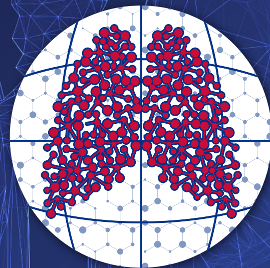

2020 HOT TOPIC MEETING:

LIQUID BIOPSY

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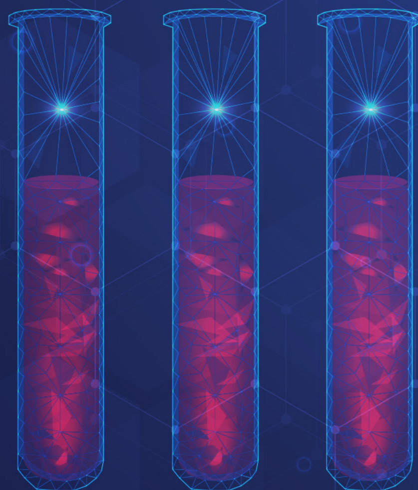
ABSTRACTS



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OA03.01
Integrating Liquid Biopsy into Clinical Practice: Which Patients with NSCLC?

Natasha Leighl
Princess Margaret Cancer Centre, Toronto, Canada

Clinical Application of Liquid Biopsy (ctDNA), October 2, 2020, 09:50 - 11:20

Liquid biopsy, or molecular profiling of cell free (cf)DNA has emerged as an important tool to help overcome many limitations with tissue-based diagnostics and radiologic monitoring in patients with advanced lung cancer. Liquid biopsy allows more frequent monitoring, and is associated with fewer complications than invasive tumour tissue biopsies, and may be cost saving compared to repeat tissue biopsy [1].

Current guidelines highlight the potential to use cfDNA assays in the diagnosis of EGFR T790M resistance after treatment of patients with advanced EGFR mutant lung cancer with first or second generation EGFR kinase inhibitors [2-4]. Studies report sensitivity of cfDNA assays in the range of 61 to 93%, with a positive predictive value that ranges between 77-95% [2,3]. If cfDNA assays fail to detect the resistance mutation, it is important to attempt tumour tissue biopsy and testing in order to rule out presence of the resistance mutation. In some centres, cfDNA testing is recommended as the initial approach to detect resistance, followed by tissue biopsy if results are negative or indeterminate [5].

Can cfDNA assays be used in primary diagnosis of patients with advanced NSCLC? Some guidelines support the use of cfDNA testing in patients with advanced NSCLC that are unfit for repeat tumour biopsy for profiling, or where tumour tissue is insufficient for molecular analysis. [3,4,6] For example, cfDNA assays to detect sensitizing EGFR mutations have a sensitivity of 60-100%, with high specificity (93-100%) [3]. Several studies have demonstrated that use of cfDNA testing in advanced NSCLC patients with insufficient tissue for complete molecular profiling can significantly increase the number of patients eligible for targeted therapy compared to relying only on tissue profiling (absolute increase of 15-19%). [5,7-9].

The clinical utility of cfDNA as part of the diagnostic work up for patients with stage IV adenocarcinoma has recently been reported [10]. Detection of biomarkers by liquid biopsy was non-inferior to tumour tissue testing: actionable biomarkers were identified for 27% of patients using cfDNA testing and 21% using tissue testing. Many patients did not have sufficient tissue for serial biomarker testing, thus cfDNA led to comprehensive testing in 95% compared to 32% with tissue testing (defined as at least one actionable alteration or results for all guideline-recommended biomarkers). Testing was also faster with cfDNA (median 9 days versus 15 days with tissue, $p<0.0001$), highly concordant with tissue (>98.2% for EGFR, ALK, ROS1, BRAF), and demonstrated 100% positive predictive value for EGFR, ALK and BRAF alterations.

Actionable genomic aberrations detected in liquid biopsy have been shown to be actionable. Data with osimertinib, capmatinib and other agents have demonstrated similar response rates to targeted therapy whether the relevant biomarker is identified through cfDNA analysis or tumour tissue profiling [5,7,11].

Caveats include the potential for negative results from liquid biopsy due to low tumour burden, non-shedding tumour cells and the potential for false negative results from pre-analytical or analytical factors. Thus patients with negative results in cfDNA analysis should reflex to tumour tissue biopsy profiling. Also

while cfDNA can detect genomic alterations, histologic transformation should be diagnosed through tissue analysis (e.g. small cell or squamous transformation after targeted therapy [Schmid]. Despite these potential limitations, liquid biopsy has emerged as an important test in the molecular diagnosis of patients with advanced lung cancer to identify actionable genomic biomarkers, and in the diagnosis of molecular resistance after targeted therapy.

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OA03.06
Quantitation of Variant Allele Frequency and Variants in the Decision Making Process: Molecular Biology Perspective and Pathology Perspective (Pathology Perspective)

Associate Professor Lynette Sholl
Brigham And Women's Hospital, Boston, USA

Clinical Application of Liquid Biopsy (ctDNA), October 2, 2020, 09:50 - 11:20

Next generation sequencing technology has enabled us to measure the proportion of a sample that contains a particular DNA variant with greater accuracy and precision than its technological antecedents including Sanger sequencing or pyrosequencing. In addition, the breadth of NGS-based panel testing renders this approach attractive for comprehensive tumor profiling in clinical practice, whether through analysis of tumor tissue or circulating tumor DNA (ctDNA) in “liquid biopsy”. The actual measure of variant fraction in a sequenced sample, reported in practice as a percentage, is known as variant allele frequency (VAF) or mutant allele fraction (MAF). In both tissue and liquid specimens (be they plasma, urine, CSF or other bodily fluid) the potential contributors to the variants detected by sequencing include: (1) the mutated allele within the tumor; (2) the nonmutated (wild type) allele within the tumor; and (3) the two (or more) alleles derived from cells other than the tumor of interest, including stroma and/or white blood cells. The VAF for tumor variants of interest will be dictated by the amount of tumor DNA in the specimen, as well as by the presence of allelic imbalance (AI). AI can occur due to gene deletion, amplification, or loss of heterozygosity, potentially at least doubling the mutant allele VAF. On average, and even in cancer patients, the majority of cell free DNA in plasma is derived from the turnover of myeloid and lymphoid cells. As a result, mutant ctDNA tends to represent a minor species; the median tumor VAF in clinical plasma-based sequencing is less than 0.5%, a value approaching the limit of detection of some validated assays.(1) While the low level VAFs frequently reported in clinical samples may lead a provider to wonder if the result is “real”, most available plasma assays utilize error correction methods and optimize technical specificity. Thus, these low-VAF alterations are in all likelihood derived from the patient (rather than from a sequencing error), however available assays cannot discern whether variants are derived from the tumor of interest or from another clonal process such as “clonal hematopoiesis”. Paired sequencing of cfDNA and blood white cell fractions have revealed that for many patients clonal hematopoiesis contributes the majority of variants and often at VAFs that exceed those of the tumor contribution.(2) Germline variants, whose average VAF in the blood is ~50%,(3) may confound the interpretation of tumor variants present at a high VAF, such as in patients with very high tumor burdens or with high level amplification of a mutated allele (for instance in some EGFR-mutated lung cancers). In summary, a variety of biological factors can influence VAFs reported in tumor sequencing; in general, clinically reported variants can be taken as technically real even at low levels but should be considered in the context of the different pathologic processes that may coexist in an individual patient.

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OA07.03
Liquid Biopsy for Detection of Minimal Residual Disease

Professor Tetsuya Mitsudomi
Kindai University Faculty Of Medicine, Osaka-Sayama, Japan

Increasing Roles for Liquid Biopsy: Moving from Research to Clinical Application, October 3, 2020, 09:50 - 11:40

Although surgery offers the best chance for cure of early-stage non-small cell lung cancer (NSCLC), many patients still have to suffer from the recurrent disease which is thought to be due to the presence of minimal or molecular residual disease (MRD) after the so-called "complete resection". Currently, platinum-doublet chemotherapy is a standard-of-care as postoperative adjuvant treatment for the completely resected stage II-III NSCLC which results in a 5% increase of the 5-year survival. However, this means that 95% of patients are either those whose disease cannot be cured by the adjuvant treatment or those who are cured by surgery alone and thus do not require adjuvant therapy. The former group of patients can be decreased by selecting patients by the relevant biomarker who will be benefited by a relevant drug. A recent report of adjuvant osimertinib for the patients with EGFR mutation is such an example. The latter group of patients will be decreased if we can identify patients who will not have recurrent disease. Attempts have been made to identify these patients by prognostic biomarkers. So many candidates have come and gone, including changes in several oncogenes/tumor suppressor genes. Knowing whether or not cancer cells remain in the body after surgery will be important information in predicting tumor recurrence. However, the number of cancer cells remaining after "curative surgery" is small, if any, and the detection of MRD has been a technical challenge.

The recent advent of next-generation sequencing is set to change this situation. Several groups of investigators and companies elaborated on the technique to increase sensitivity and specificity. For example, cancer-specific gene mutations in the circulating cell-free DNA which is circulating tumor DNA (ctDNA) could be detected in 5/5 and 22/59 patients with stage I disease by CAPP seq and TRACERx (Signatera) technology, respectively. It has been shown that there is a clear correlation between ctDNA detection and disease recurrence. Patients whose ctDNA was not detected after the surgery rarely suffers from the disease recurrence and thus have a good prognosis. This will offer a great opportunity for stratifying patients into different risk groups. Patients deemed at higher risk by the presence of MRD should have benefited by more aggressive postoperative adjuvant therapy, while those who are at low risk may omit adjuvant therapy even though they had mediastinal lymph node involvement. IT would be Furthermore when ctDNA does not decrease during adjuvant therapy, the change of drugs may be indicated to improve survival outcomes. MeRmaiD1 study currently ongoing is to compare disease-free survival of MRD-positive patients by ctDNA analysis between those who receive durvalumab and those who receive placebo, in addition to chemotherapy, which is certainly a very important approach to establish a newer generation of standard-of-care.
In this talk, I would like to summarize the current status of MRD research in lung cancer and to discuss future perspectives.

OA07.05
Liquid Biopsy for Tumor Monitoring

Natasha Leighl
Princess Margaret Cancer Centre, Toronto, Canada

Increasing Roles for Liquid Biopsy: Moving from Research to Clinical Application, October 3, 2020, 09:50 - 11:40

The role of liquid biopsy in the monitoring of advanced NSCLC continues to grow. The key challenge remains to demonstrate clinical utility of incorporating liquid biopsy monitoring into our daily practice, to demonstrate that its use improves patient outcomes. Current uses include estimation of prognosis, stratification of surveillance or treatment planning, detection of recurrence, monitoring of disease course and to be used in response assessment and treatment planning. This may include treatment change, intensification or de-escalation of therapy.

There are many members of the liquid biopsy family that have been studied in tumor monitoring. These include cell free (cf)DNA including circulating tumour (ct)DNA, which has been used to measure tumour mutation burden and changing levels of ctDNA with therapy. Peripheral blood mononuclear cells, soluble proteins, circulating tumour cells, exosomes and micro RNA expression levels have all been assessed in lung cancer [1,2].

The presence of ctDNA at baseline may be associated for worse outcome than those patients without detectable levels of ctDNA prior to treatment, suggesting that this may be prognostic and possible related to lower tumour burden in those with negative ctDNA results [3]. Clearance of ctDNA after initiation of treatment is also associated with better outcome, demonstrated across several studies of targeted therapy and immunotherapy, and a large real world study that demonstrated significant reduction in the hazard ratio for progression (0.28) and survival (0.19) [4,5]. Even in the absence of ctDNA clearance, reduction in ctDNA levels with treatment may also be associated with better outcomes [5-7]. Also evolution in T-cell clonal dynamics may be important in evaluating early response to checkpoint inhibition [8].

The association between the emergence of ctDNA changes and disease progression has been widely reported across studies of targeted and immunotherapy in lung cancer [9-11]. However, learning how to incorporate tumour monitoring into our daily clinical practice has been more challenging. The APPLE study, led by the EORTC (Dziadziuszko et al.) will help us gain further insight into the clinical utility of liquid biopsy in monitoring tumour response in those with EGFR mutant lung cancer receiving first-line gefitinib followed by osimertinib, versus traditional radiographic and clinical monitoring, versus a third arm with upfront osimertinib [12]. Similar studies are needed when deciding how to select patients for single-agent or combination checkpoint inhibition versus adding chemotherapy. Two recent studies highlight additional potential opportunities in this area. Hellmann et al explored the value of ctDNA levels in patients with advanced NSCLC receiving prolonged checkpoint inhibitor therapy [13]. Those with detectable ctDNA levels using the ultrasensitive CAPP-Seq assay were more likely to progress than those without evidence of circulating levels. In stage III lung cancer, those with detectable ctDNA after induction chemoradiation were more likely to progress than patients without detectable ctDNA levels, with a mean lead time of 4.1 months between ctDNA detection and evidence of radiographic progression [14]. Although consolidation immunotherapy improved freedom-from-progression in this group, it suggests that liquid biopsy may help identify a group at higher risk that should be considered for intensification of therapy. Randomized trials in

these and other settings are urgently needed to allow us to incorporate tumor monitoring by liquid biopsy into our daily practice.

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VIRTUAL POSTERS

Featured Posters

VP01.01
Potential of Plasma Circulating Tumor DNA as a Monitoring Tool in Patients with Advanced EGFR-Mutated NSCLC

Ms Vichitra Behel[1], Dr. Anuradha Chougule[1], Ms Kavya Nambiar[1], Ms Priyanka Bagayatkar[1], Ms Shrutikaa Kale[1], Ms Vaishakhi Trivedi[1], Dr. Pratik Chandrani[1], Dr. Vanita Noronha[1], Dr. Vijay Patil[1], Dr. Amit Joshi[1], Dr Nandini Menon[1], Dr. Abhishek Mahajan[1], Dr. Amit Janu[1], Dr. Kumar Prabhash[1]
[1]Tata Memorial Center, Mumbai, Parel, Mumbai, India

In 2018, lung cancer was reported to be the third leading cause of cancer-related deaths in India. The treatment of non-small cell lung cancer (NSCLC), which comprises 85% of all lung cancers, has revolutionized over the past decade owing to the identification of targetable driver mutations. In Indian patients, the incidence of epidermal growth factor receptor (EGFR) mutations that confer sensitivity to oral tyrosine kinase inhibitors (TKIs) is approximately 20–23%.[1–4] However, despite the use of targeted therapy, disease progression inevitably occurs.[5] Therefore, there is a need for timely disease monitoring. Plasma-based liquid biopsy has shown the potential for disease monitoring in patients with NSCLC.[6]
The aim of our study was to determine the status of EGFR mutation in plasma circulating tumor DNA (ctDNA) of patients with stage IV lung adenocarcinoma and correlate the post-treatment ctDNA status with disease progression.

This was a single-center prospective study conducted at the Tata Memorial Center in Mumbai, India on patients with histologically diagnosed lung adenocarcinoma harboring a mutation in the EGFR gene as detected by a conventional tumor tissue biopsy. Blood samples were collected from each patient at baseline and at 3–5 months post the initiation of therapy with an oral EGFR TKI. The status of EGFR mutation was determined in the plasma ctDNA obtained from the blood samples collected at both time points using the Cobas EGFR Mutation test v2 (Cobas v2; Roche Molecular Systems, Pleasanton, CA, USA). The primary endpoint of the study was progression-free survival, defined as the time to disease progression or death. The secondary endpoints were concordance between the EGFR mutation status as detected using tissue and liquid biopsy and the sensitive and positive predictive value of mutation detection using liquid biopsy.
A total of 46 patients were enrolled in the study. The demographic characteristics of the cohort are presented in Table 1. Of the 46 patients treated with oral TKIs, 10 were found to be EGFR mutation-positive at 3–5 months post the initiation of therapy, whereas 36 were EGFR mutation-negative. The median PFS for ctDNA-positive patients (7.9 months; 95% CI, 2.07–11.4) was significantly lower than that for ctDNA-negative patients (20.7 months; 95% CI, 13.9–25.6) (p=0.0119) (Figure 1). The concordance for EGFR mutation detection at baseline between tumor tissue biopsy and liquid biopsy was found to be 80% as shown in Table 2. The sensitivity and positive predictive value for liquid biopsy with tissue biopsy as gold standard were 82.2 % and 97.37%, respectively.[7]

In conclusion, EGFR mutation positivity as detected from plasma ctDNA at 3–5 months post the initiation of therapy was found to be associated with poor PFS. Thus, plasma ctDNA can be used as a monitoring tool for early detection of disease progression in patients with advanced EGFR-mutated NSCLC receiving oral TKIs.

