samples (plasma from NSCLC patients) is feasible; moreover we have analyzed a selected panel of 7 exosomal miRNAs related to NSCLC status.

**Method:** After obtaining the informed consent, blood samples (10 ml) of 12 NSCLC patients and 6 healthy volunteers were collected in the UZA-tumor biobank. Clinical data were also collected from patients' medical records. Exosomes were isolated by both Density Gradient (DG) centrifugation and Total Exosome Isolation kit (from plasma) (Invitrogen) according to manufacturers' instructions. The Total exosome RNA and protein isolation kit (Invitrogen) was used for proteins recovery from exosomes for western blot analysis for well-known exosomal markers like CD63, ALIX and TSG101. TEM (Transmission Electron Microscopy) analysis was performed in order to determine the size average of isolated exosomes.
The total exosome RNA and protein isolation kit (Invitrogen) was used to extract small-RNA from exosomal samples. The analysis of 7 miRNAs (miR-30b, -30c, -103, -122, -195, -221, -222) was performed on the LightCycler 480 (Roche) and the fold change was calculated according to the formula $2\Delta\Delta Cq$. miR-1228-3p was used has normalizer in the reaction.

**Results:** ALIX protein expression into the exosomes could be considered a reliable protein marker for exosome identification. Moreover TEM analysis has shown that these nanovesicles can be isolated in NSCLC plasma samples, suggesting that the diameter average is in exosomal range. The 7 selected miRNAs are strongly deregulated in our clinical samples and seem related to staging and in particular miR-30b and miR-30c might be related also to squamous cell carcinoma histotype.

**Conclusion:** This new liquid biopsy tool, exploiting exosomes, could represent a non-invasive test for patients' management in NSCLC; exosome analysis and exosomal miRNA profiling is feasible in NSCLC but due to the limited sample size we cannot have statistic conclusion. Further analyses are needed in order to confirm these hyphotesis.

**Keywords:** exosomes, biomarker, NSCLC, liquid biopsy

## P2.07
## Evaluation of Different Exosomal RNA Isolation Methods in NSCLC Liquid Biopsies

*Track: Biology and Pathogenesis*

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**Background:** Exosomes are biological nanovescicles (30-100 nm), with endocytic pathway origin, created from the inward budding of multivescicular bodies (MVB). It has been described that they contain lipids, proteins and several nucleic acids, among which microRNAs and lncRNAs. Most of these components have been reported to be altered in cancer and maybe exploitable as new biomarkers. However, in the literature there is no consensus about a stable endogenous control for exosomal microRNAs analysis. Here we compare three exosomal RNA isolation methods and assess the normalizer features of exosomal hsa-miR-1228-3p in a liquid biopsy sample set of Non-Small Cell Lung Cancer (NSCLC) patients.

**Method:** After obtaining informed consent, plasma sample from NSCLC patients (N=21) and healthy donors (N=6) were collected in the Oncology department of Antwerp University Hospital. Plasma samples were cleared through sequential centrifugation in order to remove cells, debris and microvesicles. After this step, exosomal RNA was extracted through two different methods: by Total Exosome Isolation kit - from plasma (Invitrogen™) coupled with Illustra RNAspin mini kit® (GE HealthCare©) and by sucrose density gradient ultracentrifugation and ExoRNEasy™ Kit (Qiagen©), according to manufacturer's specifications.
Exosomes characterization was performed through biophysical (Transmission and Scanning electron microscopy TEM-SEM) and biochemical analysis (Western blot for well-known exosomal marker as ALIX and Tsg101). Exosomal RNA quantity and quality was evaluated through spectrophotometry (Nanodrop™ ND-1000). Reverse transcription and qPCR of hsa-miR-1228-3p was performed through TaqMan microRNA assay (Applied Biosystem™). Fold of changes were calculated according to the formula $2\text{-}\Delta\Delta Ct$ using the healthy donors hsa-miR-1228-3p Ct as control. t-test analysis were performed between NSCLC samples depending on their protocol (SPSS 23 Statistics IBM®).

