between cancer and inflammation. 18F-fluorothymidine (FLT)-PET imaging may be more specific for cancer since it measures cell proliferation. Here, we used FLT-PET to assess the antineoplastic effects of radiation therapy (RT) combined with the antiangiogenic agent Enzastaurin (ENZ).
Methods: Human H460 lung cancer cells were used in an athymic nude mice xenograft model. Treatment groups (each containing 5 mice) consisted of vehicle control (DMSO), 80 mg/kg bid ENZ alone, vehicle+RT and ENZ+RT. DMSO and ENZ alone were administered for 2 days, then either sham or 2 Gy was added prior drug treatment for 5 days. After the 7 days of treatment, fixed tumor sections from each group were analyzed by IHC, using von Willebrand Factor (vWF) or Ki67 staining. Tumor blood vessels were quantified using vWF by randomly selecting 5 separate 400× fields and counting the vessels/field number. Ki67 positive cells number were scored/plotted in similar fashion to vessel quantification. Whole body FLT-PET imaging of hind limb tumors were performed before and after the 7 treatment days
Results: Ki67 proliferation index revealed that ENZ+RT group resulted in 70% ($p < 0.001$) and 30% ($p < 0.001$) reduction in proliferating cells relative to control or RT alone groups, respectively. vWF quantification showed that ENZ+RT (3; SD = 1.6) resulted in a 3-fold reduction in average number of vessels/field compared to control (10.2; SD = 2.77; $p < 0.007$) and 2-fold reduction relative to RT alone (7; SD = 2.1; $p < 0.04$). Tumor response to therapy was measured in FLT-PET imaging as change in FLT uptake. The ENZ+RT group showed the most significant attenuation of tumor avidity for FLT. When normalized to muscle uptake, only the ENZ+RT group showed a decrease below baseline measurement, which significantly correlated to IHC analysis. Furthermore, the RT+ENZ treatment resulted in a substantially decrease in tumor viability from 97% to 75%, while 90% tumor was viable before treatment
Conclusions: FLT imaging effectively depicted antineoplastic effects of RT combined with ENZ. The measurements of tumor cell proliferation by FLT-PET were significantly correlated to in vivo biomarkers such as Ki67 or vWF. These results demonstrate that FLT-PET may be important in measuring therapeutic response to anti-angiogenic agents and RT in clinical setting.

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The Centre of Biological Ressources of Nancy, France: DNA, RNA and proteines quality testing

N. Martinet, Y. Martinet, J.-M. Vignaud, S. Poulain, S. Lacomme, E. Ventard, M. Donner. *INSERM, Paris, France*

Background: Biobanks are now a keystone for the biotechnologies. In the centre for Biological Ressources of Nancy we have collected from 1989, 6,000 samples. They correspond to patients operated upon for lung tumors, mesothelioma and also from healthy controls. All are annoted with the complete clinical follow up and with the results of biological research. The collection is open to the scientific community at large and all samples need therefore to be quality controls certified. These controls are described here.
Methods: RNA was extracted from randomly selected 20 paired samples of lung cancer(tumor and healthy parenchyma) and the mRNA levels for three retinoid receptors were measured with a triplex of taqman probes. DNA was also extracted and the number of copies of the EGF receptor was measured with a duplex of taqman, one being the stable calibrator. Corresponding plasmid calibrators were included. The levels of GAPDH protein was measured with a specific ELISA in the same samples.
Results: GAPDH protein is well conserved in lung tissues but does not reflect the quality of the mRNA. The choice to measure very low expressed genes like retinoid receptors is better than to measure actin mRNA. In the lung, RARalpha is a stable calibrator. The DNAcontrol is a good quality control. Plasmids calibrators calibrate the machines result for harmonization between qPCR machines.
Conclusions: For biobank, quality controls are needed and we tested the validity of such tools. The best quality controls were for RNA and DNA but plasmid reference are also needed to calibrate qPCR machines results since they have different performances while we need controls in kit effective for all machines.

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Polymeric immunoglobulin receptor downregulation in lung cancer development

S. Ocak[1], V. Polosukhin[1], A. Gonzalez[1], T. Pedchenko[1], C. Pilette[2], Y. Sibille[1], P. Massion[1]. *[1]Vanderbilt University, Nashville TN, USA; [2]University of Louvain (UCL), Brussels, Belgium*