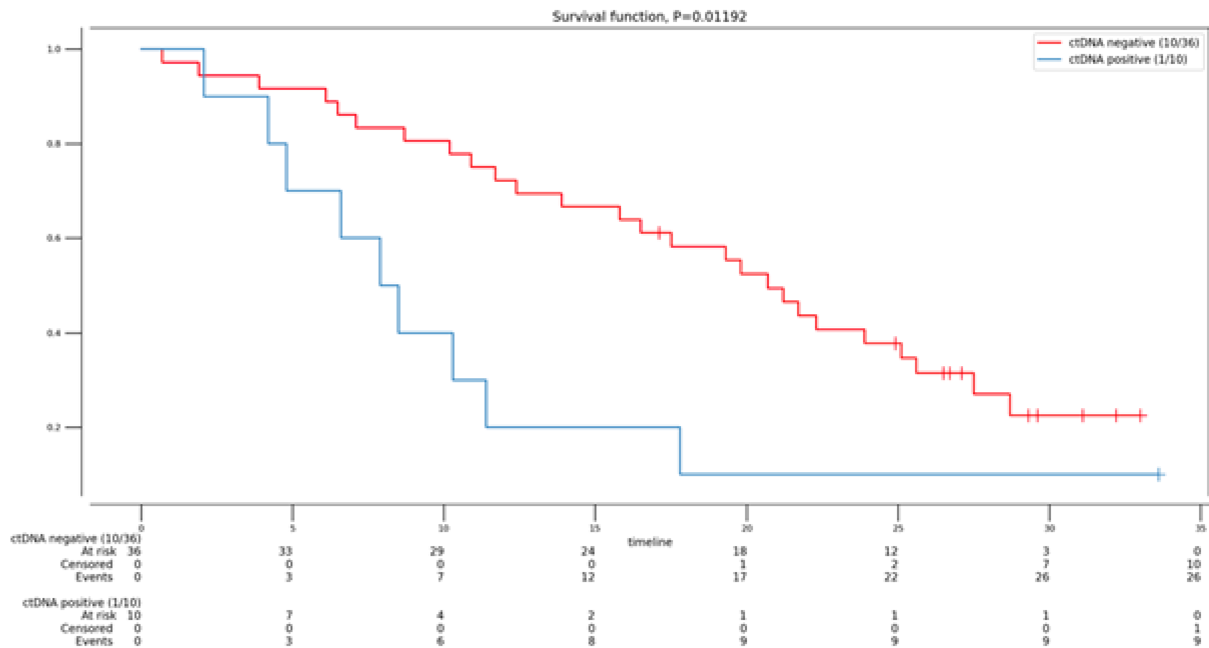


Table 1: Demographic details

Patient characteristics	No. of patients (%)
Age, years	
Median	55
Range	35–75 years
Sex	
Male	28 (60)
Female	18 (40)
Histology	
Adenocarcinoma	46 (100)
Disease stage	
Stage IV	46 (100)
Smoking status	
Non-smoker	32 (69.5)
Smoker	14 (30.5)
ECOG PS	
1	40 (87)
2	6 (13)
Comorbidities	
Diabetes mellitus	3 (6.5)
Hypertension	2 (4.3)
None	42 (91)

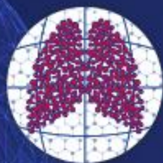
Table 2: Concordance for mutation detection at baseline between tumor tissue biopsy and liquid biopsy

	EFGR mutation +ve on tissue biopsy	EFGR mutation -ve on tissue biopsy
EFGR mutation +ve on liquid biopsy	37	1
EFGR mutation -ve on liquid biopsy	8	0

VP01.03
Treatment Induced Tumor Lysis to Enhance Sensitivity of ctDNA Analysis and its Prognostic Value: A First-in-Human Pilot Study

Dr. Daniel Breadner[1,2], Dr. Mark Vincent[1,2], Dr. Rohann Correa[1], Ms. Morgan Black[1], Mr. Andrew Warner[1], Dr. Clive Morris[3], Dr. Gregory Jones[3], Dr. Alison Allan[1,2], Dr. David Palma[1,2], Dr. Jacques Raphael[1,2]
[1]London Regional Cancer Program, London, Canada, [2]Western University, London, Canada, [3]Invivata Inc., Research Triangle, United States

Background: Blood based liquid biopsies examining circulating tumour DNA (ctDNA) have increasing applications in non-small cell lung cancer (NSCLC). Limitations in sensitivity remains a barrier to ctDNA replacing tissue-based testing. There is a paucity of data regarding the dynamics of ctDNA levels in the hours to days following a new treatment. We hypothesize that chemotherapy or radiation will yield an increased abundance of ctDNA in plasma by inducing tumor lysis, allowing for the detection of genetic alterations that were occult in baseline testing. **Methods:** Two prospective cohorts of 20 patients (pts) with stage III/IV NSCLC were enrolled. Cohort 1 (C1) contained pts starting the first cycle of platinum doublet chemoradiation (C1a, n=10) or the first cycle of platinum doublet cytotoxic chemotherapy ± immunotherapy without radiation (C1b, n=10). Cohort 2 (C2) contained pts receiving palliative radiation. Two baseline samples were collected, the first ≤ 14 days prior to starting treatment and one immediately prior to treatment. In C1, subsequent samples were collected 3, 6, 24 and 48 hours post initiation of chemotherapy. Pts in C2 had samples collected immediately prior to radiotherapy fractions 2, 3, and 4. Samples were analyzed for ctDNA using the 36-gene amplicon-based NGS Inivata InVisionFirst®-Lung assay. **Results:** A total of 40 pts were enrolled, 1pt only had baseline samples collected and is excluded from the post-treatment analysis. Detectable ctDNA was present at baseline in 32 pts (80%), 4 additional pts (50% of the 8 without detectable ctDNA at baseline) had detectable ctDNA in post treatment samples. Seven of the patients with detectable ctDNA at baseline (23%) had new genetic alterations detected in post treatment samples. A total of 11/39 pts (28%) had new genetic alterations detected in the post treatment samples. Mutant molecule numbers increased with treatment in 24 of 31 (77%) pts with detectable ctDNA, C1 - 13 of 19 pts (68%) and C2 - 11 of 20 pts (55%). ctDNA levels peaked a median of 7 hours (IQR: 2 – 26 hours) after the initiation of chemotherapy and a median of 2 days (IQR: 1-3 days) after radiation was commenced. The percentage increase in ctDNA levels was a median of 43% (IQR: -20 to +125%) in C1. C2 had a median increase of 12% (IQR: -12 to +104%). The ability of acute changes in ctDNA levels to predict response to treatment and prognosticate recurrence and survival will also be presented. **Conclusion:** ctDNA levels increase in the hours to days after starting treatment. ctDNA testing in the acute post treatment phase can yield results that were not evident in pretreatment testing. Application of this principle could improve ctDNA utility as an alternate to tissue-based testing and improve sensitivity for the detection of treatment-resistant clones.



VP01.04
Circulating Tumor Cells and Tissue AXL as the New Prognostic Biomarkers in Resected Non-Small Cell Lung Cancer Patients.

Mr Diego De Miguel Perez[1,2,3], Ms Clara Isabel Bayarri-Lara[4], Mr Francisco Gabriel Ortega[2], Mr Alessandro Russo[1], Ms María José Moyano Rodriguez[4], Ms Maria Jesus Alvarez-Cubero[2], Ms Elizabeth Maza Serrano[2,5], Mr José Antonio Lorente[2,3], Mr Christian Rolfo[1], Ms María José Serrano[2,5]
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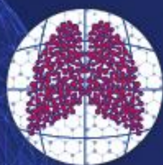
Background: The prognosis of early stage non-small cell lung cancer (NSCLC) patients is quite disappointing. Despite surgical resection, the benefit of current adjuvant therapy is relatively small. This, might be caused by the complex heterogeneous dynamic nature of tumors and the molecular differences between histological subtypes, that the traditional tissue biopsy might not be able to reflect. Moreover, lung adenocarcinoma (ADC) and squamous cell carcinoma (SCC) have distinct clinico-pathological characteristics and novel therapeutic strategies are under active evaluation in the adjuvant setting. Thus, there is an urgent need to identify novel prognostic and predictive biomarkers that would aid in the clinical management of the patients and potentially improve their survival and quality of life. For that, we investigated the prognostic impact of circulating tumor cells (CTCs) and gene and miRNA tissue expression in resectable NSCLC patients. **Patients and methods:** We assessed the association between CTCs subpopulations and outcome in resected early stage ADC at three different time-points (CTC1-3) (before surgery, after one month, and after six months) in comparison to SCC patients. Furthermore, gene and miRNA tissue expression, immunoprofiling and epithelial-to-mesenchymal transition (EMT) markers were correlated with the outcome. **Results:** ADC (n=47) and SCC (n=50) cohorts revealed similar number of CTCs but different dynamics along the treatment (p=0.033); SCC patients showed statistical reduction (p=0.001) not observed in ADC patients (p=0.378). Moreover, these histological subtypes manifested different tissue expression profiles, resulting also in the presence of different CTCs subpopulations. In ADC, miR-155 correlated with tissue AXL and IL6R expression, which were related to the presence of EMT CTC1 (p=0.014 & p=0.004). The presence of epithelial CTC2 and CTC3 correlated with higher tumor stage (p=0.006) and lymph node affectation (p=0.017) respectively in ADC. In the multivariate analysis, epithelial CTC2 was an independent prognostic factor for relapse-free survival (p=0.034), while epithelial CTC3 (p=0.017) and tissue AXL (p=0.017) were independent prognostic for overall survival only in ADC patients. Neither the surgery nor the adjuvant treatment influenced the prognosis of these patients. **Conclusions:** Our study demonstrated the prognostic impact of tissue AXL expression and the presence of epithelial CTCs after surgery only in ADC patients, who interestingly showed no CTC-reduction during the follow-up. Moreover, our results could justify the inclusion of tissue AXL expression and the characterization of EMT CTC as potential biomarkers for treatment selection of ADC patients and set the basis for the use of AXL inhibitors or immunotherapy as new adjuvant treatments.

IASLC  2020 HOT TOPIC MEETING: LIQUID BIOPSY

VIRTUAL MEETING

OCTOBER 2-3, 2020 | WORLDWIDE VIRTUAL EVENT

ADC
SCC
40%
38%
Adjuvant therapy
Surgery and tissue collection
Adjuvant therapy (platinum doublets x4)
Regular follow-up for 5 years
DAY 1
DAY 0
1 MONTH
6 MONTHS
Blood draw (CTC1)
Blood draw (CTC2)
Blood draw (CTC3)
Operable lung cancer patients (stage I-IIIA)
Real-time liquid biopsy
RNA
microRNA
q-PCR
Tissue expression

ADC
p=0.378
SCC
p=0.001
CTC1
CTC2
CTC3

ADC resected patients
RFS CTC2 +
RFS CTC2 -
Follow-up
Adjuvant therapy
Follow-up (months)

Overall survival (%)
CTC3- & AXL low
CTC3+ or AXL high
HR=9.42 (p=0.002)
Median OS (95%CI), months
CTC3- & AXL low = Undefined
CTC3+ & AXL high = 19 (13.1-24.9)
Follow-up (months)

VP01.05
Extracellular Vesicle miRNAs and Autophagy-Activated CTCs are Predictive and Prognostic Biomarkers in Advanced Non-Small Cell Lung Cancer Patients.

