condition of co-cultivation with macrophages from mice without therapy. TAs+PO244 therapy decreased population of LLC cells in proliferative pool (G2/M+S phase) to 40%, whereas control rates were 65% and 60% in LLC cells without co-culture and LLC cells with macrophage co-culture from mice without therapy, respectively. As the adhesive potential inversely correlates with cell ability to migrating, the *in vitro* data indicated that migration and tumor infiltration can be activate when tumor growing *in vivo*. We have shown it in combined therapeutic scheme application of TA and PO244 on LLC. Monotherapy by TA stimulates tumor infiltration by lymphocytes insignificantly, whereas in combined therapy with PO244 this parameter is increased 2.4 times ($p<0.05$).

Conclusion: Cytotoxic/cytostatic influence, which was expressed in increasing of apoptotic level and decreasing of cell population of proliferative pool was defined after co-cultivation of macrophages from LLC-bearing mice treated by TAs+PO244 with primary LLC culture. This effect can be one of the possible mechanisms of TAs+PO244 impact on the lung cancer.

Keywords: lung cancer, Immunotherapy, Ligands of Toll-like receptors

P2.01-092
PRMT5 is a Poor Prognostic Marker for NSCLC and Inhibition of PRMT5 Results in Increased Lung Cancer Sensitivity to Cisplatin and Radiotherapy

Topic: Targets for Treatment Prediction

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Background: Protein arginine methyltransferase 5 (PRMT5), a member of the protein arginine methyltransferase family, has important regulatory function in many cellular processes through epigenetic control of target gene expression. Because of its overexpression in a number of human cancers and its essential role in cell proliferation, transformation and cell cycle progression, PRMT5 has been recently proposed to function as an oncoprotein in cancer cells. In this study, we explore prognostic and predictive value of PRMT5 expression in lung cancer. Impact of PRMT5 inhibition in the setting of radiation therapy and platin-based chemotherapy was investigated.

Methods: PRMT5 expression levels in lung tumors as well as their paired normal tissue obtained from TCGA public databases were compared. The impact of PRMT5 expression on lung cancer patient survival was investigated by using "Director's challenge Consortium for the Molecular Classification of Lung Adenocarcoma" and JBR10 datasets. SiRNA designed to target PRMT5 was used to transiently knockdown (KD) PRMT5 expression in several lung cancer cell lines. Clonogenic survival assays of lung cancer cell lines with increasing doses of cisplatin or radiation were performed in cells with normal endogenous PRMT5 expression or in cells after siRNA knockdown. Impact of PRMT5 knockdown in cell cycle, apoptosis, DNA damage response was investigated through cell cycle analysis, Annexin/PI flow cytometry, ɣH2A foci measurements in lung cancer cells with normal or reduced PRMT5 expression.

Results: PRMT5 expression is significant higher in lung tumors compared to parired normal tissue in TCGA datasets (LUAD and LUSC) with p value ≤ 0.0001. Patients with high PRMT5 expression portend lower overall survival at 3 years ($p=0.02$) from director's challenge lung cancer study. Patients with low PRMT5 expression had significantly better DFS at 5 years ($p=0.3$) if they received cisplatin while patients with high PRMT5 expression did not benefit from cisplatin treatment ($p=0.7$). In several lung cancer cell lines, we observed >90% PRMT5 KD in transiently transfected cells at 48 h and 72 h post transfection as verified by western blot analysis. This inhibition of PRMT5 activity achieved by transient KD lead to a significant decrease in colony survival after radiation and cisplatin. There is an increase of cell population in G1 arrest in PRMT5 transient KD cells.

