days, median time to progression 122 days and median survival 211 days. No significant correlations were found between BF and MRglu per scan as well as between the change in BF and MRglu over time. The residual MRglu after 3 weeks of treatment predicted survival (P = 0.03; 95% CI, 2.18 to 430197). Martingale residual plots showed that the effect of BF is not simply linear. However, the change in BF between baseline and 3 weeks post-treatment showed that a decrease might be indicative of time to progression (P = 0.006; 95% CI, 1.0 to 1.06) and survival (P = 0.076; 95% CI, 1.0 to 1.05).
Conclusions: 18FDG-PET and H2(15)O-PET seem valuable biomarkers in monitoring early response to antiangiogenic treatment in patients with NSCLC. Due to the limited number of patients and the relative short time to follow up, results have to be interpreted with care. Residual MRglu was able to predict survival, whereas tumor blood flow seems to be a promising biomarker for monitoring treatment response.

P16

Biological profiles of two ERBB2-amplified human breast cancer xenografts diversely sensitive to Trastuzumab

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Background: Trastuzumab is a recombinant monoclonal antibody directed against the human growth factor receptor-2(ERBB2/HER2), overexpressed in 25% of breast cancers. However, only 35% of patients with ERBB2-positive cancer respond to trastuzumab and 50% of patients achieving an initial response escape to trastuzumab. Only few ERBB2-breast cancer cell lines are available for preclinical studies. Here, the purpose is to approach the mechanism of trastuzumab resistance using two new models of human breast cancers xenografts (HBCx) with ERBB2 gene amplification, sensitive or resistant to trastuzumab, respectively.
Methods: Tumor samples were directly implanted into nude mice from patients and expanded as xenografts (Marangoni et al, Clin Cancer Res 2007). CGHarray detected ERBB2 amplification and exons of ERBB2 gene were sequenced. Tumor growth and responses to trastuzumab (10 mg/kg ip weekly) were determined. Gene, protein expression and phosphorylation were evaluated by Q-RTPCR and Western Blot, respectively. Coimmunoprecipitation assays were performed for ERBB2/ERBB3 heterodimerisation. ERBB2-positive BT474 cell line was from ATCC.
Results: Both HBCx-13 and HBCx-5 xenografts were canalar infiltrating cancers, HBCx-5 being mucinous. p53 was mutated in HBCx-13 and not in HBCx-5, PTEN expression was higher in HBCx-13 than in HBCx-5. Both displayed an high ERBB2 amplification in CGHarray. No mutation was detected in exons 14, 15, 16, 19 and 20 coding for ERBB2 extracellular domain and tyrosine kinase domain. HBCx-13 was exquisitely sensitive to trastuzumab while HBCx-5 was resistant. AKT and ERK phosphorylation was increased in both HBCx whereas it decreases in BT474 cells after trastuzumab treatment. Trastuzumab induced ERBB2/ ERBB3 dimerisation in both xenografts while it decreased in BT474.
Conclusions: These data indicate that trastuzumab resistance of HBCx-5 is not associated with changes in heterodimerization, ERBB2 mutations in analyzed exons or p53 mutations. The difference observed in PTEN expression could explain the differences in trastuzumab responses. It is showed that these xenografts and BT474 cells have different ways of response to trastuzumab. These new models of ERBB2 amplified breast cancers are the opportunity to explore mechanisms of resistance to Trastuzumab and to test new compounds.

P52

Activated CD8+ T cells radiosensitize EMT-6 mammary carcinoma cells through secretion of interferon-gamma