Sr Diego De Miguel Perez[1,2,3], Ms Rosario Guerrero Tejada[4], Sr Alessandro Russo[1], Mr Antonio Martínez-Única[4], Sr Brandon Cooper[1], Ms Rena Lapidus[1], Sr Muthukumar Gunasekaran[5], Sr Jose Antonio Lorente[2,3], Sr Jose Exposito[4], Ms María José Serrano[2], Sr Christian Rolfo[1]
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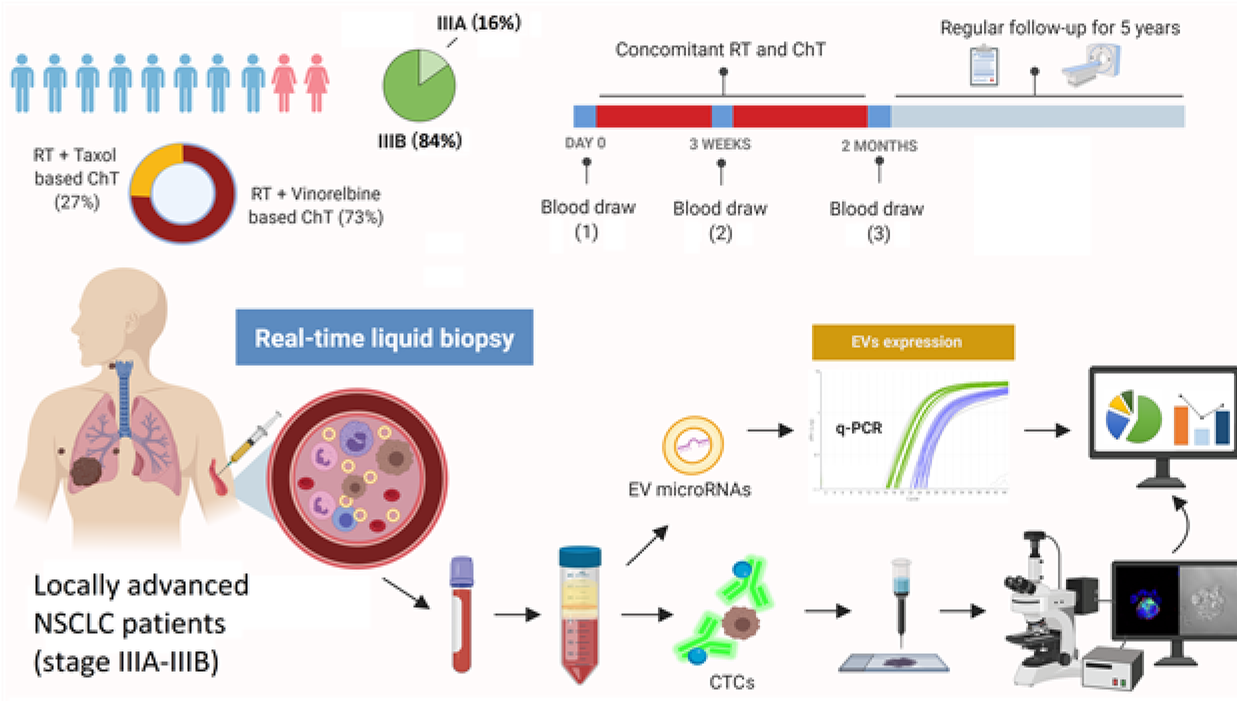
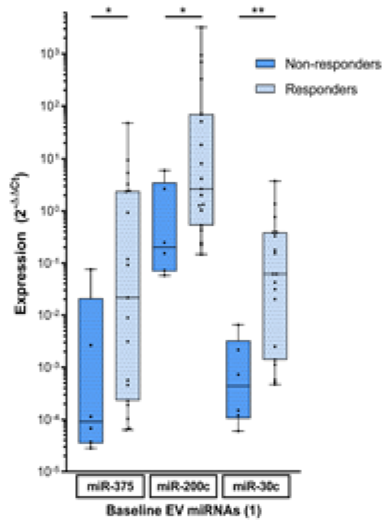
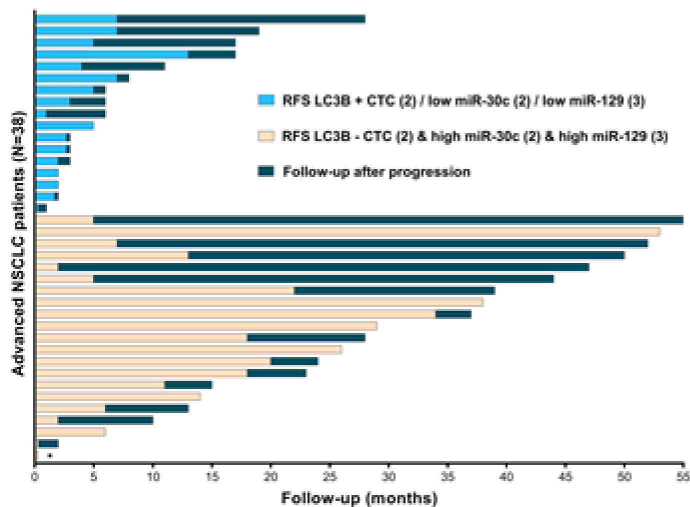
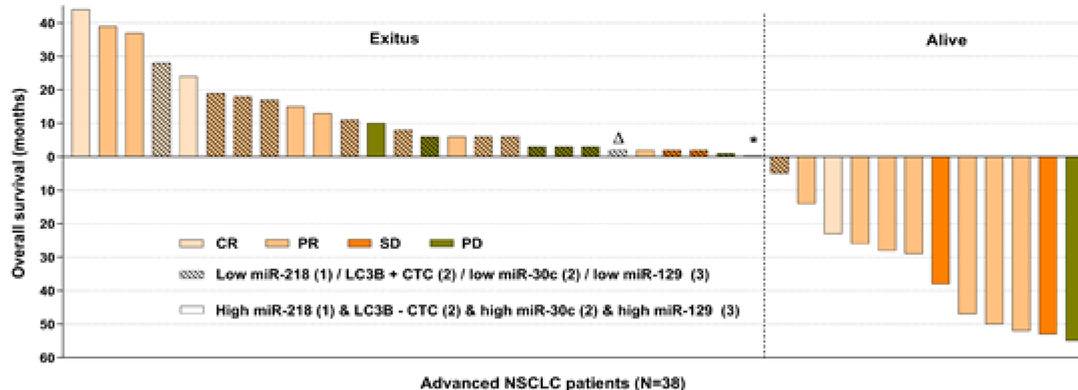
Background: Most non-small cell lung cancer (NSCLC) patients are diagnosed at advanced stages, where the survival rate is low. Concomitant chemo-radiotherapy is the standard of care in these patients; however, progression is early developed. Treatment failure might be caused by the heterogeneous and dynamic nature of tumors that the traditional tissue biopsy may not reflect, resulting in an inaccurate patient stratification. Thus, new reliable prognostic and predictive biomarkers are needed to identify those patients who will benefit from these regimens. miRNAs, known as important regulators of cancer genes, can be selectively encapsulated into extracellular vesicles (EVs) by cancer cells, playing an important role in cell-to-cell communication and acting as cancer biomarkers. Similarly, circulating tumor cells (CTCs) shredding from tumors are a key event in the metastatic process and have been postulated as potential prognostic factors in these patients. However, little is known about the release of heterogeneous subpopulations of CTCs that can be found in a single patient. Despite recent evidence has linked the upregulation of autophagy to an increased resistance to cisplatin, vinorelbine or radiotherapy treatments, there are still controversial reports showing a dual role of autophagy in tumor cell survival. Thus, further research is needed. **Objectives:** To identify the role of specific EV-miRNA signatures and autophagic CTCs as biomarkers in advanced NSCLC patients. **Methods:** This prospective study included 38 locally advanced NSCLC patients under concomitant radio-chemotherapy. Peripheral blood samples were collected before (1), during (2), after the treatment (3). Epithelial CTCs were immunomagnetically isolated and autophagic activity was characterized by immunofluorescence of the LC3B marker. EVs were isolated by ultracentrifugation methods and characterized according to ISEV recommendations. miRNA levels were analyzed by RT-PCR. **Results:** The increase of EV-miR-375 and miR-200c (1-3) correlated with the reduction of autophagic CTCs. High levels of EV-miR-375, miR-200c, and miR-30c (1) identified non-responders to the treatment with an AUC=0.939. Moreover, the multivariate analysis, including the presence of autophagic-activated CTCs and EV-miR-30c (2), identified patients with shorter relapse-free survival and overall survival. The miRNA target analysis showed that miR-375, miR-200c, and mir30c shared targeted genes involved in the autophagy activation and cell division, being involved in radio-chemotherapy resistance. **Conclusion:** This is the first report showing that EV-miRNAs and autophagic-CTCs are predictive and prognostic biomarkers in advanced NSCLC patients. Their identification could provide real-time tumor information for treatment stratification of NSCLC patients and might also benefit new combinatory strategies based on autophagy inhibition or immunotherapy.


IIIA (16%)
IIIB (84%)
RT + Taxol based ChT (27%)
RT + Vinorelbine based ChT (73%)
Concomitant RT and ChT
Regular follow-up for 5 years
DAY 0
3 WEEKS
2 MONTHS
Blood draw (1)
Blood draw (2)
Blood draw (3)
Real-time liquid biopsy
EVs expression
q-PCR
EV microRNAs
CTCs
Locally advanced NSCLC patients (stage IIIA-IIIB)


Non-responders
Responders
Expression ($2^{-\Delta\Delta Ct}$)
miR-375
miR-200c
miR-30c
Baseline EV miRNAs (1)


RFS LC3B + CTC (2) / low miR-30c (2) / low miR-129 (3)
RFS LC3B - CTC (2) & high miR-30c (2) & high miR-129 (3)
Follow-up after progression
Advanced NSCLC patients (N=38)
Follow-up (months)


Exitus
Alive
Overall survival (months)
CR
PR
SD
PD
Low miR-218 (1) / LC3B + CTC (2) / low miR-30c (2) / low miR-129 (3)
High miR-218 (1) & LC3B - CTC (2) & high miR-30c (2) & high miR-129 (3)
Advanced NSCLC patients (N=38)

VP01.06
Identification of New Biomarkers in Non-Small Cell Lung Cancer (NSCLC) by Analyzing Exosomes Cargo

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Introduction: Non-smal cell lung cancer (NSCLC) is one of the cancer types with the highest incidence and mortality. More than 60% of patients are diagnosed in advanced stages, and tissue biopsy is unavailable in many cases. Exosomes are membranous vesicles (40-150 nm) that carry biological information to distant tissues, being able to regulate several tumor processes. The objective of this study was to analyze NSCLC exosomes cargo for searching new biomarkers that could improve NSCLC clinical management. **Methods:** Biomarker screening was carried out in exosomes secreted by tumor cell cultures (cell lines and primary cultures derived from resected NSCLC patients). Secreted exosomes were isolated by ultracentrifugation, and characterized through nanovesicles tracking analysis (NTA), electron microscopy and immunoblotting. Exosomal DNA mutations were determined by Digital PCR, and gene expression was carried out with Transcriptomic microarrays ($p \leq 0.01$). Expression of selected biomarkers was evaluated in (TCGA) database and assessed by RTqPCR in our resected NSCLC training cohort (paired tumor/normal tissue). Prognostic value was determined by Kaplan-Meier curves, $p<0.05$. **Results:** Exosomes size was around 130 nm and exhibited specific surface markers (TSG101, CD9). Mutational analysis of EGFR, RAS and ALK genes showed the same pattern in exosomes and the cells of origin. Transcriptomic analysis revealed a significant differential expression between adenocarcinoma (ADC) vs squamous cell carcinoma (SCC) derived exosomes. Concretely, XAGE1B and AQP4 were overexpressed in ADC, whereas CABYR and RIOK3 were overexpressed in SCC ($p<0.01$). These results were confirmed in TCGA (n=706) and in our NSCLC patient cohort (n=186) ($p<0.05$). Furthermore, our cohort revealed an association between XAGE1B expression and prognosis in ADC group (n=74; Overall Survival: NR months vs 49.6, p=0.013). **Conclusion:** Exosomes are a source of biomarkers for the study of NSCLC, which provide valuable information of tumor characteristics, especially in cases where it is not possible to obtain a tissue sample.

Supported by GV/2018/026, PI18/00266, AMACMA & AECC Valencia

VP01.07
Longitudinal Disease Monitoring of Circulating Tumor DNA in EGFR T790M Mutation-Positive Non–Small-Cell Lung Cancer Patients Receiving Treatment with Osimertinib (WJOG8815L)

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Purpose: The purpose of this clinical phase II study (WJOG8815L) was to assess the clinical utility of monitoring with circulating tumor DNA (ctDNA) for genetic alterations during the treatment with osimertinib in non-small cell lung cancer (NSCLC) patients. **Experimental design and Methods:** Plasma samples were longitudinally collected at the following time-points during treatment with osimertinib: pre-treatment (Pre), on day 1 of treatment cycle 4 (C4), on day 1 of treatment cycle 9 (C9), and at diagnosis of disease progression or treatment discontinuation (PD/stop). The plasma ctDNA was analyzed for actionable mutations and the T790M mutation of EGFR and other genetic alterations by cobas EGFR Mutation Test v2 (cobas), droplet digital PCR (ddPCR), and targeted deep sequencing using next-generation sequencing (NGS). The mutation burdens of the actionable and T790M mutation of EGFR were determined in terms of the mutant fractions (MFs units, copies/µL, and mutant allele frequency (%) by cobas, ddPCR, and NGS, respectively). **Results:** Plasma samples (n = 169 points) were obtained from a total of 52 eligible patients. Actionable and T790M mutations of EGFR were detectable in the ctDNA by all the assays before the start of treatment. A decrease in the MFs of both actionable mutations and the T790M mutation of EGFR was observed during treatment. In contrast, a rebound of the MF at PD/stop was observed only for the actionable mutations, suggesting that osimertinib targeted T790M mutation-positive tumors and tumors with actionable EGFR mutations of EGFR. The median values of the MFs of the EGFR mutations were used as the cutoff points to categorize the patients into the MF-high and MF-low groups. Significant differences in the response rates were observed between the MF-high and MF-low groups at C4 as classified according to the median MF values determined by all three assays for actionable EGFR mutations (the response rates, for example, were 37.5% and 76.7% in the MF-high and MF-low groups, respectively, as categorized according to the results of ddPCR), but not at Pre or C9. On the other hand, no significant differences in the response rates were observed between the MF-high and MF-low groups for the EGFR T790M mutation at any time-point. Significant differences in the duration of progression-free survival (PFS) were also consistently observed between the MF-high and MF-low groups at C4 and C9 (as determined by all three assays) for actionable mutations. The most significant difference in the PFS was observed between the MF-high and MF-low groups for actionable mutations at C4, as categorized according to the results of ddPCR ($P < 0.0001$).

Conclusion: ctDNA monitoring of actionable mutations of EGFR at C4 is suitable for predicting the treatment outcomes in NSCLC patients receiving osimertinib.

VP01.08
Pilot Study of a Novel Liquid Biopsy Test to Discriminate Benign vs. Malignant Processes in Subjects with Indeterminate Pulmonary Nodules

Dr Joshua D Kuban[1], Mr Shahram Tahvilian[2], Mrs Lara Baden[2], Dr Claudia Henschke[3], Dr David Yankelevitz[3], Mr Daniel Leventon[2], Ms Rebecca Reed[2], Mrs Ashley Brown[2], Ms Allison Muldoon[2], Dr Michael J Donovan[4], **Dr Paul C Pagano[2]**
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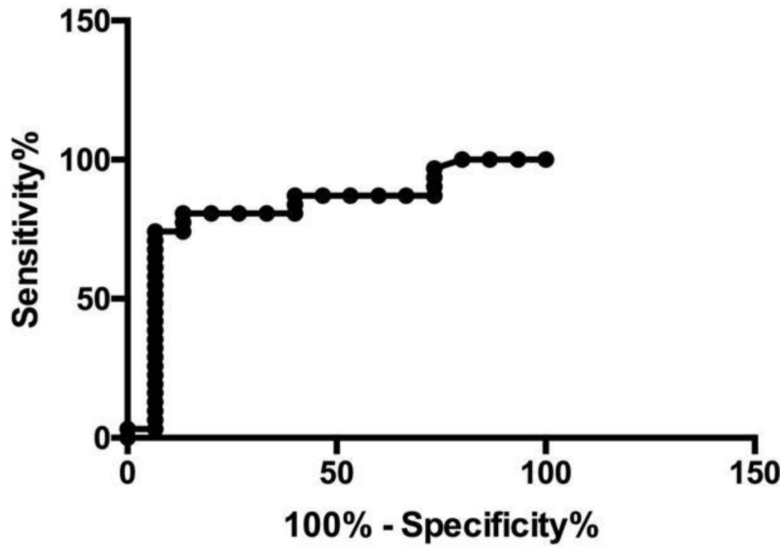
Introduction: Computed tomography (CT scan) is the standard approach for interrogating pulmonary nodules suspicious for lung cancer based on radiological and clinical features as important factors in the biopsy decision process. It is estimated ~40% of biopsies for suspicious pulmonary nodules yield a benign diagnosis and these biopsies potentially lead to complications including infection, pneumothorax, hemorrhage and even death. Novel blood-based assays are clinically needed to improve specificity. LungLB™ is a liquid biopsy being developed as an aid in the clinical assessment of patients with indeterminate nodules suspicious for lung cancer. **Methods:** Blood samples taken at the time of biopsy were sent blinded from MD Anderson to LungLife's CLIA laboratory. Eligible patients were scheduled for percutaneous biopsy as per standard practice, without regard to nodule characteristics. The LungLB™ test is a 4-color fluorescence in-situ hybridization assay used to detect early circulating tumor cells from peripheral blood draw. CTC were defined as having a gain in two or more channels. LungLB™ is being developed as an aid in the clinical assessment of patients with indeterminate nodules suspicious for lung cancer. **Results:** We report data from a blinded analysis of 46 patients receiving a LungLB™ blood draw concurrent with nodule biopsy (n=31 malignant, n=15 with benign nodules) ranging in size from 0.3 to 4.4 cm. Using ROC analysis, we achieved a sensitivity of 81% and specificity of 87% (threshold 2.17 CTC/10,000 cells analyzed) with an AUC of 0.823 (Figure 1). Clinical factors commonly used in malignancy prediction models were also assessed and were found to be non-informative (Table 1), suggesting data reflect "real world" scenarios and have no demonstrable selection bias. **Conclusion:** Preliminary performance of the LungLB™ assay suggests the test may be useful as an adjunct to the clinical assessment of indeterminate lung nodules. Expanded multi-site validations are currently underway.



