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)
Radiotherapy/radiobiology

900 ORAL

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.

901 ORAL

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.

902 ORAL

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

and analysed using Allred scoring in epithelium and mast cell counts per high powered field in epithelial and stromal compartments.
Results: Paired samples were available from 4 patients who had received radiotherapy 1.5−23 years prior to mastectomy. Of the transcripts differentially expressed in the epithelial compartment, KIT was reduced in the irradiated samples, and its ligand KITLG (stem cell factor, KIT ligand) was increased although statistical significance was not achieved. Preliminary validation with immunohistochemical staining in 4 sample pairs confirmed a striking reduction in the expression of c-Kit in the lobular epithelial cells of the previously irradiated breast compared to the unirradiated breast ($p = 0.01$). Preliminary data suggest an increase in c-Kit positive mast cell numbers in both the epithelial and stromal compartments, confirming also that the epithelial cells are responsible for the reduction in expression levels of c-Kit.
Conclusions: c-Kit expression is reduced in normal epithelium of irradiated human breast and c-Kit positive mast cell numbers may be increased in both stromal and epithelial compartments. This is of particular interest because of known involvement of mast cells in many fibrotic conditions, and previously only animal data has been reported for radiation fibrosis. We plan further immunohistochemical analysis for both c-Kit and its ligand stem cell factor in an extended sample set.

903 ORAL

Long-term risk of contralateral breast cancer in relation to treatment

M.J. Hooning[1], B.M.P. Aleman[2], M. Hauptmann[2], J.G.M. Klijn[3], R. Noyon[1], M. Stovall[4], F.E. van Leeuwen[1]. *[1]Netherlands Cancer Institute, Epidemiology, Amsterdam, The Netherlands; [2]Netherlands Cancer Institute, Radiation Oncology, Amsterdam, The Netherlands; [3]Erasmus MC Daniel den Hoed Cancer Center, Medical Oncology, Rotterdam, The Netherlands; [4]M.D. Anderson Cancer Center, Radiation Physics, Houston, USA*

Purpose: To assess long-term risk of contralateral breast cancer (CBC) in a predominantly young breast cancer (BC) population, focusing on the effect of different radiation regimens, chemotherapy and family history of BC.
Methods: We studied incidence of CBC in 7221 1-year survivors of breast cancer who were treated between 1970 and 1986. Treatment-specific risk of CBC was evaluated in Cox proportional hazards regression models.
Results: RT-associated risk of CBC increased with decreasing age at first treatment (for age >35: hazard ratio (HR) = 3.4, 95% CI, 0.8 to14.8; for age >45: HR = 1.14, 95% CI, 0.83 to 1.55; Ptrend < 0.05). Among women irradiated before age 45 those who had postlumpectomy RT experienced 1.5-fold (95% CI, 1.1 to 2.1) increased risk of CBC compared with those who had postmastectomy RT. The joint effects of postlumpectomy RT (HR = 1.35) and positive family history for BC (HR = 1.21) on risk of CBC were greater than expected when individual risks were summed (HR = 3.26, 95% CI, 1.91 to 5.58). Young irradiated patients with positive family history developed predominantly medially located CBCs (82% vs 42% in patients without family history; P = 0.01). Treatment with adjuvant chemotherapy (cyclophosphamide, methotrexate and fluorouracil) exerted a protective effect on the risk of developing a CBC in the first 5 years of follow-up.
Conclusions: Young BC patients treated with postlumpectomy RT experience increased risk of CBC, specifically in case of a positive family history of BC. This finding questions the rationale for breast-conserving therapy in mutation carriers and warrants further research.

904 ORAL

Breast cancer risk in 5-year survivors of Hodgkin's lymphoma, the influence of treatment and premature menopause

F. Van Leeuwen[1], M.L. De Bruin[1], M.B. van 't Veer[2], E.M. Noordijk[3], J.M. Zijlstra[4], J.W.W. Coebergh[5], H. van den Berg[6], B.M.P. Aleman[7]. *[1]The Netherlands Cancer Institute, Department of Epidemiology, Amsterdam, The Netherlands; [2]Erasmus MC Daniel den Hoed Cancer Center, Department of Hematology, Rotterdam, The Netherlands; [3]Leiden University Medical Center, Department of Radiotherapy, Leiden, The Netherlands; [4]VU University Medical Center, Department of Hematology, Amsterdam, The Netherlands; [5]Comprehensive Cancer Centre South/Eindhoven Cancer Registry, Cancer Registry, Eindhoven, The Netherlands; [6]Emma Children Hospital Academic Medical Center, Department of Pediatric Oncology, Amsterdam, The Netherlands; [7]Netherlands Cancer Institute, Department of Radiotherapy, Amsterdam, The Netherlands*