**Results:** TEM and SEM analysis shown that the isolated nanovesicles have a diameter around 30-100 nm and cup shape appearance, according to the literature. Western blot analysis demonstrated the presence of well-known exosomal markers ALIX and Tsg101 and absence of Calnexin (negative control). ExoRNEasy™ kit seems to provide the highest yield of RNA. Real time PCR analysis of hsa-miR-1228-3p has shown no significant

differences between the different exosomal RNA extraction methods.

**Conclusion:** Among these methods, ExoRNEasy$^{TM}$ kit seems to provide the highest yield of RNA. No significant differences were found between sample groups of hsa-miR-1228-3p expression among all the used methods and we suggest that hsa-miR-1228-3p should be considered as a stable endogenous control for exosomal microRNA analysis.

**Keywords:** exosomes, miRNA, exosomal isolation, NSCLC biomarker

## P2.08
## Gene Fusions Detected in Non-Small Cell Lung Carcinoma (NSCLC) and Small Cell Lung Carcinoma (SCLC)

*Track: Biology and Pathogenesis*

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**Background:** Gene fusions, first thought to be limited to hematologic malignancies and soft tissue sarcomas, are taking on more importance in many solid tumors. For instance, functional ESR1 fusions in breast cancer are now associated with resistance to hormonal therapy. In NSCLC, *ALK*-, *RET*-, and *ROS1*-rearrangements are frequently targeted with available kinase inhibitors. However, much remains unknown regarding other chromosomal gene rearrangements. The purpose of this study is to report fusion assay results performed at a multi-omic tumor profiling facility to identify potentially novel or uncommon fusions in a variety of thoracic carcinoma.

**Method:** In total, 356 NSCLC and 20 SCLC were profiled using the ArcherDx FusionPlex Assay at a CAP/ISO/CLIA-certified laboratory (Caris Life Sciences) using FFPE specimens. Fifty-two genes were analyzed for potential gene fusions. Fusion-positive specimens were confirmed using in-situ hybridization and/or Sanger sequencing. Depending on available specimen, tumor samples were evaluated by immunohistochemistry (IHC), and next generation sequencing (NGS) for co-occurring biomarkers.

**Results:** Overall, 32 fusion transcripts in 31 of 356 NSCLC specimens contained a previously reported or novel fusion (8.7%). Fusion transcripts were found in adenocarcinoma (78.1%, 25/32) followed by SCC (15.6%, 5/32) and carcinoma, NOS (6.3%, 2/32). One NSCLC specimen contained two co-occurring fusions (EML4-ALK, PRKCG-PRKCB) and 40.6% (13/32) were either ALK (n=8), *RET* (n=2), or *ROS1* (n=3) rearrangements. More than once detected fusions included *MSMB* (n=3), *ERG* (n=2), *MAST2* (n=2), and *PRKCA* (n=2). Notable fusions included *BRD4*, *FGFR3*, *MET*, and *NTRK3* detected in single cases. Sequencing analysis by NGS revealed no co-occurring deleterious mutations in *BRAF, EGFR, ERBB2, MET, NRAS*. However, *KRAS* G12 mutations were detected in 22.6% (7/31) fusion-positive specimens, all of which were adenocarcinomas. PD-L1 expression was detected in 30.4% (7/23) of fusion-positive specimens. Only one fusion (*SYN2-PPARG*) was identified in SCLC.

**Conclusion:** ArcherDx FusionPlex Assay is a laboratory validated assay for detection of fusions involving ALK, RET, and ROS1, and some additional directly targetable fusions. The presence of mutant KRAS and/or PD-L1 in fusion-positive NSCLC could be used for novel drug combinations. These results could be useful to direct patients to clinical trials for relevant drugs. Further studies are warranted to explore the role of fusions in driving various cancers.

**Keywords:** NSCLC, SCLC, fusion gene, FFPE

## P2.09
## cMET in NSCLC: Expression, Amplification and Mutations

*Track: Biology and Pathogenesis*

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**Background:** Targeted therapies have gained a lot of attention in non-small cell lung cancer, including several cMET inhibitors against non-small cell lung cancer (NSCLC). Nowadays, cMET amplification is used as a standard biomarker for patient selection; however there is still discussion about the cut-off value. More recently, splicing variants of cMET, which show exon 14 skipping, are gaining importance since it has been shown that patients harboring this mutation can