Parameter (mean)	Benign n=15	Malignant n=31	P value
Age (years)	69.5	68.9	0.796
Gender (% female)	47%	42%	
Smoking history (pk/yr)	31.6	22.6	0.092
Nodule size (cm)	2.19	1.94	0.247
Nodule size range (cm)	0.8 – 3.4	0.3 – 4.4	
Nodule location (n)			
Right upper lobe	5	10	
Right middle lobe	0	1	
Right lower lobe	1	3	
Left upper lobe	5	10	
Left lower lobe	4	7	
Cells Analyzed	32,460	33,631	>0.999

VP01.09
Clinical Utility of Circulating Genetically Abnormal Cells in Differential Diagnosis of Benign from Malignant Pulmonary Nodules Sized between 5-10mm

M.D. Jiayuan Sun[1], M.D. maosong Ye[2], Mr. xiaoxuan Zheng[1], Mr. juncheng Zhang[3], Dr. Xin Ye[3], Dr. Chuoji Huang[3], M.D. Ruth L. Katz[4], M.D. Chunxue Bai[2]
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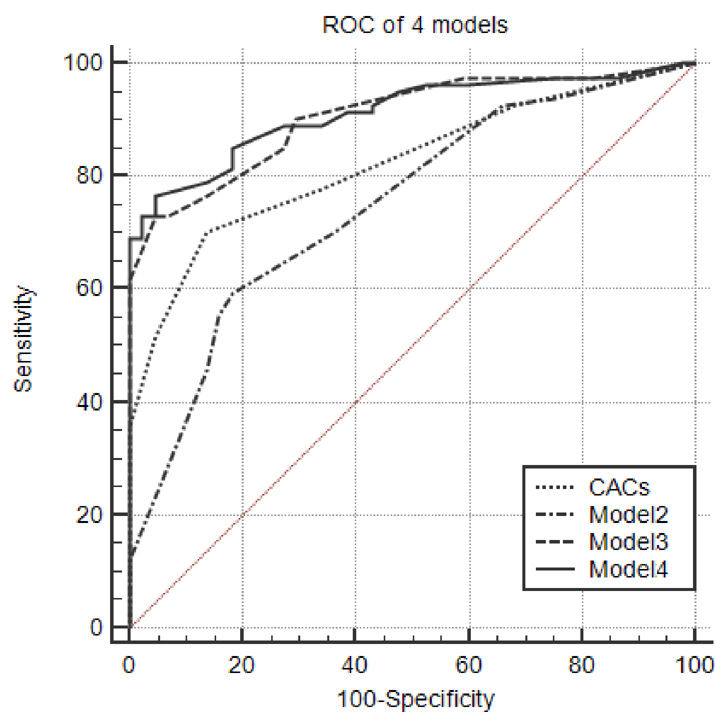
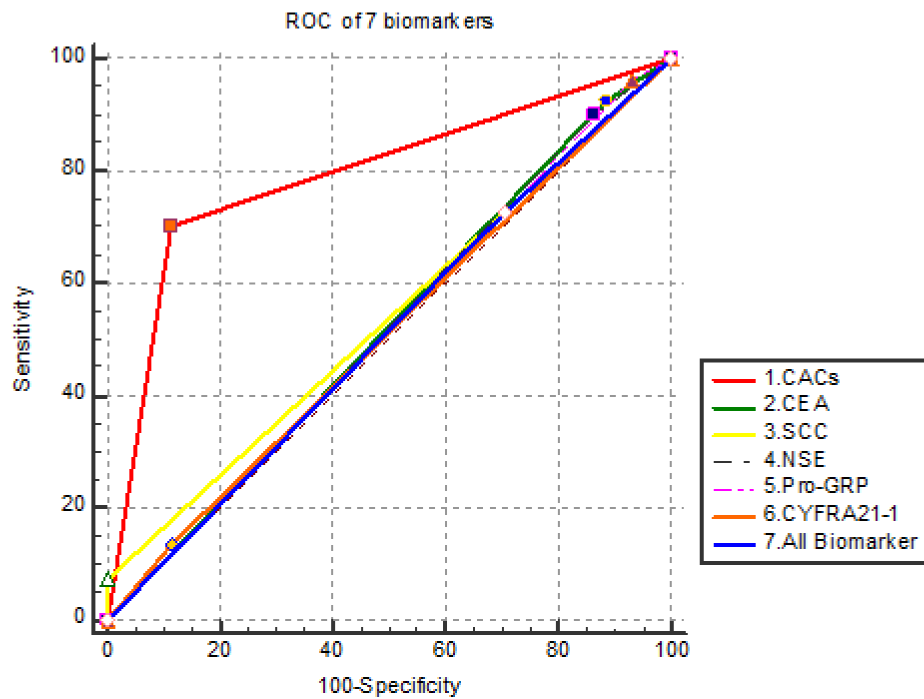
Purpose: While the low-dose computed tomography (LDCT) is wildly used in lung cancer screening, an increasing number of patients with between 5 to 10 mm diameter pulmonary nodules were detected. However, clinical guidelines still recommended the observational follow-up, which meant the existing diagnostic methods were not effective enough to tackle with relevant problems and would result in delayed diagnosis. Therefore, there is a need for complementary biomarkers along with LDCT to screen early lung cancer. We conducted a Multi-center Chinese Pulmonary Nodule Detection (MCPND) Trial (ChiCTR2000030437) in China. The diagnostic performance of circulating genetically abnormal cells (CACs) was evaluated in blood samples collected from patients with pulmonary nodules between 5-10 mm before lung lobectomy. **Patients and Methods:** 125 peripheral blood samples from participants, including 81 patients with lung cancer and 44 donors with benign lesions. All participants with pulmonary nodules were identified by CT sized between 5-10mm in diameter. The results also confirmed with histopathological diagnosis after surgical resection. Blood samples were processed and targeted chromosomal loci (3p22.1, 3q29, 10q22.3, and CEP10) were visualized by FISH–based assay. The diagnostic performance of CACs was evaluated in a blinded validation group. The levels of serum-based biomarkers (CEA, NSE, SCC, Pro-GRP, and CYFRA21-1) and clinical variables like patients' gender, nodule size, position and so on, were also measured. **Results:** The diagnostic performance of CACs for lung cancer detection was 70.4% for sensitivity and 86.4% for specificity using associated criterion suggested by Youden index. Area under the ROC curve (AUC) was 0.795 (P=0.0141; 95%CI (0.714, 0.862). However, the sensitivity of serum-based biomarkers is 27.2% when one or more biomarkers is counted as positive. The AUC of CACs can rise to 0.913 when combined with clinical variables. **Conclusion:** This validation study showed a high diagnostic value of CACs. It could assist the doctors and radiologists to differentiate benign from malignant small pulmonary nodules after LDCT in a noninvasive way.



ROC of 4 models
Sensitivity
100-Specificity
0
20
40
60
80
100
CACs
Model2
Model3
Model4



ROC of 7 biomarkers
Sensitivity
100-Specificity
0
20
40
60
80
100
1.CACs
2.CEA
3.SCC
4.NSE
5.Pro-GRP
6.CYFRA21-1
7.All Biomarker

CACs data and clinical variables	AUC	95% CI
model1.CACs quantitative data	0.824	0.746-0.886
model2.clinical variables	0.750	0.664-0.823
model3.CACs qualitative data +clinical variables	0.907	0.842-0.951
model4.CACs quantitative data +clinical variables	0.913	0.850-0.956

Table1. comparison of 4 models by AUC

POSTERS

VP01.10
Mutational Landscape and Tumor Mutation Burden of Indian NSCLC Patients

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Background: Understanding the mutational landscape in Indian patients with non-small cell lung cancer (NSCLC) by next generation sequencing is critical to appropriate treatment planning and delegation. Mutation analysis by NGS provides all the details on alterations and tumor mutational burden (TMB) that could be useful clinically and help identify patients with higher likelihood of response to immunotherapy. Cell-free DNA (cfDNA) has shown great potential in pre-clinical cancer detection and post diagnosis surveillance. Several studies have assessed the prognostic and predictive value of cfDNA in non-small cell lung cancer (NSCLC). High tumor mutational burden (TMB) is an emerging biomarker of sensitivity to immune checkpoint inhibitors and has been shown to be more significantly associated with response to PD-1 and PD-L1 blockade immunotherapy. **Method:** The study cohort comprised of 121 primary tumor samples and 385 cfDNA samples from patients with NSCLC. Targeted next generation sequencing was carried out using a customized panel of cancer-related genes. Appropriate optimized gene panels were used for mutation profiling of tumor DNA from tissue samples and plasma respectively. **Results:** Mutations were detected in 105 of 121 tissue samples and 233 of 385 cfDNA samples. EGFR was the most commonly mutated gene in both tissue (47%) and cfDNA (33%) followed by TP53 (tissue: 34%; cfDNA: 27%), KRAS (tissue: 9%; cfDNA: 7%), BRAF (tissue: 8%; cfDNA: 2%), PIK3CA (tissue: 8%; cfDNA: 4%), MET (tissue: 8%; cfDNA: 2%) and RB1 (tissue: 4%; cfDNA: 3%). TMB calculation for 51 tumor tissue samples showed median TMB of 8.27 mutation/Mb. TMB of >10 Mutations/Mb was observed in 19 patients (37%) and the subset of NSCLC patients with high TMB (>10 Mutations/Mb) likely have enriched tumor specific neoantigens and increased tumor immunogenicity, which can improve the response to cancer immunotherapy. **Conclusion:** This is the first study to describe the landscape of tissue based TMB in Indian patients with NSCLC. High TMB was observed in 37% of Indian NSCLC patients. TMB could be used as a biomarker for immunotherapy and would facilitate treatment in this patient cohort with a more favorable prognosis on treatment with immunotherapy.

VP01.11
Detection of Resistant T790M Mutation of EGFR from Pleural Effusion and Other Body Fluids in Lung Adenocarcinoma Patients

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Background: The concept of 'liquid biopsy' as a surrogate for tumor tissue is not restricted to blood-derived samples but also includes other malignant body fluids such as CSF, pleural fluid, and ascites. Specific applications of liquid biopsies in patients with lung cancer metastases, who have developed progression/recurrence during EGFR TKI treatment is unexplored in India. This study aimed to detect EGFR gene mutations from different types of body fluids from patients with metastatic lung adenocarcinoma.
Methods: In the period of December 2017 to June 2018, randomly, 11 patients' ctDNA of malignant body fluids were tested simultaneously with their respective plasma ctDNA for EGFR mutation detection by Real time PCR. Further, the results, of these were compared with their original clone as a benchmark.
Results: In the group of sensitizing mutations (Exon 19deln and Exon 21 L858R), at progression plasma ctDNA was positive in 63%, whereas 100% positive in other body fluids (CSF, pleural effusion, ascites, pericardial effusion), there by showing 63 vs 100 (%) concordance respectively with their original clone of the FFPE samples. In the group of acquired resistance mutation of exon 20 T790M, 3/11 (27.27%) were detected in plasma ctDNA whereas 9/11 (81.8%) were detected in other body fluids. Thus, the results suggest that malignant body fluid supernatant free DNA has a higher detection rate in picking up resistance exon 20 T790M mutation as compared to plasma free cfDNA samples. **Conclusion:** The encouraging results suggests that ctDNA of other than plasma ctDNA can be used to detect EGFR mutations and that their mutation status may be useful as a predictor of the response to TKI.

VP01.12
Molecular Characterization of Lung Cancer Brain and Leptomeningeal Metastases via Clinical Next Generation Sequencing of Cell-Free DNA From Cerebrospinal Fluid

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Background: Lung Cancer is among the most common solid tumors to involve the brain and leptomeninges. Its diagnosis is often challenging due to the risks associated with biopsy procedures and the low sensitivity of conventional non-surgical diagnostic modalities. Cerebrospinal fluid (CSF) liquid biopsies have emerged as an accessible and rich alternate source of tumor DNA with potential to completely alter the evaluation and management of metastatic central nervous system (CNS) malignancies. Here we present the results of CSF liquid biopsies from patients with NSLC submitted for routine molecular testing using MSK IMPACTTM (Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets, targeting 468 cancer-related genes) in 40 clinical samples. **Methods:** CSF samples submitted to the pathology department as part of routine assessment for suspected metastatic lung cancer were identified. cfDNA from CSF samples were extracted and sequenced using MSK-IMPACTTM. Results of paired cfDNA from supernatant and gDNA from cell pellet sequencing were compared and further correlated to previously characterized tumor samples, when available. **Results:** In all, 40 CSF patient samples were studied (37 adenocarcinoma, 1 squamous cell carcinoma, 2 neuroendocrine tumors). Median nucleic acid yields for cfDNA and gDNA were 1.25 ng (range 0.0 ng-253.5 ng) and 65.4 ng (range 0.0 ng-1719.5 ng), respectively. Sequencing success rate was 80% (32/40 cases). Somatic alterations (mutations, fusions and copy number alterations) were observed in cfDNA in 84.4% (27/32) of successful sequenced cases. 92.6% (25/27) cases demonstrated a driver alteration among cfDNA variants. Variants consistent with a therapy-related resistance mechanism were demonstrated in 6/27 samples (22.2%). Among cases with cytologic evaluation of CSF, 95.5% (21/22 samples with abnormal cytology were positive for tumor variants, as were 25% (4/16) of cytologically normal samples. In all 36/40 cfDNA and matched cell pellet gDNA specimens were available for testing; mutations were more commonly detected in cfDNA compared to gDNA (63.8% [23/36] vs 41.7% [15/36]). cfDNA also harbored 1.7X more variants than corresponding positive gDNA (6.36 vs 3.81, $p=0.01$) with higher median variant allele fraction. Tumor variants detected in CSF cfDNA were used to confirm, as well as independently establish a diagnosis and guide treatment decisions. **Conclusion:** Molecular profiling of CNS metastases of NSCLC based on CSF liquid biopsies is feasible and highly successful. cfDNA is markedly superior to gDNA from cell pellets, facilitating the capture of genetic alterations at high VAF even in the context of a negative cytology.

VP01.13
Shifting Paradigms in the Detection of Primary EGFR Mutations

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Background

Epidermal growth factor receptor (EGFR) TKI (tyrosine kinase inhibitors) are the cornerstone of treatment in cases of advanced non small cell carcinoma (NSCLC). The gold standard for detection of the same involves genotyping of tumor tissues.Owing to difficulties in accessibility of tumor due to its location, size, and relation to important structures, the adequacy of tissue is a major concern. Economic constraints pose a further challenge. The emergence of liquid biopsy, has shown promise in overcoming these obstacles, and in earlier detection of sensitizing EGFR mutations helping in rendering prompt therapy. Whether the same can replace tissue genotyping has been a question posed in many studies in literature, although strict recommendations are still lacking. This study is an early experience of primary EGFR mutation detection by liquid biopsy and tissue genotyping from a resource constrained country which aims to review the concordance, the turnaround times of both techniques and response outcomes. **Methods:** Thirty six patients of newly diagnosed NSCLC underwent liquid biopsy for EGFR mutation detection, of which 26 patients also underwent tissue genotyping. Liquid biopsy was performed using EGFR Mutation Test v2 (cobasv2;Roche Molecular Systems, CA,USA) and tissue genotyping was performed using Therascreen EGFR Mutation detection kit as per manufacturer recommendations. Clinical data was retrieved from medical record archives and appropriate statistical analysis was performed. **Results:** Thirty six newly diagnosed biopsy proven NSCLC, adenocarcinoma patients underwent liquid biopsy for EGFR mutation detection. The median age was 56 years (range: 35-77 years) with a male preponderance. Mutations in EGFR gene were detected in 19 (52.7%) cases, the most common being an in frame deletion in exon 19 (12 cases, 33.33%), followed by L858R mutation in 4 cases, L861Q in two cases and exon 20 insertion in 1 case. 17 cases did not show any EGFR mutations. The median semi quantitative index of the mutation detected was 12.35 on cobas v2. Of these 26 patients underwent tissue genotyping and there was 100% concordance with the mutation subtypes detected. The median turn around time for liquid biopsy was 49 hours, as compared to 216 hours for the tissue genotyping. **Conclusion:** From our early experience, it is clear that liquid biopsy can be used as “first tool” to detect EGFR mutations, with good sensitivity, specificity as well as semi quantitative results. It can also detect rare and uncommon mutations , and low level mutations with a limit of detection of 10 mutant copies per 990 wild type copies. The easy accessibility of peripheral blood, faster turnaround time as evidenced by our experience, and lower cost of the technique makes it a promising surrogate for detection of primary EGFR mutation in resource constrained (economic and tissue constraints) settings.

