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.

**Conclusions:** These results confirm those previously reported in the literature and show the therapeutic potential of rabbit polyclonal antiferritin Ab in relapsed or refractory HL. On the basis of all these results, MATBioPharma proposed to test a radioimmunotherapy with polyclonal antiferritin antibodies (Abs) in patients with refractory or relapsed HL. The treatment is constituted with chelated rabbit polyclonal antiferritin Abs to be loaded with $^{111}$Indium for the diagnosis of the tumour(s) by immunoscintigraphy and with $^{90}$Yttrium for the treatment of the tumour(s). A phase I study is still ongoing at the Institut Curie/Centre René Huguenin (France) to evaluate the safety and tolerability of ascending doses of $^{90}$Yttrium antiferritin until the maximum tolerated dose (MTD) is reached and to select a dose for further investigation (one dose step below MTD). A pharmacokinetics is concomitantly performed to determine dose linearity and pharmacokinetic parameters of increasing $^{90}$Y-Ab and Ab. The second dose level will be completed in the third quarter 2007 and available data on immunoscintigraphy, safety, and efficacy of included HL patients will be provided for the 7th International Symposium on HL.

**P1**

**Urokinase-type plasminogen activator receptor variants in serum and plasma of women with various breast lesions**

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**Background:** The urokinase-type plasminogen activator receptor (uPAR) can be detected in blood in both intact and cleaved soluble forms. Several studies reported that an increased level of uPAR in blood is correlated with poor prognosis in various cancers. We examined whether the soluble form of intact uPAR, suPAR (I−III) and the cleaved soluble forms, suPAR (II−III) and suPAR (I), could be detected in blood samples from women with different breast lesions.
**Methods:** Preoperative serum and plasma was taken from 10 patients in each of the following diagnostic groups: benign breast cancer, carcinoma in situ of the breast, local malignant breast cancer, locally advanced breast cancer and metastatic breast cancer. The protein levels of the various uPAR variants were determined using specific designed time-resolved fluorescence immunoassays (TR-FIA). TR-FIA 1 quantifies non-occupied uPAR(I−III) while TR-FIA 2 measures non-occupied uPAR(I−III) and uPAR (II−III). The levels of uPAR (II−III) can be calculated by substracting the concentrations measured by TR-FIA 1 from those measured by TR-FIA 2. TR-FIA 3 quantifies the liberated uPAR(I).
**Results:** The levels of soluble uPAR forms in EDTA plasma correlated well with those in serum except for uPAR (I). We found a trend of increased serum and in particular plasma uPAR (I−III), uPAR (II−III) and uPAR (I−III) + uPAR (II−III) levels with the degree of severity of the breast lesion. This observation was most striking for plasma uPAR (I−III) + uPAR (II−III) levels ($p = 0.006$). We have not been able to demonstrate a significant trend for serum or plasma uPAR (I), however this could be a type II error.
**Conclusions:** The present results indicate that soluble uPAR variants could distinguish breast lesions of different severity and as such could be potentially prognostic markers in breast cancer.

**P79**

**Assessing the utility of matrix-degrading enzymes as a biomarker for disease status and therapeutic efficacy in lung cancer**

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**Background:** The matrix-degrading function of several members of the matrix metalloprotease (MMP) family has been implicated as an important step in tumor growth, invasion, and distant metastasis formation. MMPs have been correlated with angiogenesis and tumor progression in several different tumor types. Here, for the first time, we examined the urine of advanced lung cancer patients for the presence of MMP activity to determine whether these markers of invasion could be detected and, if so, whether their levels correlate with disease status and therapeutic efficacy of chemotherapy.
**Methods:** Subjects with small cell lung cancer (SCLC) or stage IIIB/IV non-small cell lung cancer (NSCLC) were enrolled under an IRB-approved protocol for specimen collection from January-July 2007. Serial urine specimens were obtained prior to initiation of chemotherapy and prior to subsequent cycles until documented progression, study withdrawal, or death. Urine samples normalized for protein content were assessed by gelatin zymography and quantitated by densitometry.
**Results:** Amongst 10 SCLC subjects analyzed thus far, 8 had detectable MMP-2 activity in the urine and 7 had MMP-9. In addition 5/10 SCLC subjects had larger molecular weight MMP species. Patients with more localized disease (stage IIIB or limited-stage SCLC) had only MMP-2 detectable. Among 12 NSCLC subjects, MMP-2 was readily detectable in 8/13 and MMP-9 was present in 5/13. Initial serial measurements in 5 subjects show 2−5 fold increases in MMP levels during the first months of therapy.
**Conclusions:** MMP-activity was detectable in a majority of subjects with either SCLC or NSCLC. The limited subset assessed suggests increased levels and numbers of MMP species in widely metastatic disease compared to those with more limited disease. In addition, intra-subject increases in MMP activity were seen in subjects who had initiated chemotherapy, although the relationship of this change to tumor status has yet to be determined. Full longitudinal analyses through the completion of treatment will be presented.
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**P75**

**Incidence of familial breast cancer and BRCA mutations in Iranian and Ukrainian breast cancer patients**

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**Background:** Breast cancer (BC) is the most commonly diagnosed cancer in Iranian women, and is the leading cancer cause of death in this population but in Ukraine, its incidence rates are 3 times higher than Iran. Both environmental factors and genetics have an impact on the risk of BC. Approximately 90% of breast cancers are sporadic while the remaining 10% are inheritable. Germline mutations in breast cancer-associated gene 1 (BRCA1) have been detected in approximately one-half of the familial breast cancer (FBC) and most of the familial breast/ovarian cancer cases. An accurate evaluation of the penetrance of BRCA1 and BRCA2 mutations is essential to the identification and clinical management of families at high risk of breast cancer. In this article, we consider incidence of FBC and BRCA mutation in Iranian and Ukrainian BC patients.
**Methods:** Patient samples were drawn from four medical centers in Iran and Ukraine. We retrieved 203 formalin-fixed, paraffin-embedded tissue blocks from women with breast cancer diagnosed, the age of 25−80 years for the years 2004 and 2006. All cases were reviewed using a special questionnaire, which allowed taking into account the presence or absence family history of breast cancer and also other pathology information. Verification of every cancer reported in a relative was sought through pathology reports, hospital records. Multiplex PCR was used to detect the simultaneous detection of three common mutations. For each BRCA mutation, three primers (one common, one specific for the mutant, and one specific for the wild-type allele) were used.
**Results:** Incidence of familial breast cancer was 32.1% and 28.6% in Iranian and Ukrainian patients respectively. There were no significant differences between cases with regard to incidence of FBC in these populations. The proportion of cases with one of three BRCA mutations (5382insC) was 9% in Iranian breast cancer patients and 9% in Ukrainian breast cancer patients. The hereditary proportions were higher than this for women with at least 1 first-degree relative with breast cancer (19%) in Iranian and Ukrainian patients. There was no statistical difference between Ukrainian and Iranian women with breast cancer diagnosed at age <50 years in term of 5382insC incidence.
**Conclusions:** Breast cancer risk was strongly related to age, with more than 80% of cases occurring in women over 45 years old. The highest number of cases of familial breast cancer diagnosed was in the 41−50 age group. The relative risk of breast cancer conferred by a first-degree relative with breast cancer was dramaticaly decreased by age. The findings of the present study suggest that 5382insC mutation and family history may have an impact on the incidence of breast cancer in women but because of high relative risk of BC in Ukraine than Iran (nearly 3 times), it is suggest that environmental factors are of greater importance than genetic factors. Our analysis shows testing of 5382insC mutation in breast cancer can be utilized as one of prognosis factors of FBC development risk in these populations.