Background: Female Hodgkin's lymphoma (HL) survivors are at increased risk of breast cancer (BC) up to 25 years after treatment, especially those irradiated to the breast area at young ages. We assessed the cumulative risk after 25 years and the influence of gonadotoxic therapy on the risk of BC in patients irradiated to the breast area.
Methods: We performed a cohort study in 1155 women, treated for HL in the period 1965–1995 before age 51 (32% (RT), 8% CT, 60% RT+CT). We compared the incidence of BC with the general population and calculated standardized incidence ratios (SIRs) and absolute excess risks (AERs). We assessed absolute risk at 30 years using Kaplan-Meier risk estimation and competing risk techniques. Cox regression analyses was performed to study therapy-effects in relation to gonadotoxicity.
Results: During follow-up (median 18.2 years), 100 women, of whom 99 were irradiated to the breast area, developed BC (SIR 5.4 [95%CI 4.4−6.6], AER 54 per 10,000 patients per year). The risk remained high after prolonged follow-up (>30 years after treatment SIR 8.7 [4.2−16.0]). Although women treated before age 21 experienced the highest risk (SIR 16.9 [11.1−24.9], the risk among women aged 31−40 at treatment was still elevated (SIR 2.9 [1.8−4.5]). The cumulative risk (Kaplan-Meier) for BC 30 years after first treatment was 22%, whereas the cumulative incidence accounting for death as a competing risk was 17% at that time. Among women irradiated to the breast area, treatment with procarbazine ($\leqslant 8.4$ g/m^2: HR 0.6 [0.3−1.1], >8.4 g/m^2: HR 0.4 [0.1−1.0]), as well as RT to the ovaries (HR 0.3 [0.0−1.1]) lowered the risk for BC. In addition, women who retained normal ovarian function $\geqslant$16 years after treatment were at an increased risk for BC compared to those with <8 years of intact ovarian function (HR 5.4 [2.1−13.8]). Smoking and use of oral contraceptives did not influence the risk of BC, whereas obese women had a higher risk for BC (HR 1.8 [1.0−2.9]).
Conclusion: The risk of BC remains elevated up to >30 years after treatment, which suggests need for lifetime surveillance. The Kaplan-Meier method substantially overestimated the absolute risk of BC after HL compared with the method accounting for death as a competing risk. Gonadotoxic therapy lowers the risk of BC in patients irradiated to the breast area.

905 ORAL

Risk analysis in breast cancer patients younger than 45 years: which risk parameters gain in importance after breast conserving surgery (BCS), systemic therapy (ST) and radiation therapy (RT)?

J. Hammer[1], C. Track[2], D.H. Seewald[1], J. Feichtinger[1], H.D. Thames[3], W. Langsteger[4], A.L. Petzer[5], K. Spiegl[1], E. Bräutigam[1], S. Pöstlberger[6]. *[1]Barmherzige Schwestern Hospital, Radiation Oncology, Linz, Austria; [2]Barmherzige Schwestern Hospital, Radiation Oncology and Breast Health Center, Linz, Austria; [3]M.D. Anderson Cancer Center, Biostatistics and Applied Mathematics, Houston TX, USA; [4]Barmherzige Schwestern Hospital, Nuclear Medicine, Linz, Austria; [5]Barmherzige Schwestern Hospital, Internal Medicine/Hematology and Oncology, Linz, Austria; [6]Barmherzige Schwestern Hospital, Surgery and Breast Health Center, Linz, Austria*

Background: We evaluate residual risk after breast conserving surgery (BCS), chemo- and/or hormone-therapy (ST) and radiotherapy (RT) in women younger than 45 years.
Materials and Methods: From 1984 to 1997, 220/1635 patients with breast cancer who underwent BCS and ST, and RT in our institution presented younger than 45 years (pre-menopausal). Recursive partitioning analysis was carried out for the endpoints local recurrence (LR) and disease free survival rate (DFR). Covariates included were age, T-stage, N-stage, ratio of involved lymph nodes and excised nodes (n-ratio), location of the index tumor, ER/PR status, and menopausal status. The relative hazard ratio (RHR, HR relative to median patient) was estimated in sub-groups of at least 20 patients.

Table A.

Risk group	n	n-ratio	PR	RHR
Low	98	<0.16	pos	0.32
Intermediate	90	<0.16	neg	1.16
High	32	<0.16	any	2.88

Table B

Risk group	n	n-ratio	loc	T-stage	RHR
Low	106	<0.09	lat	any	0.48
Low-interm	66	<0.09	med/centr	any	1.16
High-interm	25	$\geqslant$0.09	any	T1	1.51
High	23	$\geqslant$0.09	any	T2	3.42