VP01.14
Utility of Liquid Biopsy in Diagnosis and Treatment Response in EGFR Mutant NSCLC Patients with Leptomeningeal Involvement

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Introduction: Leptomeningeal Metastasis (LM) occurs in roughly 3-4% of patients with Non-Small Cell Lung Cancer (NSCLC) and increases to up to 9% in patients with EGFR mutations. The diagnosis of LM remains challenging and are based on neurological, radiological and cerebrospinal fluid (CSF) analysis. Clinical symptoms may be non-specific, and gadolinium enhanced magnetic resonance imaging of the brain and spine is difficult to interpret. Cytological assessment of the CSF has limited sensitivity and often requires multiple sample collection attempts. Although cytology CSF evaluation remains the golden standard for diagnosing leptomeningeal disease, the assessment of treatment success remains challenging since these methods are more qualitative than quantitative in nature. This calls for a more quantitative and robust assay to diagnose and measure treatment response in patients with LM. Liquid biopsies are non-invasive, cost-effective, clinically widely used and allows for the analysis of Circulating Tumor Cells (CTCs) and Cell Free DNA (cfDNA). Here we present the results of a prospective study, evaluating CTCs and cfDNA obtained from the CSF in patients with EGFR mutated NSCLC with leptomeningeal disease, undergoing treatment with an experimental EGFR Tyrosine Kinase Inhibitor (TKI). METHODS: CSF was collected at baseline and at different time intervals throughout treatment from patients enrolled Clinical Trial KD-019-206, a Phase II Multicenter Study of Tesevatinib in subjects with NSCLC EGFR Activating Mutations, Prior Treatment with a TKI and Brain- or Leptomeningeal Metastases (NCT02616393). CSF was analyzed by cytology and CTCs were identified by Biocept's Target Selector™ platform. cfDNA was quantitatively analyzed for absolute counts of EGFR mutations (Del19, L858R, T790M) using the Target Selector™ single gene qPCR assays based on Biocept's proprietary Switch-Blocker enrichment technology. RESULTS: We analyzed 15 evaluable patients with 28 unique sample collections. Standard cytology was compared with Target Selector™ CTC detection. Target Selector™ detected CTCs in 21 samples (75%) and cytology was positive in 15 (54%) samples. For 8 patients with matched treatment response, cytology and Target Selector™ data, correlations were done with CTC density and EGFR mutation copy number. In 4 (67%) patients with a Stable Disease (SD) throughout treatment, mutant EGFR copy number demonstrated a downward trend. One patient demonstrated a Partial Response (PR), which was paralleled with a striking decrease in EGFR Deletion Exon 19 Mutation copy number, as well as a decline in CTCs. In two patients with a LM-Progressive Disease (PD), EGFR-Del19 mutation copy number showed an increase. Alterations in CTCs density and EGFR copy number mutations were observed before a treatment response was detected by traditional clinical measures, such

as RECIST evaluations. **Conclusion:** These data suggest that liquid biopsy analyses of the CSF in patients with LM disease, may provide a sensitive method for the diagnosis of LM, the detection of actionable biomarkers such as EGFR and the potential to assess a treatment response. Alterations in CSF CTC density and EGFR copy number have the potential to indicate an antineoplastic response prior to traditional clinical measures. Larger clinical trials are needed to fully define the clinical impact of our observations.

VP01.15
Analysis of Plasma-Derived Extracellular Vesicle (EV) Cargo Using the nCounter Nanostring Platform

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Introduction: Although genetic and transcriptomic analysis of tumor tissue can provide prognostic information and may guide treatment decision making, some tumor types, such as lung cancer, are difficult or even impossible to biopsy. In addition, repeated sampling to monitor therapy response is unattainable. Liquid biopsies, including plasma-derived extracellular vesicles (EVs), have shown potential to be used as minimally invasive, safe and sensitive alternative for tissue biopsies. The molecules found within EVs, such as mRNA, can be used for intercellular communication and may provide a rich source for biomarker discovery. In addition, the lipid bilayer of EVs protects its cargo, and allows the use of biobank stored samples. However, lack of standardized methods and clinical validation prevents the implementation of EV testing in daily clinical practice. The nCounter NanoString platform has been extensively used for genetic and transcriptomic tumor characterization, but studies that perform liquid biopsy-based nCounter analysis are still limited. The use of both a standardized platform, such as nCounter, and analytically and clinically validated protocols for EV-mRNA may provide the basis for acceptance of EV-based assays in daily cancer care. We performed a proof-of-concept study, testing and optimizing EV-mRNA extraction and consequent analysis on the nCounter platform. **Methods:** EVs were isolated from 600 uL plasma of 19 cancer patients and 10 healthy controls using the miRCURY Exosome Serum/Plasma Kit. We compared different EV-mRNA extraction methods, including automated RNA extraction- and Trizol-based methods. Hereafter, the protocol for pre-amplification of EV-mRNA was optimized. All samples were analyzed, using the optimized workflow, with the Human Immunology V2 mRNA Panel, including 600 mRNA targets, and differential expression (DE) analysis was performed. Since plasma samples are highly valuable, we finally tested the minimal required plasma input for EV-mRNA analysis on nCounter. **Results:** Testing of several RNA extraction methods revealed that, although Trizol-based extraction outperformed other methods, no extraction method yielded a quantity of RNA that was sufficient for direct hybridization on nCounter. Optimization of the pre-amplification protocol revealed that an amplification of 10 cycles was sufficient for downstream EV-mRNA analysis on the nCounter platform. In addition, supernatant collected during EV isolation was analyzed, and results showed that the RNA targets were derived from within the EVs. On average, 337 mRNA targets were detected within the EVs, with no significant differences between cancer patients and healthy donors. DE analysis was performed and indicated that this workflow can be used for biomarker assay development. Interestingly, most DE mRNAs were shown to be lower expressed in cancer patients. The minimum required input test revealed that 150 and 500 uL input plasma yielded similar amounts of detected gene targets, although the total amount of counts was two-fold higher when plasma input was 500 uL. **Conclusion:** This proof-of-concept study shows, for the first time, that the nCounter NanoString platform can be used for plasma-derived EV-mRNA analysis from cancer patients and healthy controls. Future studies will focus on the development and validation of biomarker assays based on plasma-derived EV-mRNA.

VP01.16

A Pilot of Diagnostic Circulating Tumour (ct) DNA Next Generation Sequencing (NGS) Assessment in Patients with Suspected Advanced Lung Cancer During the COVID-19 Pandemic

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Background: COVID-19 has reduced diagnostic procedure availability. Non-invasive ctDNA-NGS is highly concordant with tissue-NGS, with fast turnaround times. **Methods:** A pilot study assessing the diagnostic potential of Guardant360™ ctDNA-NGS was performed in patients with radiological-suspected advanced-stage lung cancer, during COVID-19 at RMH. The first 12 evaluable patients were enrolled April-June 2020. Twenty patients are planned; ≥10% expected to have results allowing treatment without further tissue testing. **Results:** Demographics are shown in Table 1; 50% were never-smokers, 67% had stage IV disease. Table 2 outlines ctDNA-NGS results; 75% were informative, (reporting any genomic variant). Of 3 patients with uninformative results, 2 had stage III disease, 1 had stage IV NSCLC with neuroendocrine differentiation (false-negative: 25%). Five (42%) patients had actionable variants; 2 (17%) changed management. One patient with no druggable variants on ctDNA-NGS declined biopsy and pursued supportive care. Median time to ctDNA-NGS result was 8.5 days (range 5-13). Eleven patients underwent biopsy; 7/11 required ≥1 biopsy. 8/9 patients with informative ctDNA-NGS underwent subsequent biopsies: 4 confirmed malignancy, 2 were insufficient for diagnosis, 2 were benign; 2 patients had discordant results (informative ctDNA-NGS, benign histology). One patient had EML4-ALK fusion on tissue but not ctDNA-NGS, another had EML4-ALK fusion detected on ctDNA-NGS while awaiting biopsy. In 3 patients with tissue molecular results, median time between ctDNA and tissue result was 25 days (range -2-39). **Conclusions:** In this small sample, ctDNA-NGS could not be used to substitute tissue biopsy. Guardant360™ ctDNA-NGS increased the rapid detection of actionable variants compared to tissue testing, and led to timely treatment decisions when biopsy access was limited; thus, should be considered in parallel with, not after, tissue diagnosis in suspected metastatic lung cancer. Implementation of this strategy could result in better care for a substantial number of patients.

Table 1. Patient characteristics

	Evaluable patients * N = 12 N (%)	**Informative ctDNA NGS N = 9 N (%)**	**Uninformative ctDNA NGS N = 3 N (%)**
Age (years)			
Median (range)	72 (51 – 87)	73 (56 – 87)	51 (51 – 72)
Sex			
M	7 (58%)	4 (44%)	3 (100%)
F	5 (42%)	5 (56%)	0

Ethnicity			
Caucasian	7 (58%)	6 (67%)	1 (33%)
Asian	4 (33%)	3 (33%)	1 (33%)
Middle Eastern	1 (8%)	0	1 (33%)
Smoking			
Never (0-99 cigarettes per lifetime)	6 (50%)	4 (44%)	2 (67%)
Ex-smoker	4 (33%)	3 (33%)	1 (33%)
Current smoker	1 (8%)	1 (11%)	0
NA	1 (8%)	1 (11%)	0
ECOG			
0	0	0	0
1	9 (75%)	7 (78%)	2 (67%)
2	1 (8%)	0	1 (33%)
3	2 (17%)	2 (22%)	0
4	0	0	0
Previous diagnosis of lung cancer			
No	12 (100%)	9 (100%)	3 (100%)
Yes	0	0	0
Stage based on imaging			
1 – 2	1 (8%)	1 (11%)	0
3	3 (25%)	1 (11%)	2 (67%)
4	8 (67%)	7 (78%)	1 (33%)
Sites of metastases			
Nil	4 (33%)	2 (22%)	2 (67%)
Brain	3 (25%)	3 (33%)	0
Pleura	3 (25%)	2 (22%)	1 (33%) **
Bone	3 (25%)	2 (22%)	1 (33%) **
Liver	2 (17%)	2 (22%)	0
Adrenal	1 (8%)	0	1 (33%)**
Tissue sampling for advanced NSCLC			
No	1 (17%)	1 (11%)	0
Yes	11 (83%)	8 (89%)	3 (100%)
Repeat biopsy required			
N	4 (33%)	2 (22%)	2 (67%)
Y	7 (58%)	6 (67%)	1 (33%)
NA	1 (8%)	1 (11%)	0
Histology / Cytology result			
Benign • Anthracosis and fibrosis (excisional	2 (17%)	2 (22%)	0

lymph node biopsy) • Anthracosis and histiocytosis (excisional lymph node biopsy)			
Insufficient tissue (all awaiting repeat biopsies)	3 (25%)	2 (22%)	1 (33%)
Malignant	6 (50%) • Adenocarcinoma (4) • NSCLC with neuroendocrine differentiation (1) • Poorly differentiated carcinoma (1)	4 (44%) • Adenocarcinoma (3) • Poorly differentiated carcinoma (1)	2 (67%) • Adenocarcinoma (1) • NSCLC with neuroendocrine differentiation (1)
NA	1 (17%)	1 (11%)	0

* Two of the first 14 patients enrolled were not evaluable as they were already previously biopsy-confirmed to have lung cancer, therefore are not included in the analysis.
** One patient had metastasis to pleura, bone and adrenal gland. The other two patients did not have metastases.

Table 2. ctDNA-NGS results.

	All patients N=12 N (%)	**Malignant on biopsy N = 6 N (%)**	**Benign on biopsy N = 2 N (%)**	**Indeterminate on biopsy N = 3 N (%)**	**No biopsy N = 1 N (%)**
Informative (detected ≥1 genomic variants)					
No	3 (25%)	2 (33%)	0	1 (33%)	0
Yes	9 (75%)	4 (67%) • MET exon 14 skipping • ARID1A Q1212L • EML4-ALK fusion, TP53 R181C • KRAS G12C, STK11 P281fs,	2 (100%) • DDR2 R752C, ALK I1383T • EGFR splice site SNV, FGFR2 H210Q	2 (67%) • JAK2 V617F • TP53 D249S, FGFR1 amplification	1 (100%) • KRAS G12C, TP53 G244C, TP53 T118fs

		TP53 R273L			
Actionable variants					
No	7 (58%)	3 (50%)	2 (100%)	2 (67%)	0
Yes	5 (42%)	3 (50%) • MET exon 14 skipping • EML4-ALK fusion • KRAS G12C	0	1 (33%) • JAK2 V617F	1 (100%) • KRAS G12C
ctDNA-NGS results changed systemic therapy					
No	10 (83%)				
Yes	2 (17%) • Tepotinib for MET exon 14 skipping • Alectinib for EML4-ALK fusion				
Variants detected on ctDNA NGS, not detected on tissue					
No	1 (8%)				
Yes	2 (17%)* • MET exon 14 skipping • EML4-ALK fusion*				
NA *	9 (75%)				
Variants detected on tissue, not detected on ctDNA NGS					
No	2 (17%)				
Yes	1 (8%) • EML4-ALK fusion				
NA	9 (75%)				

* Tissue molecular test results for patient with EML4-ALK pending at time of analysis

VP01.17
Clinical Utility of ctDNA Next Generation Sequencing (NGS) for Target Identification in Diagnostic and Acquired Resistance Settings in Metastatic NSCLC – A Single Centre Experience

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Background: Advances in targeted therapeutics have resulted in increasing numbers of molecular biomarkers needing testing in metastatic NSCLC, resulting in greater requirements for broader genotyping. Relative benefits of ctDNA-NGS for target identification over current standard-of-care tissue and single-gene ctDNA genotyping outside clinical trials are not fully understood. This abstract has also been submitted to the ELCC 2020 meeting. **Methods:** A retrospective case-notes review of patients with metastatic NSCLC undergoing prospective molecular tumour genotyping by comprehensive ctDNA-NGS (Guardant360 73 gene panel) as part of routine clinical care at a single cancer centre. ctDNA-NGS testing was performed for up-front target identification or at acquired TKI resistance. Variants were tiered according to AMP/ASCO/CAP consensus recommendations. Primary objective: proportion of informative ctDNA NGS tests, defined as reporting a genomic variant. **Results:** 54 ctDNA-NGS tests from 47 patients were included. ctDNA-NGS was informative in 30/34 cases (88.2%) in the up-front setting and 18/20 cases (90%) in the acquired resistance setting. In the up-front setting, number of Tier I variants identified when ctDNA-NGS was performed in addition to tissue testing increased by 69%, from 13 to 22 patients. In the acquired TKI resistance setting, ctDNA-NGS identified a Tier I resistance variant in 35% (7/20) of cases, compared with only 15% (3/20) identified by standard-of-care molecular testing alone. 78% of non-informative or false-negative ctDNA-NGS tests occurred in patients with intrapulmonary-only or CNS-only disease. For paired ctDNA-NGS and tissue samples, time from sampling to report was significantly shorter for ctDNA-NGS than for tissue genotyping (9 vs. 18 days, p=0.0015). **Conclusion:** We demonstrate the utility of ctDNA-NGS as complementary to standard-of-care molecular genotyping in metastatic NSCLC, by increasing the proportion of patients identified with actionable genomic variants in a rapid and minimally invasive manner. Appropriate patient selection is key to ensure maximal clinical benefit and optimal use of resources.