Conclusion: High PRMT5 expression is associated with worse survival in lung cancer patients. Inhibition of PRMT5 in lung cancer cells results in sensitization to cisplatin and radiotherapy,

P2.01-093
Exo-ALK Proof of Concept: Exosomal Analysis of ALK Alterations in Advanced NSCLC Patients

Topic: Targets for Treatment Prediction

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Background: A subset of NSCLCs (approx. 5%), present alterations in ALK gene. This produces abnormal ALK proteins that induce cells to grow and spread. Different generation of ALK inhibitors are available for targeted therapy and their indication depends on the detection of ALK alterations in the tissue. Thus, it is mandatory to develop new techniques that allow us to demonstrate ALK alterations in peripheral blood. The purpose of this study is to analyze the feasibility to determine ALK alterations in exosomes (Exo-ALK) in NSCLC patients and determine the sensitivity and specificity of the technique.

Methods: This study is performed in blind in a cohort 19 NSCLC with and without known alterations of ALK in tumoral tissue. ALK-positive tissue samples were identified by FISH or IHQ and patients were included independently of stage and time of disease. Exosomal RNA is isolated by exoRNeasy Serum/Plasma (Qiagen) and retrotranscripted by ProtoScript II First Strand cDNA Synthesis kit. The ALK gene present in the exosomes was determined by NGS and bioinformatic analysis by OncoDNA. Samples and data from patients included in the study were provided by the Biobank of the University of Navarra and were processed following standard operating procedures approved by the Ethical and Scientific Committees, were provided also by UZA Biobank and by the University of Naples Federico II.

Results: The analyzed samples have been 16 ALK-EML4 tissue positive patients and 3 ALK-EML4 tissue negative, defined in this case by FISH. After analysis, we have been able to detect 9 positive ALK-EML4 patients, 8 negative samples and 2 samples where the RNA was degraded. Looking at the clinical data, the 9 positive samples detected in the exosomal RNA were positive also for ALK-EML4 translocation in the tissue, and comparing the 8 negative samples, 3 were tissue negative and 5 tissue positive. These data show a specificity of 64% and a specificity of 100%. No correlation has been found comparing naïve patients with treated patients.

Conclusion: Exosomes are raising as one of the most promising tools to understand the tumor due to their stability in the blood and their similarity to the cells of origin. Our preliminary results show a high specificity and sensitivity for a proof of concept analysis. Further studies with a bigger number of patients and a cross validation analysis are required, but as we represent in this abstract, exosomes can represent an important tool for the clinical management of this specific NSCLC population.

Keywords: liquid biopsy, non-small cell lung cancer, exosomes, ALK alterations

P2.01-094
Stromal Antigen 1 (SA-1), a Cohesin, is a Novel Proto-Oncogene Regulating Chromatin in Non-Small Cell Lung Cancer (NSCLC)

Topic: Miscellaneous

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Background: The molecular composition NSCLC is heterogeneous and clinically manifested as differential therapeutic responsiveness. It is increasingly appreciated that changes in high order chromatin (HOC) structure may play an important role in controlling gene expression and may be one of the fundamental events in carcinogenesis. However, while several HOC regulators are altered in lung cancer (e.g. Arid1a) from the cancer genome atlas work (TCGA), these occur in a minority of tumors suggesting involvement of other modulators. Recently, SA-1 (Stag-1), a member of the cohesin family, has been shown to be a HOC regulator in cancer via controlling chromatin looping and hence gene expression. Since no previous reports on cohesin in lung cancer, we therefore, focused on the role of SA-1 in NSCLC.

Methods: We performed immunohistochemical analysis (IHC) of 190 cancers and compared to benign tissue through standard techniques. We also extracted SA-1 data from the TCGA databases (*Nature* 2012 & 2014).

Results: SA-1 was markedly (~2-3 fold) overexpressed in all types of NSCLC ($p<0.01$) versus benign tissue (Figure 1). This increase was striking at stage 1 NSCLCs with minimal further increase noted at higher stages. TCGA data demonstrated amplification/mutation in ~17% of squamous but only 3% of adenocarcinomas. This suggested that epigenetic regulations was paramount. Kaplan-Meier analysis showed major impact of SA-1 alterations on survival. For instance, in squamous cancers, median disease-free survival with versus without SA-1 amplification was 8 vs 38 months, respectively.