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Background: Activated CD8+ T cells were shown to be major mediators of anti-tumor immunity, while their effect on radiosensitivity has not yet been explored. The aim of this study was to examine the cytolytic and radiosensitizing activities of CD8+ cells in 1% oxygen, modeling the immunosuppressive and radioprotective microenvironment of solid tumors.
Methods: Splenocytes were isolated from BALB/c mice and activated with immobilized anti-CD3 and soluble anti-CD28. CD8+ T cells were selected by immunomagnetic beads. Syngeneic EMT-6 mammary carcinoma cells were irradiated in 1% oxygen and their radiosensitivity was assessed by colony formation assay. Radiosensitization was determined as a dose enhancement ratio at the level of a surviving fraction of 0.1. To exmanine the cytolitic activity, tumor cell viability was accessed by a 3 h tetrazolium based MTT assay.
Results: Expanded CD8+ T cells secreted high levels of IFN-gamma and increased the radiosensitivity of syngeneic EMT-6 mammary carcinoma cells up to 1.8-fold. This radiosensitization was abrogated by IFN-gamma immunoneutralization and by the metabolic iNOS inhibition in tumor cells. While considerable radiosensitizing effects were observed at a CD8+/EMT-6 cell ratio below 1/1, the cytotoxicity of CD8+ T cells was impaired by hypoxia even at a 10/1 ratio. RT-PCR, FACS and ELISA data in agreement revealed down-regulation of IFN-gamma in hypoxic CD8+ cells. In contrast, hypoxia transcriptionally up-regulated iNOS in EMT-6 tumor cells that were exposed to IFN-gamma+/CD8+ T cells. The latter was essential for preserving the radiosensitizing effects under hypoxic conditions.
Conclusions: Our results for the first time demonstrate the radiosensitizing properties of activated CD8+ cells. This finding warrants further validation of T cell immunity as a prognostic determinant of tumor radioresponse and indicates a rationale for exploring the radiosensitizing potential of immunostimulating strategies.

P65

Radiosensitization by histone deacetylase inhibitors +/− demethylating agents in head and neck cancer cell lines

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Background: Promoter hypermethylation and histone deacetylation are the most important epigenetic changes identified in cancer. Of major interest is the reversibility of these processes that has resulted in the implementation of several new drugs in cancer therapy. The aim of this project was to evaluate the radiosensitizing potential of the demethylating agent decitabine (DAC), either alone or in combination with the histone deacetylase inhibitors (HDAC-I) trichostatin A (TSA) and LBH589 (Novartis) in several head and neck cancer cell lines. Furthermore, a possible relationship between the radiosensitivity and the methylation profile of each individual cell line was investigated.
Methods: For seven head and neck cancer cell lines, hypermethylation of several genes was assessed by conventional methylation-specific PCR (MSP) and by epi-array ("base5-platform", Oncomethylome Science). This included genes involved in response to irradiation (ATM, PARP3, . . .) as well as genes known to be frequently methylated in this cancer type (p16, MGMT, RASSF1A, DAPK, . . .). Sensitivity of all cell lines to radiotherapy +/− optimized doses of DAC +/− TSA or LBH589 was determined by colony assays.
Results: The investigated cell lines show diverse methylation profiles both with conventional and with array-MSP. So far, some cell lines seem to be radiosensitized by the HDAC-I LBH589 and/or TSA. No radiosensitization by DAC has been identified yet.
Conclusions: In this project, the radiosensitizing potential of DAC +/− TSA or LBH589 as well as a link between radiosensitivity and methylation profile of several head and neck cancer cell lines is investigated. The final results of this project will be presented at the time of the congress.

P38

Radioimmunotherapy (RIT) of refractory or relapsed Hodgkin's lymphoma (HL) with ^{90}Yttrium-labelled antiferritin antibody

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Background: The aim of this study was to evaluate the safety and efficacy of radiolabelled DTPA-chelated rabbit polyclonal antiferritin antibody (Ab) in relapsed or refractory HL.
Methods: The protocol included a first intravenous injection of ^{111}Indium-labelled antiferritin Ab followed by immunoscintigraphy at 4, 48, and 72 hours and intravenous injection of ^{90}Yttrium-labelled antiferritin Ab in the case of tumour targeting.
Results: Ten patients were included in the study: median number of chemotherapy regimens: 3; number of autografted pts: 8; number of previously irradiated pts: 9; response to last chemotherapy: 6 PR and 4 progressions. All immunoscintigraphies showed tumour targeting. Nine patients were treated, as the last patient died from progressive HL before therapeutic injection. Median injected activity was 12 MBq/kg (0.32 mCi/kg). Among the ten patients who were included in the study, 1 CR and 6 PR were observed (ORR 70%) with a median duration of response of 8 months (range: 7−12 months). Toxicity was mainly haematological, with grade 1 or 2 neutropenia and anaemia, and grade 2 and 3 thrombocytopenia. The pharmacokinetic study showed that the half-lives of ^{111}Indium and ^{90}Yttrium were almost identical.