A combined analysis of the risk alleles TGFbeta1 (CT, TT), XRCC1 (GA, AA), ATM (GA, GG), and SOD2 (CT, CC) revealed a positive correlation between the number of risk alleles and the probability to develop a grade 2/3 fibrosis, with no fibrosis grade 2/3 in patients without any risk allele but 80% of the patients carrying four risk alleles. For the analysed SNP in the XPD gene no effect on the development of fibrosis was apparent. Comparison of chromosomal damage and risk alleles in 47 out of the 69 patients showed a strong increase in the number of chromosomal damage with risk genes, particularly with the SNP in the SOD2 gene, implying a relation of these genes to individual radiosensitivity. On the other hand, a strong reduction in the number of chromosomal damage was evident in patients carrying a SNP in the XRCC1 gene, indicating the involvement of this gene in other pathways responsible for the development of fibrosis.
Conclusion: A combination of SNPs in so called risk alleles result in a higher susceptibility to late effects after irradiation in breast cancer patients. Thus, polymorphisms in specific genes might be useful to identify patients with an increased risk to develop late tissue effects after radiotherapy.

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Expression of KIT (c-Kit; CD117) is reduced after radiation in normal human breast tissue: a study using cDNA array analysis of microdissected samples

C. Westbury[1], J.S. Reis-Filho[1], T. Dexter[1], A. Mackay[1], K. Fenwick[1], D. Steele[1], S. Parry[1], A. Nerurkar[2], C. Isacke[1], J. Yarnold[3]. [1]*Institute of Cancer Research, Breakthrough, London, United Kingdom;* [2]*Royal Marsden Hospital, Pathology, London, United Kingdom;* [3]*Institute of Cancer Research, Radiotherapy, London, United Kingdom*

Background: Gene expression profiling of normal tissues after curative radiotherapy was carried out to investigate the pathogenesis of late radiation injury in humans.
Materials and Methods: Irradiated and non-irradiated normal breast tissue was collected from patients undergoing bilateral mastectomy for ipsilateral tumour relapse or prophylaxis following radiotherapy for breast cancer. Using P.A.L.M. laser capture microdissection of frozen sections, breast tissue was separated into an epithelial compartment (terminal duct lobular units and ducts) and a stromal compartment (remaining tissue). RNA was extracted, amplified and hybridised to a 20k cDNA array against a breast tissue reference RNA. Using 2 statistical methods, paired SAM analysis and a Fisher's exact test (R 2.0.1 software), expression profiles of irradiated vs non-irradiated breast were compared for each tissue compartment. Immunohistochemical staining for c-Kit was performed in paraffin sections