Table 1. Baseline characteristics for all patients included in the analysis (n=47)			
Median age at diagnosis (range)		61 (31-79)	
		n	%
Gender			
Male		16	34.0
Female		31	66.0
Smoking			
Never smoker		25	53.2
Ex/current smoker		22	46.8
Histology			
Adenocarcinoma		44	93.6
Pleomorphic carcinoma		2	4.3
Squamous		1	2.1
M Stage descriptor at time of ctDNA NGS testing			
M1A		13	27.7
M1B		5	10.6
M1C		29	61.7
Intracranial-only metastases			
Yes		4	8.5
No		43	91.5
Intrapulmonary-only metastases			
Yes		12	25.5
No		35	74.5
Known tissue variants at time of ctDNA NGS			
No		28	59.6
Yes		19	40.4
	EGFR Del19	11	23.4
	EGFR L858R	1	2.1
	EML4-ALK fusion	1	2.1
	ROS1 fusion	1	2.1
	TP53 variant	1	2.1
	EGFR Del19 and T790M	1	2.1
	EGFR Del19, T790M and C797S	1	2.1
	EGFR Del19 and EGFR Ins20	1	2.1
	EGFR Ins20	1	2.1

VP01.18
Characterization of Peripheral T-Cell Receptor β Chain Repertoite in Advanced Non-Small Cell Lung Cancer Patients Treated with Pembrolizumab

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Background: Anti-PD1 therapy with pembrolizumab has been demonstrated to improve the clinical outcome in patients with high programmed death ligand 1 (PD-L1) expression in advanced non-small cell lung cancer (NSCLC). However, not all patients benefit from this treatment and PD-L1 testing alone is not yet an adequate biomarker for patient selection. Consequently, the identification of predictive biomarkers and more accurate understanding of the immune system are necessary. The aim of this project is to characterize peripheral T-cell receptor β chain (TCR-β) repertoire in advanced NSCLC patients treated with first-line pembrolizumab. **Methods:** Peripheral blood samples were collected before treatment and at first response assessment from 17 advanced NSCLC patients who received pembrolizumab as first-line treatment. TCR-β libraries were prepared with Oncomine TCR-β SR (RNA) assay and sequenced on Ion GeneStudio S5 Series (Thermofisher Scientific). Clone richness, evenness, Shannon diversity, convergence and TCR-β variable region (TRBV) genes usage were tracked and evaluated in pre and postreatment samples for association with clinico-pathological characteristics, using non-parametric tests and median as cut-off. Prognostic value was determined by Cox regression analysis and Kaplan-Meier curves with log-rank test, $p<0.05$. **Results:** Most of the patients in this cohort were smokers (64.7%) and male (82.4%). PD-L1 expression≥50% was found in all patients. According to RECIST 1.1, 64.7% of patients presented clinical benefit (complete or partial response, and stable disease), while 35.3% progressed in the first imaging evaluation. Analysis of TCR-β repertoire in baseline samples revealed that smokers showed significantly lower clone richness (p=0.005) when compared to non-smokers. Additionally, patients with higher evenness before treatment showed improved progression-free survival (PFS) compared to patients with lower evenness (p=0.033). Data of TRBV genes usage revealed that higher expression of TRBV4-1 was significantly correlated with brain metastasis (p=0.011) and bone metastasis (p=0.032). Furthermore, higher expression of TRBV4-3 was associated with clinical benefit at first response assessment (p=0.040) and longer PFS (p=0.018) than those with lower expression. **Conclusion:** Our preliminary results provide a more accurate understanding about the TCR-β repertoire among advanced NSCLC patients. The analysis of the variable region of TCR-β suggests that TRBV expression pattern might be applied as a prognostic/predictive biomarker in patients treated with pembrolizumab. Nonetheless, further studies in an extended cohort will be needed to clarify the prognostic and/or predictive utility of the TCR-β repertoire in NSCLC patients.

Funded by CB16-12-00350 from CIBEROnc, PI18/00226 from ISCIII, Arnal Planelles Foundation and AMACMA

VP01.19
Predictive and Prognostic Role of Soluble Biomarkers in Advanced Non-Small Cell Lung Cancer (NSCLC) Patients Treated with anti-PD1/PDL1 Immunotherapy

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Introduction: Immunotherapy with anti-PD1/PDL1 antibodies has become a standard treatment for advanced NSCLC, with higher efficacy in patients with PDL1 positive tumors. However, the difficulty to achieve tumor samples for PDL1 testing requires the search of new biomarkers. This study aims to assess the utility of circulating biomarkers for predicting response to anti-PD1/PDL1 therapies in NSCLC. **Methods:** Blood samples from 50 NSCLC patients (pts) were collected before second-line treatment with anti-PD1/PDL1 antibodies. Plasma biomarkers' levels were measured by Multiplex bead-based assays (ProcartaPlex, Thermo Fisher). Non-parametric tests were used to correlate clinico-pathological characteristics with analytical variables dichotomized using median as cut-off. To evaluate survival, Cox Regression analysis and Kaplan Meier curves with log-rank test were performed. **Results:** Soluble sCD137, sGITR and sIDO correlated significantly with response (p=0,016, p= 0,018, p=0,030, respectively), while sPDL1 showed a trend. Three groups of pts were established according to high or low levels of these 4 markers as following: G1, pts with low levels of 3 or 4 markers; G2, pts with low levels of 2 and high levels of 2 markers; G3, pts with high levels of 3 or 4 markers. G1 pts showed significant lower response rate (p= 0.011), and G3 patients had better OS (NR vs. 8.6 vs. 6.33 months, p= 0.010). Pts with higher sPDL1 had a significant increase in PFS (NR vs. 3 months, p=0.017), while a trend toward an increase in OS (NR vs. 8.27 months, p= 0.081) compared to those with lower levels. Multivariate analysis for OS revealed that combination of 4 soluble markers has an independent prognostic value in this cohort (HR=0.595, p= 0.04). **Conclusion:** Circulating biomarkers can be reliable detected in plasma of advanced NSCLC pts. sCD137, sIDO, sGITR and sPDL1 seem to be related to anti-PD1/PDL1 response or PFS, and combination of these soluble markers may be an independent prognostic factor for OS.

Supported by grant PI18/00226 from ISCIII, CB16/12/00350 from CIBEROnc, F. Arnal Planelles and AMACMA

VP01.20
The Prognostic Impact of Blood Tumor Mutational Burden (bTMB) in the First-Line Treatment of Advanced Non-Small Cell Lung Cancer (NSCLC): A Systematic Review and Meta-Analysis of Randomized Trials

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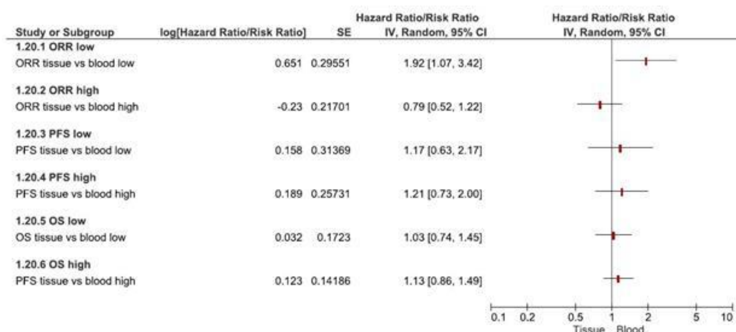
Background: PD-L1 expression and tissue tumor mutational burden (tTMB) have been deeply investigated as immune checkpoint inhibitors (ICIs) positive predictive biomarkers in the frontline setting of advanced Non-Small Cell Lung Cancer (NSCLC). In the era of precision oncology, careful attention should be paid on the emerging role of TMB estimated by circulating tumor DNA in blood (bTMB), a minimally invasive approach that could similarly predict survival in patients receiving ICIs showing good correlation with tTMB. **Methods:** The purpose of this meta-analysis, including eleven different cohorts of seven randomized controlled trials (KEYNOTE-042, CheckMate-227, KEYNOTE-189, KEYNOTE-407, KEYNOTE-021 cohort G, CheckMate-026, MYSTIC), was to compare tissue and blood major survival outcomes among first-line Immuno-Oncology (IO) treatment strategies (single-agent ICI, ICIs plus chemotherapy and combination ICIs) within two subgroups (TMB-low and -high) versus standard platinum-based chemotherapy (CT). We estimated and indirectly compared pooled hazard ratios (HRs) and relative risks (RRs). **Results:** Indirect comparisons for efficacy outcomes showed that ICIs were associated with a statistically significant advantage over CT alone in terms of objective response rate (ORR; RR 1.89, 95% CI 1.26-2.84), progression-free survival (PFS; HR 0.56, 95% CI 0.48-0.65) and overall survival (OS; HR 0.76, 95% CI 0.65-0.89), strongly suggesting the use of IO regimens strategy within the TMB-high subgroup. Namely, indirect comparisons according to tTMB and bTMB showed no significant differences in major clinical outcomes (Image 1). **Conclusions:** Despite some limitations and awaiting the final results of ongoing prospective studies of bTMB as a predictive biomarker for ICIs, our results proved similar efficacy outcomes between bTMB and tTMB. In this scenario, diagnostic accuracy studies should be encouraged for the clinical implementation of bTMB in the first-line management of metastatic NSCLC patients.



VP01.21
Molecular Features of Gene Alteration Profiles in Circulating Cell-Free DNA of Advanced Non-Small Cell Lung Cancer Patients

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Introduction: Circulating cell-free DNA (cfDNA) includes tumor-derived DNA (tDNA) in cancer patients. Therefore, cfDNA has been used clinically in gene mutation testing as a liquid biopsy method, which has the advantage of being less invasive than tumor biopsy. The aim of this study was to detect the features of the gene alterations detected in cfDNA derived from tumors. **Patients and Methods:** Six patients with primary lung cancer who died due to cancer progression and underwent autopsy were enrolled in this study from 2011 April to 2017 September. cfDNA was isolated from blood samples collected within 3 months before death. tDNA was extracted from visible lesions at autopsy. Gene alteration profiles were obtained using next generation sequencing both in cfDNA and tumor lesions including metastases. Tumor genotyping assays via NGS were performed using the GeneRead™ DNAseq Targeted Panels V2 Human Comprehensive Cancer Panel (Qiagen), which cover 160 major oncogenes and tumor suppressor genes. **Results:** From the six patients, DNAs were collected from 19 total tumor samples (6 primary tumors and 13 metastatic lesions) and 6 cfDNA samples, and was used for NGS analyses. The mean number of gene alterations detected per patient was 253 (range 99–1918) in the tDNA samples and 218 (range 56-362) in the cfDNA samples, respectively. The features of the tDNA gene alterations detected in cfDNA, compared with those not detected in cfDNA, included a higher rate of being present in multiple tumors (67% truncal mutations, 36% shared mutations and 4% individual mutations) and a higher variant allele frequency (47.6% (range, 2.1–100.0) vs. 4.1% (range, 1.1–100.0) for tDNA alterations detected in cfDNA vs. not detected in cfDNA, respectively). **Conclusion:** These results suggest that essential gene alterations enriched in cfDNA help characterize cancer cells, and that genetic testing using cfDNA has an advantage over that using tissue biopsy, which only samples a small portion of the tumor.

VP01.22
The VALUE of Routine Liquid Biopsy for NSCLC Patients in a Public Healthcare System

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Background: Next-generation sequencing (NGS) of cell-free DNA (cfDNA) obtained from blood samples may improve diagnostic testing in patients with advanced NSCLC, with faster turnaround time (TAT) and potential cost savings compared to standard tissue profiling. We examined the clinical utility and treatment outcomes with liquid biopsy in patients with advanced NSCLC from the perspective of the Canadian public healthcare system. **Methods:** Patients with advanced non-squamous NSCLC at 6 cancer centres across Canada (BC, Alberta, Ontario, Quebec) were recruited (NCT03576937). Cohort 1 (N=150) included treatment naïve patients with measurable disease and ≤10 pack year smoking history. Cohort 2 (N=60) included patients with known oncogenic drivers that progressed on tyrosine kinase inhibitors (TKIs). Patients progressing on EGFR kinase inhibitors were required to have documented absence of EGFR T790M or to have failed prior osimertinib. Consenting patients had a peripheral blood draw and cfDNA NGS analysis using Guardant360™ (G360, Guardant Health), a validated assay that detects alterations in 74 cancer-associated genes, prior to starting initial treatment (Cohort 1) or next line of therapy (Cohort 2). Standard of care tissue profiling was performed per institutional standards. Pre-specified study endpoints include the number of actionable genomic alterations identified through G360 (EGFR, ALK, BRAF, ERBB2, KRAS G12C, MET amplification/exon 14 skipping, RET, ROS1, NTRK1), result TAT, time-to-treatment initiation, objective response rate, progression-free survival and patient-reported quality of life (EQ-5D). **Results:** Between February 2019 and July 15, 2020, 148 patients (98 female, 51 male) were accrued to Cohort 1 and 60 (40 female, 20 male) to Cohort 2 with G360 results. After excluding variants of unknown significance and synonymous alterations, 117 patients in Cohort 1 (79%) had ≥1 alteration detected by G360 (301 alterations detected in 35 genes). Of these, 281 alterations were considered actionable with FDA-approved drugs and/or available clinical trials. Actionable targets included EGFR (31.7%), ERBB2 (3.2%), MET (3.2%), ALK (1.4%), KRAS G12C (1.1%), and ROS1 (0.4%), total 41%. Additional clinically relevant alterations included TP53 (27.8%), KRAS non-G12C (3.9%), PIK3CA (3.9%) and BRAF nonV600E (1.1%). In Cohort 2, 53 patients (85%) had ≥1 alteration detected, with a total of 165 alterations in 28 genes including EGFR (45.8%, 5 were C797S), ALK (fusions 3.3%; mutations 2.0%), BRAF (V600E 0.7%; other 3.3%), MET (amplification 2.0%; exon 14 0.7%), FGFR3 (0.7%) and RET (0.7%) as well as non-driver mutations. Twenty-six patients (13.5% in Cohort 1; 10% in Cohort 2) had no alterations detected by G360. In samples with alterations detected, the median number of alterations per patient was 3 (range 1-17). The median time to reporting of G360 was 7 days (range 5-27). Time to treatment initiation, turnaround time and differences between actionable results in liquid versus tissue biopsy will be presented. **Conclusion:** Over 87% of advanced NSCLC patients had detectable cfDNA results, and 115 had actionable mutations identified by G360. Liquid biopsy is an important and timely method of molecular diagnosis in newly diagnosed patients with advanced NSCLC and in the setting of targeted therapy resistance.

VP01.23
Non-invasive Diagnostic Triaging of Suspected Lung Cancers based on Immunocytochemistry Profiling of Circulating Tumor Cells

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Background: Diagnosis of lung cancer in suspected individuals is based on histopathological examination (HPE) of tumor tissue obtained by an invasive biopsy. However, lung biopsies are associated with various procedural risks and may often not be possible owing to anatomical considerations as well as patient co-morbidities. It is also estimated that around 60% of all lung biopsies are benign, indicating a disproportionate number of individuals who undergo an invasive biopsy that is subsequently deemed unnecessary. We present a non-invasive approach for diagnostic triaging of symptomatic individuals with suspicious findings on radiological scans (such as lung nodules in Low Dose Computed Tomography, LDCT) of suspected of lung cancer. **Methods:** We collected peripheral blood from 1256 individuals (827 males and 429 females) including 682 previously diagnosed cases of lung cancer as well as from 574 individuals who were suspected of lung cancer, underwent blood collection prior to undergoing a biopsy, then underwent an invasive biopsy and were subsequently diagnosed with lung cancer based on HPE of tumor tissue. Peripheral blood mononuclear cells (PBMCs) were isolated from blood samples and treated with an epigenetically activating medium which induces cell death in normal (non-malignant) hematolymphoid cells as well as epithelial cells in peripheral blood, but selectively confers survival privilege on apoptosis resistant Circulating Tumor Cells (CTCs). In a subset of 305 samples, including 146 previously known cases and 159 suspected cases, CTCs were profiled by immunocytochemistry (ICC) using IVD approved antibodies to detect lung-specific markers (Napsin-A, TTF-1 and p40). ICC findings were compared with the HPE results to determine concordance. **Results:** Detection of CTCs was concordant with presence of malignancy in 642 (94.1%) of the 682 previously diagnosed cases (retrospective group) and was predictive of malignancy in 550 (95.8%) of the 574 suspected cases (prospective group) with an overall concordance of 94.9% among the 1256 cases. In a subset of 305 samples, ICC profiling with Napsin-A, TTF-1 and p40 was concordant with prior diagnosis in 126 (86.3%) of 146 previously known cases and was prospectively concordant with subsequent HPE analysis in 146 (91.8%) of the 159 suspected cases of lung cancer with an overall concordance of 89.2%. **Conclusion:** The study shows a high sensitivity in CTC detection by ICC profiling. This approach can be used for non-invasive triaging of individuals suspected of lung cancer for further evaluations. In cases where a diagnostic biopsy is unviable, a liquid biopsy based approach can non-invasively provide relevant diagnostic direction.