Background: Epithelial transport of polymeric immunoglobulin A (pIgA) from submucosal tissues to mucosal lumen is mediated by the polymeric immunoglobulin receptor/secretory component (pIgR/SC). Although pIgR/SC is downregulated in lung cancer, its implication in tumorigenesis remains unknown. Our hypothesis is that downregulation of pIgR/SC is an early event in lung tumor progression and that pIgR/SC contributes to cell proliferation, apoptosis and migration.
Methods: Expression levels of pIgR/SC were evaluated by Western blot (WB) and immunohistochemistry (IHC). WB was performed on 2 normal bronchioepithelial and 8 lung cancer cell lines, and on normal and cancer tissues from 4 different patients. IHC was performed on 11 normal lung tissues, 10 preinvasive lung tissues and 162 lung cancer tissues assembled in tissue microarrays (TMA). Immunostaining was scored by 2 pathologists. The scoring index (0 to 300) was created as product of staining intensity (0: no, 1: low, 2: moderate, 3: strong) and percentage of positive cells (0 to 100%).
Results: WB showed low expression of pIgR/SC in 10/10 lung cell lines and downregulation in 2/2 squamous cell carcinoma (SCC) and in 1/2 adenocarcinoma (ADC) tissues, compared to normal lung tissues from same patients. IHC on normal lung tissues (10/11) revealed a strong staining for pIgR/SC in mucous and serous cells in surface and glandular epithelium (mean score, MS = 250), a weak staining in ciliated cells (MS = 50) and an expected absence of staining in alveolar cells. Preinvasive lung lesions showed no pIgR/SC expression (4 low and 6 high grades). IHC on lung TMA displayed pIgR/SC positive staining only for 10/59 ADC (17%, MS = 48), for 4/11 bronchioloalveolar carcinoma (36%, MS = 61), and for 4/63 SCC (6%, MS = 21). There was no staining for all of the 12 large cell lung cancers, 2 small cell lung cancers, 5 large cell neuroendocrines and 10 carcinoids.
Conclusions: Our results demonstrate downregulation of pIgR/SC expression in a continuum from normal epithelium to invasive lung cancer, in a majority of lung tumors. In addition, absence of pIgR/SC expression in preinvasive lesions suggests that pIgR/SC downregulation is an early event during lung cancer development. Based on these results, we are currently evaluating whether pIgR/SC downregulation represents a marker of tumorigenesis process or could participate in regulation of proliferation, apoptosis and migration in normal, transformed and cancerous airway epithelial cells.

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The use of a multi-analyte immunoassay panel (MAIP) to detect potential prognostic biomarkers associated with 2-month progression free survival rate in patients treated with Enzastaurin as 2nd and 3rd line therapy of NSCLC

A. Cleverly[1], G. Bepler[2], Y. Oh[3], H. Burris[4], R.S. Herbst[3], M. Lahn[5]. *[1]Eli Lilly and Company, Erl Wood, UK; [2]H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL, USA; [3]M.D. Anderson Cancer Center, Houston, TX, USA; [4]The Sarah Cannon Cancer Center, Ashville, TN, USA; [5]Eli Lilly and Company, Indianapolis, IN, USA*

Background: Since the development of tumor markers several attempts have been made to discover other plasma or serum based markers to either predict therapy response to anti-tumor treatments or to better anticipate the prognosis of patients with cancer. The advent of flow cytometry has allowed the development of assessing serum or plasma markers using multiplex immunoassay approaches, as applied in a previously presented study.
Methods: Plasma samples were collected from patients participating in a single arm, phase II study of single agent with enzastaurin as a 2nd and 3rd line treatment for NSCLC. Samples collected at baseline, prior to cycle 1, cycle 2, and cycle 3 (1 cycle = 28 days) were banked and stored at −80ºC, prior to analysis by standard procedures by Rules Based Medicine (Austin, TX). Univariate mixed effect analyses were performed to determine if there was any evidence of changes in analyte concentrations across cycles or between sub-groups of patients categorized by their progression free survival time – greater than or less than 2 months. For this initial screen for possible markers, it was decided not to adjust results for multiplicity of tests, thus recognizing the potential for false discoveries in the listed results.
Results: 55 patients were enrolled and received 500 mg oral enzastaurin/day. No tumor responses were observed. Thirty-six patients progressed and 19 (35%) patients were free from progression (FFP) at 2 months. 13 analytes were associated with the patients who were FFP $\geqslant$2 months. Majority of the biomarkers, such as IL-6, IL-8, CRP, alpha-1-antitrypsin, complement-3, fibrinogen, growth hormone, PAI-1, TIMP-1 and VEGF, were reduced in patients who were FFP $\geqslant$2 months, while apolipoprotein-A1, IL-7 and TNF-beta increased.
Conclusions: Since this study was not a randomised study and because there was no observed tumor response as defined by RECIST, it was not possible to associate a specific marker with enzastaurin activity. However, the difference in some markers when associated with the 2-mo freedom from progression, suggests that these markers may be prognostic for patients in 2nd/3rd line NSCLC.