VP01.24
Circulating Free DNA Expression in Lung Cancer Surgical Patients

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Circulating free DNA expression in a curative surgical setting needs to be performed, to define biomarkers, to evaluate residual disease and stratify risk of relapse (prognostic value). **Aim:** Investigate the added value of circulating free tumor DNA (cfDNA) in early stage lung cancer patients submitted to surgery and evaluate its expression across time. Evaluate cfDNA concentration according to clinical and pathological features. **Methods:** Classify patients according to clinical and histological characteristics. Obtain tumor specimen, peripheral blood samples before surgery (PS), pulmonary vein (PV), at surgical discharge (SD) and first follow-up (F1). Ion Ampliseq Colon and Lung panelTM will be used for tissue biopsies and Oncomine Lung circulating free tumor DNA assay for circulating free tumor DNA (cfDNA) samples. All amplified products will be used to prepare libraries and sequenced using the ion PGMTM or S5TM system. The QuantStudio 3D Digital PCR systemTM will be used to confirm selected results. All statistical analyses were performed using the GraphPad Prism 8.1.2. Wilcoxon-matched pairs signed rank tests were used to infer the difference in quantity of cfDNA (ng/mL) present in the blood of lung cancer patients at different collection times points as mentioned above. Paired T-tests was performed. **Results:** The study randomized 47 patients but only 38 patients were studied. All tissue samples were analyzed for tumor mutations and allele frequency was quantified. Quantitative differences in cfDNA concentrations obtained from plasma were analyzed. Differences of expression were noted between PS, PV, at SD and F1. This evidences that more cfDNA is shedded directly form the tumor bed into the vein that drains blood directly from the tumor when compared to peripheral blood samples obtained before surgery (PV>PS; $p=0.004$), and greater increase was observed after surgery in samples obtained at discharge (SD>PV; $p=0,0003$). At F1 lower cfDNA concentrations were identified when compared to SD. At SD cfDNA concentration has a higher statistical significance when compared with PS, PV and F1 ($p<0.0001$, $p=0.0002$, $p<0.0001$ respectively). **Conclusion:** The presence of cfDNA post-surgery at SD is a negative factor favoring the presence of residual disease. A prognostic impact concerning disease outcome and a predictive impact on a surgical curative treatment.

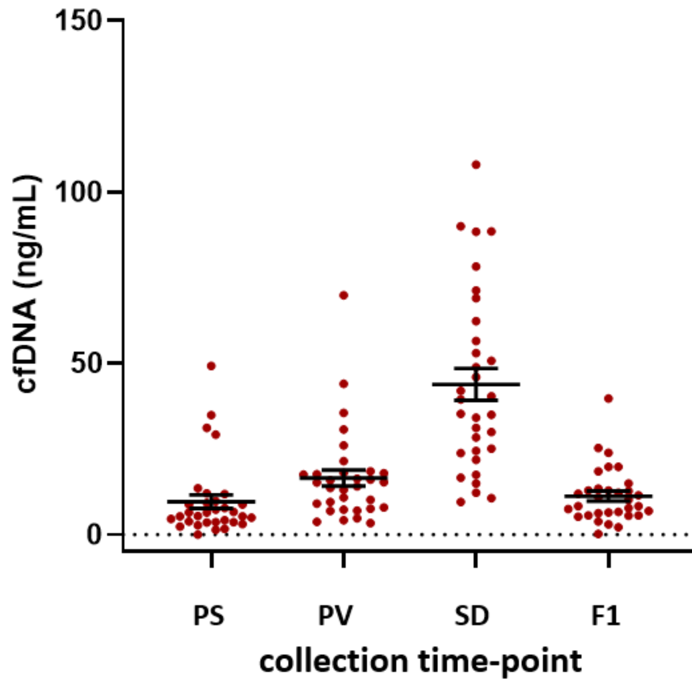

Table1: CfDNA concentration at different collection time points

	PS	PV	SD	F1
Number of values	32	32	32	32
Minimum	0,000	3,390	9,500	0,2000
25% Percentile	3,600	7,600	23,94	5,750
Median	5,920	14,65	37,31	9,250
75% Percentile	9,650	17,95	60,85	13,28
Maximum	49,20	69,80	108,0	39,70
Range	49,20	66,41	98,50	39,50
Mean	9,626	16,51	43,83	11,23
Std. Deviation	11,01	13,37	26,34	8,015
Std. Error of Mean	1,947	2,363	4,657	1,417

VP01.25
The Role of Liquid Biopsy in Non-Small Cell Lung Cancer Basal Setting Patients: Focus on KRAS Mutations.

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Background: Non-small cell lung cancer (NSCLC) represents the most leading cause of death for cancer worldwide. A vast majority of these patients (25-30%) harbored Kirsten Rat Sarcoma Viral Oncogene Homolog (KRAS) point mutations. Despite the classical negative prognostic role, KRAS point mutations, and in particular exon 2 p.G12C (accounting for about 13% of advanced stage NSCLC patients), have been recently acquired a predictive role due to the promising results of a novel tyrosine kinase inhibitor (AMG510, Amgen, Thousand Oaks, CA).[1, 2] Tissue samples represent the “gold standard” material for molecular analysis, However, in a not negligible percentage of patients, tissue is not available. In this setting liquid biopsy may represent an alternative valid source of nucleic acids to perform molecular assessment. Here, we reviewed the results obtained on plasma samples of naïve to any treatment advanced stage NSCLC patients, by adopting a next generation sequencing (NGS) custom panel (SiRe®). **Materials and Methods:** SiRe® panel allows the simultaneous analysis of 568 clinical relevant hotspot mutations in six genes (EGFR, KRAS, NRAS, BRAF, c-Kit, and PDGFRA).[3] On the overall, circulating tumor DNA (ctDNA) extracted from 194 plasma samples of advanced stage NSCLC patients, were analyzed. **Results:** On the overall, 36 (18.6%) KRAS mutated cases were detected, with an overall median allelic frequency of 5.0% (ranging between 0.2% and 46.8%). As expected among these KRAS mutated patients, the exon 2 p.G12C was the most common detected mutation (13/36, 36.1%). **Conclusions:** In conclusion, we confirmed the technical feasibility of SiRe® NGS panel to assess KRAS mutational status on ctDNA.

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VP01.26
The Role of Liquid Biopsy in Non-Small Cell Lung Cancer Basal Setting Patients: Focus on EGFR Mutations.

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Background: A not negligible percentage of non small cell lung cancer (NSCLC) patients do not always have sufficient tissue material for testing epidermal growth factor receptor (EGFR) mutations and other relevant predictive biomarkers.[1] In this setting, circulating tumor DNA (ctDNA) may represent a valid tool to overcome this limitation. In fact, ctDNA extracted from plasma may be used as a valid surrogate for EGFR mutational testing, in patients with tissue unavailability.[2] However, due to ctDNA low concentration into the bloodstream, the detection of gene mutations may be challenging. Thus, highly sensitive molecular approaches should be adopted in this setting. For this purpose, we have recently developed and validated a next generation sequencing (NGS) narrow panel, namely SiRe®.[3] **Materials and Methods:** In sixty four advanced stage NSCLC patients, naïve to any treatment and without tissue availability for EGFR testing, ctDNA extracted from plasma was analyzed by using our custom NGS narrow panel, in order to evaluate EGFR and other relevant biomarker status. EGFR mutations were further confirmed by using a digital PCR (dPCR) approach. **Results:** Sixty-three out of 64 (98.4%) cases were successfully analyzed by our custom NGS panel. On the overall, considering clinical relevant biomarkers, 5 (8.0%), 13 (20.6%), 2 (3.2%) patients harbored EGFR, KRAS and BRAF mutations, respectively. All EGFR mutations were confirmed by dPCR. **Conclusions:** Our data showed that SiRe® NGS panel represents a robust analytical tool for ctDNA mutational status analysis in basal setting.

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VP01.27
The Role of Liquid Biopsy in Lung Cancer: Focus on Prevention.

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Background: In the current era of personalized medicine, liquid biopsy plays a key role as a diagnostic tool in the clinical management of cancer patients.[1] Despite tissue specimen still represents the gold standard material for molecular analysis, circulating tumor DNA (ctDNA) extracted from plasma was introduced in clinical practice for non-small-cell lung cancer (NSCLC) patients to assess epidermal grow factor receptor (EGFR) molecular status in order to administrate tyrosine kinase inhibitors (TKIs) for several reasons.[2] In addition liquid biopsy may play a relevant role to monitor acquired resistance mutation after a first line of treatment.[3] The present study focused the attention on the diagnostic potential of liquid biopsy in balanced tertiary screening modeling. **Materials and Methods:** Advanced stage NSCLC patients underwent liquid biopsy analysis within the last five years were retrieved from the digital archives of the Predictive diagnostic laboratory of the University of Naples “Federico II”. Laboratory data were collected through the software SPSS. Non-parametric analysis was performed in order to test the differences between “wild type” patients or not. A multivariate logistic model was performed in order to assess the effect of mutation, age, and gender on the tumour progression. **Results:** On the overall, 515 total cases (almost of all plasma or peripheral blood), were retrieved for the analysis. The average age reported was 66.3 years, and the 25° percentile is 59 years. As far as cases are concerned, 221 and 294 patients were at basal or progression settings, respectively. Patients at progression odds ratio to have a tumour progression of 4.15 (95%IC 2,7-6,3), compared with those on basal setting, regardless of gender and age. The detected mutations were 131 from different types of pulmonary carcinomas. **Conclusions:** Our data showed that liquid biopsy may play a key role in balanced tertiary screening modeling.

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VP01.28
Lung Cancer Testing Using Sputum: A Non-Invasive Liquid Biopsy

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Background: Liquid specimens from organ sites offer the potential to sample cells directly to assess organ function and disease state. Sampling the fluid associated with an organ has a significant advantage because the prevalence of abnormal cells is often higher than otherwise found in whole body specimens such as blood; thus offering the possibility to develop high sensitivity tests for early stage cancer associated with a specific organ, promoting more efficient therapeutic triage. The Cell-CT® computes 3D isometric, sub-micron resolution images of single cells, allowing robust orientation-independent measurements of 3D morphometric biomarkers or phenotypes. AI algorithms based on features computed from cells are incorporated into the LuCED® lung test using sputum to generate a score that automatically identifies potentially abnormal cells and otherwise characterize the cellular material. In this abstract, we propose the consideration of a sputum liquid biopsy specimen to detect stage 1 lung cancer. **Methods:** Sputum specimens from 15 donors with biopsy confirmed stage 1 NSCLC and 17 cases that were negative for cancer were processed by the LuCED® Lung Test using the Cell-CT platform. Negative cases were understood to be free of confounding factors for specificity such as COPD and unresolved nodules though they may have had substantial smoking history. **Results:** Sensitivity for stage 1 lung cancer on NSCLC cases: 87%. Specificity for cancer negative cases: 88%. **Conclusions:** The analysis shows the potential of LuCED to detect stage 1 lung cancer with high sensitivity and specificity based on the non-invasive sputum liquid biopsy sample. Positive indications on control cases may be due to unresolved pre-cancerous conditions.

VP01.29
NGS-Based Liquid Biopsy as a Non-Invasive Tool to Identify Targetable Alterations in Non-Small-Cell Lung Cancer (NSCLC) Patients

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Introduction: Despite the development of targeted therapies for Non-Small Cell Lung Cancer (NSCLC), current diagnostic practices present certain limitations, as tissue samples are often unavailable for molecular testing. In this regard, liquid biopsies have several advantages, as they are non-invasive and easy-to obtain, allowing testing at different disease moments and monitoring alterations along treatments.
The objective of this work was to assess the utility of employing Next-Generation Sequencing (NGS) in different fluid samples to detect targetable alterations in NSCLC patients. **Methods:** 66 advanced NSCLC patients were included in this study. Total cell-free nucleic acids (cfNA) were isolated from 58 blood, 6 pleural fluid, 1 cerebrospinal fluid and 1 ascitic fluid samples. NGS was performed using Oncomine Pan-Cancer Cell-Free Assay (Ion Torrent) and Ion GeneStudio S5 Series (Ion Torrent). Variant calling was performed with Ion Reporter Software (ThermoFisher Scientific), and its significance was assessed using publicly available databases. Statistical analyses were performed using SPSS software (IBM). **Results:** cfNA isolation yielded successfully in 83.3% of the cases (cfDNA average was 62.10ng [12.29-1108.50]).
53 patients had sufficient cfNA concentration to be sequenced and passed all the quality controls. From these, 81.1% were stage IV, 75.5% Adenocarcinoma and 76.0% smokers or former smokers.
49.1% patients were newly diagnosed, from which 69.2% presented at least one alteration, being TP53 the most frequently mutated gene, followed by KRAS (mainly p.G12C and p.G12V). 12% of the patients presented actionable alterations level 1, and 46.2% level 1-4 according to oncoKB database. The most remarkable targetable alterations found were EGFR p.L858R, BRAF p.V600E and a fusion in RET gene.
23.1% of the patients were EGFR mutated at diagnosis, which were sequenced to identify resistance mechanisms upon progression to 3rd generation TKIs or 1-2nd generation in case no T790M mutation was detected by dPCR. 80% of these patients presented the EGFR sensitizing mutation. Furthermore, mutations in genes such as TP53, KRAS, BRAF and HER2, as well as amplifications in EGFR, MET, ERBB2, MYC and CDK4 genes were found as resistance mechanisms. **Conclusion:** These results support the use of NGS-based liquid biopsies that enhance personalized diagnosis and targeted therapies in NSCLC patients. This study was

supported by CB16/12/00350 and P118/00226 from ISCIII, AMACMA and AECC Valencia Scientific Foundation.

VP01.30
Use of an Artificial Intelligence-Derived Algorithm for Accurate FISH Probe Detection in a Liquid Biopsy Test for Lung Cancer

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Introduction: Fluorescence in-situ hybridization (FISH) is a commonly used technique in clinical diagnostics. Current DNA FISH algorithms bin cells based on specific signals and then employ an expert reviewer to confirm and report results. The challenge is there are significant limitations in the traditional FISH (image analysis) techniques, leading to misclassification which is suboptimal for rare-cell (i.e. circulating tumor cell, CTC) identification. LungLB™ is a FISH-based liquid biopsy test which uses DNA FISH to confirm identification of CTC as an aid in the clinical assessment of patients with indeterminate nodules suspicious for lung cancer. The LungAI™ software is being developed to more accurately identify FISH signals in clinical samples compared to a trained operator. **Methods:** Blood samples were processed in LungLife's CLIA laboratory. The LungLB™ test is a 4-color FISH assay used to detect early circulating tumor cells from a peripheral blood draw. Images of cells were acquired using an automated fluorescent microscope and CTC were defined as having a gain in two or more channels. An algorithm to identify FISH probes, the basis of the LungAI™ software, was developed using deep learning based on convolutional neural networks (U-Nets) using >4000 ground-truth probe images manually annotated by domain experts. Each case contains an average of 15,000 probe images. The algorithm was tested on 20 clinical samples and compared to existing microscope software. Improvements in CTC classification and detection were quantified by measuring concordance between CTC counts and matching exact CTC images from LungAI™ software versus traditional FISH analysis techniques with trained operators. **Results:** Traditional image analysis programs will mis-classify a significant number of normal cells as CTC, due to probe signals that appear split or broken, in order to minimize missed CTC. With LungAI™ software we report >60% reduction in the number of mis-classified CTC ($P<0.0001$, Wilcoxon matched-pairs signed rank test), resulting in improved specificity and greatly reducing the quantity of cells a licensed technician must review and verify. In half of biopsy-confirmed lung cancer cases, additional CTC were identified that were previously missed using existing microscope software for FISH. While this did not change the LungLB™ test result for all cases, one case was changed from a false negative to a true positive, suggesting the use of LungAI™ may enhance assay sensitivity. **Conclusion:** Preliminary performance of the LungAI™ software suggests the AI-derived algorithm may be useful in enhancing performance of the LungLB™ test for identifying CTC. Expanded cohort studies and algorithm validations are underway.

VP01.31
Circulating Tumor Cell as a Predictive Marker for Immunotherapy in Advanced Non-Small Cell Lung Cancer

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Background: Circulating tumor cells (CTCs) have potential to provide minimally invasive way for the response monitoring of various cancer. This study aimed to investigate the feasibility of CTCs as a predictive marker for anti-PD-1/PD-L1 immunotherapy in patients with advanced non-small cell lung cancer (NSCLC). **Methods:** This study included patients with advanced NSCLC receiving PD-1 or PD-L1 inhibitors as second- or third-line from July 2019 to February 2020. Blood was collected in a K2-EDTA tube before each injection from cycle 1 to 4 (C1 to C4), and CTCs were isolated and enriched by using CD-PRIMETM system which is antibody-independent size-based isolation method. By Bioview CCBS system, total CTCs (tCTCs) were identified by a sum of single positive cells (sCTC, EpCAM+CK+CD45-) and double positive cells (dCTC, EpCAM+CK+CD45+). **Results:** Among 36 response-evaluable patients, objective response rate and disease control rate were 27.8% (10/36) and 63.9% (23/36), respectively. Baseline CTC counts were not significantly different according to best response. The sCTC count difference from C1 to last cycle showed a correlation with the percentage of RECIST (Spearman`s rho, rs=0.567, p=0.007). Patients with partial response showed lower sCTC count at C3 (median, 2.3 vs. 6.4, p=0.078) and C4 (1.6 vs. 4.9, p=0.087) than those with progressive disease (PD). The subgroup with high sCTC increase from C1 to C3 (cutoff: 87.5%) showed higher PD rate than the subgroup with low sCTC increase or decrease (60.0% vs. 7.7%, p=0.044). The subgroup with high dCTC increase from C1 to C2 (cutoff: 200%) showed higher PD rate than the subgroup with low dCTC increase or decrease (75.0% vs. 16.7%, p=0.008). Patients with high increase of sCTC (C1 to C3) and dCTC (C1 to C2) showed worse median progression-free survival than those with low increase or decrease of sCTC (2.1 vs. not reached, p=0.078) and dCTC (1.9 vs. not reached, p=0.002). **Conclusion:** The sCTC count during treatment and high increase of sCTC and dCTC from baseline could be potential biomarkers to predict the disease progression and poor survival in patients with advanced NSCLC who received anti-PD-1/PD-L1 immunotherapy.

VP01.32
Real-World Data: Importance of Monitoring Blood-Based KRAS Mutations and Immune Status in Patients with advanced NSCLC

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Background: Until recently, KRAS mutation proteins were undruggable and patients with KRAS mutant non-small cell lung cancer (NSCLC) have shown a poor prognosis. With the promising results of new KRAS-targeting therapies currently in development, there is a need for rapid turnaround, blood-based KRAS mutation testing to identify treatment-eligible patients and to help expedite time-to-treatment. When coupled with blood-based proteomic immune profiling, these liquid biopsy results may improve treatment guidance given the established role of inflammation in disease progression. This analysis reports the analytical, and real-world clinical performance of blood-based testing for the three most common KRAS mutations in NSCLC, G12C, G12V and G12D (KRAS G12C/V/D), and immune status profiling for patients with NSCLC enrolled in the INSIGHT observational study (NCT03289780). **Methods:** The INSIGHT observational study has enrolled over 3000 subjects to date from 33 US sites. The all-comers design allows enrollment of NSCLC patients with all stages and lines of therapy. Subjects are followed for up to 18 months and subject characteristics, therapeutic decisions, staging, disease monitoring metrics, and available biomarker data are collected. Subjects received blood-based genomic and proteomic testing (Biodesix Lung Reflex, Boulder, CO) prior to therapy initiation. Circulating tumor DNA analysis was performed using Droplet Digital™ PCR and proteomic immune profiling was based on MALDI-TOF mass spectrometry and a machine learning algorithm for classification (K-Nearest Neighbor). A total of 1975 subjects with at least one year follow up were included in this INSIGHT interim analysis. Overall survival between groups was compared using Cox proportional hazard (CPH) analysis. **Results:** Subjects from the INSIGHT observational study were provided with genomic and proteomic testing results with an average turnaround of less than 46 hrs. 1751 subjects received KRAS G12C/V/D mutation testing. Of these subjects, 11.2% across all stages and 13.8% with stage IIIB/IV NSCLC were found to be positive (KRAS mut+). The KRAS G12C variant was found in 6.0% of tested subjects and 54.0% of all KRAS mut+ subjects. KRAS mut+ subjects receiving first-line platinum doublet chemotherapy (n=39) experienced significantly shorter overall survival (OS) with a median OS (mOS) of 3.7

months compared to 13.0 months for subjects who tested negative for KRAS G12C/V/D (KRAS mut-, n=317) (HR=0.45, CPH p<0.001). The proteomic test further stratified OS of KRAS mut+ subjects receiving chemotherapy with mOS of 2.3 months in KRAS mut+/proteomic poor subjects (HR=0.34, CPH p=0.010). While subjects receiving immunotherapy (n=266) did not experience significantly different OS (HR=1.09, CPH p=0.700) when stratified by KRAS mutation status, the proteomic classifier significantly stratified OS of KRAS mut+ subjects receiving immunotherapy (n=60) with mOS of 6.3 months and mOS not reached for proteomic poor and good subjects, respectively (HR=0.41, CPH p=0.016). **Conclusion:** The presence of a detected KRAS mutation was prognostic of poor outcome in subjects with advanced stage NSCLC receiving first-line platinum-based chemotherapy, but not for subjects who received an immunotherapy containing regimen. Proteomic testing further stratified KRAS mut+ subjects demonstrating that combined blood-based genomic and proteomic testing provides clinically meaningful information for predicting response to treatment and could help guide treatment selection.

VP01.33
The Turnaround Time Impact of Liquid Biopsy in Comparison with Tissue Biopsy in Advance NSCLC

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Background: Molecular profiling is a backbone step in diagnosing NSCLC. Tissue based analysis is preferred, however liquid biopsy may provide a salvage approach in case of tissue exhaustion and can even be faster than tissue based analysis. Therefore, this pilot study aimed to evaluate the impact of NGS-based liquid biopsy in addition to tissue-based analysis as well as to assess its effect on time to report and time to treatment in naïve NSCLC patients. **Methods:** This is a multicenter study performed in Israel presenting the preliminary results of 25 naïve metastatic NSCLC patients. A request for tissue-based molecular profiling were sent in parallel to a request for liquid NGS platform, using the Gaurdant360 test. The latter includes a panel of 74 cancer related point mutations and InDels, 18 amplification genes and 6 fusion genes, in addition to MSI status. Tissue-based analysis was based on local standard of care, which was immunohistochemistry for ALK, ROS1 and PCR or amplicon based NGS for EGFR. **Results:** This study describes a cohort of 25 patients, including 36% females with a median age of 69.1 (48-87) and 48% prior smokers, summary in Table 1. In one patient, pathologic analysis was not performed due to insufficient tumor sample. Two patients had undetectable mutations by liquid biopsy. Turnaround time analysis revealed that the median range (days) from the pathological diagnosis to receiving the tissue report on the last biomarker was 21.5 (7-45) days. While the median ranges from blood draw to receiving the cfDNA findings was 10 (7-19) days. Median estimates revealed that cfDNA findings were available 5 days before the last biomarker result. In 5 pts where the oncologist sent for cfDNA analysis before/at the time of confirmed diagnosis, cfDNA findings were available 13 days before the last biomarker result.
Actionable genes were identified in 11 tissue biopsies, while 14 were identified by liquid biopsy. Liquid biopsy was able to identify patients carrying mutations in PIK3CA, RET fusion and MET genes that were not tested by the local labs. One patient carrying ALK fusion and one EGFR were detected by tissue biopsy and were missed by liquid biopsy. Preliminary analysis of 20 patients' therapies shows that 16 pts received treatment (2 were lost to follow up, 2 died before treatment initiation), of whom 6 received biomarker-based treatment (4 osimertinib, 1 alectinib, and 1 enrolled in the LOXO trial). Clinical outcomes were available for 14 of the treated pts: 9 (64%) had partial response; 3 (21%) stable disease; and 2 (14%) progressed/died. **Conclusions:** This study suggests that NGS-base liquid biopsy improves time to report in patients with advanced NSCLC in comparison to tissue-based molecular analysis.

Table 1. Findings summary and demographics

	N=25	%,(Min-Max)
Gender		
Female	9	36%
Male	16	64%
Age (ys)	69.1	(48-87)
Smoking status		
Former smokers	12	48%
Current smokers	2	8%
Never smokers	8	32%
Unknown	3	12%
Driver Mutations	**Tissue**	**Liquid**
ALK	2	1
EGFR	8	7
KRAS		5
ROS1	1	1
MET		2
RET		1
PIK3CA		2
Turnaround Time, days	**Median**	**(Min-Max)**
Liquid biopsy - Time to result		
From blood draw to results	10	(7-19)
From pathological diagnosis to blood draw	7	(-15-46)
From pathological diagnosis to liquid results	20	(-5-54)
Tissue biopsy - Time to result		
From pathological diagnosis to tissue results	21.5	(7-45)
Molecular tissue biopsy results to liquid biopsy results	5	(-29-29)

VP01.36
Gene Mutation Profiles in Liquid Biopsies upon Development of Resistance to Afatinib

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Introduction: EGFR-mutant lung cancers are genomically heterogeneous in terms of compound EGFR mutations and mutations in other genes. The purpose of this study was to explore gene mutations in circulating cell-free DNA that are related to resistance to afatinib. We have previously presented the tentative results at AACR Annual Meeting 2020, and now present the updated data. **Patients and Methods:** Patients with EGFR-mutant non-small cell lung cancer who received afatinib monotherapy were enrolled in this trial. Plasma samples were longitudinally collected before afatinib treatment, during treatment, and at the time of development of afatinib resistance. Written informed consent was obtained from all patients. Cell-free DNA was extracted from plasma using the QIAamp Circulating Nucleic Acid Kit (QIAGEN). Mutation profiles were determined by next-generation sequencing and analyzed using a human lung cancer panel (QIAGEN). **Results:** A total of 71 patients were enrolled in this study. Plasma samples at the time of resistance to afatinib were collected from 38 patients. EGFR sensitive mutation were found in 9/38 patients (23.7%) at the time of resistance. In 3 of the 9 patients, T790M, EGFR resistant mutation was detected. In 21 patients whose samples were collected both before treatment and at the resistance, the number of gene mutations except for EGFR mutation was lower at the time of resistance (0-8) than before treatment (0-22) ($p=0.04$). But in the 5 cases who were detected EGFR mutation both before and after treatment, there was no significant difference ($p=0.63$). In the 5 cases who were detected EGFR mutation both before and after treatment, TP53 was detected in all the 5 cases, and SETD2, NTRK3, FGFR1, MAP2K1, APC, NRAS, and JAK2 were detected more frequently after treatment than before treatment. **Conclusion:** The number of gene mutations detected in cfDNA at the time of resistance tends to be lower than before treatment. However, there are newly detected mutations in them. The mutations may be related to the development of resistance for afatinib.

VP01.37
Clinical Utility of Personalised Somatic Genomic Rearrangement Biomarkers after Surgical Resection of Non-Small Cell Lung Cancer

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Introduction: Although surgical resection is the curative treatment for some patient with early stage non-small cell lung cancer patients, 30% to 55% relapse after the surgery within 5 years. Currently, PET/CT scans are the only widely available method for relapse monitoring. Recent studies have indicated that monitoring of circulating tumour DNA (ctDNA) levels is more sensitive and specific than CT scans in many tumour types for recurrence detection. However, only relatively few NSCLC cancer patients contain a recurrent mutation for ctDNA monitoring. Personalised somatic genomic rearrangements, which can be identified from the tumour tissue by whole genome sequencing (WGS), can be utilised as biomarkers for the detection of ctDNA to enable monitoring of cancer especially for patients without clear truncal mutations. Droplet digital PCR then can be used for relapse monitoring making our approach more cost-effective with high sensitivity and specificity for routine diagnostic testing. **Methods:** Blood samples are collected before surgery and at 15 time points after surgery over 3 years. Tumour DNA is extracted from FFPE tumour tissue for WGS. Somatic rearrangements were then identified from the WGS data using the genomic rearrangement caller, GRIDSS. After filtering the results, the top three to five ranked somatic rearrangements by GRIDSS from each sample were chosen as markers for primer design and validation. PCR primers were designed to bind with the flanking regions of the breakpoint for each somatic rearrangement marker. The primers were validated using droplet digital PCR. DNA extracted from the buffy coat was used as a germline DNA control. Two somatic rearrangement markers showing the best ddPCR results were chosen for longitudinal ctDNA monitoring using plasma DNA. **Results:** We present results illustrative for three patients. For patient 1, both primers were strongly positive in FFPE tumour DNA and negative in buffy coat DNA. This indicates that the genomic rearrangement markers are somatic, and the primer sets work well on the target genomic rearrangements. After 6 months of surgery, the CT/PET scan showed a suspicion of relapse which was confirmed later by core biopsy. A strong positive signal was seen for both markers at this timepoint indicating that our approach can identify the tumour relapse accurately. The negative result on both markers at the 12-month timepoint shows that there was complete molecular response for the radical radiotherapy that was perform after 9 months of the original surgery. The other two patients remained negative during the monitoring period. **Conclusion:** Biomarkers designed from somatic genomic rearrangements can be used to monitor the tumour relapse and treatment response accurately. Personalised somatic genomic rearrangement is a powerful biomarker for disease monitoring after surgical resection of non-small cell lung cancer.

VP01.38
Ultra-Rapid Detection of Circulating EGFR Mutations from Plasma under 3 hours and without Prior Cell-Free DNA Extraction

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Background: Liquid biopsy using circulating cell-free DNA (cfDNA) offers a non-invasive alternative to tissue-based genotyping in patients with advanced non-small cell lung cancer (NSCLC). However, current liquid biopsy methods require extensive technical support and can take up to 1-2 weeks for report generation. Here, we describe our clinical validation of a fully automated, cartridge-based assay for detecting cell-free EGFR mutations from plasma under 3 hours and without the need for prior cfDNA extraction. **Method:** We evaluated the performance of the Idylla ctEGFR mutation assay using an integrated, real-time PCR-based system (Biocartis) for qualitative detection of 42 EGFR mutations in exons 18, 19, 20, and 21. Testing was performed on 2 ml of plasma without prior cfDNA extraction. All plasma samples were also tested using a validated next-generation sequencing (NGS) assay and confirmed to harbor known EGFR mutations. All mutation calls from the Idylla assay were manually reviewed using the Idylla Explore interface. **Result:** Twenty-seven plasma samples were collected from patients with advanced NSCLC. Concurrent plasma NGS revealed 31 EGFR mutations, including 17 exon 19 deletions, eight p.L858R mutations, and six p.T790M mutations. The median variant allele frequency (VAF) as quantified by orthogonal NGS was 3.5% with a range of 0.2 - 76.9%. Overall, 26 out of 31 variants (84%) were called by the ctEGFR Idylla assay based on the automated calling algorithm. Sensitizing mutations were detected at VAF as low as 0.2%. Five variants not called included three subclonal p.T790M variants (0.4% VAF each) and one p.L858R and one exon 19 deletion at 1.4% and 1% VAF, respectively. Manual review of the PCR tracings allowed the detection of these two sensitizing mutations, thus raising the final detection rate to 90%. Notably, we detected an p.L858R mutation that was confirmed on tumor tissue but missed by plasma NGS. The technical hands on time averaged 2 minutes, and time from setup to instrument report generation was 140 minutes. **Conclusion:** The Idylla ctEGFR mutation assay enables sensitive detection of canonical sensitizing EGFR mutations from 2 ml of plasma. The convenience of blood testing coupled with the ultra-rapid turn-around time (<3 hours) may facilitate same-day treatment decisions in the clinic. Further optimization and validation are underway, with the potential for adding other driver mutations to the ultra-rapid screening panel.

VP01.39

A ‘Plasma-First’ Molecular Profiling Approach Complements Actionable Mutation Detection in Suspected Lung Cancer Patients

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Introduction: Tissue biopsy and associated molecular profiling is an integral part of the diagnostic approach to suspected lung cancer. It is however invasive, slow and associated with limitations of tissue heterogeneity. The ‘plasma-first’ paradigm of liquid biopsy may complement diagnosis in providing additional information and may be particularly helpful in patients from whom obtaining tissue is challenging. **Methods:** Patients with suspected lung cancer (n=71, median age=65) were recruited prospectively between 2013-2015 at the Department of Respiratory Medicine, Changi General Hospital, Singapore. Blood was collected first, followed by baseline tissue sampling by bronchoscopy or effusion collection within 48 hrs. Matched plasma cfDNA testing on 1 to 9 mL plasma was done using an ultrasensitive amplicon-based next-generation sequencing (NGS) panel including EGFR, BRAF, KRAS, ERBB2 and MET genes. Targeted EGFR diagnostic testing for lung cancer was done in a CAP-accredited clinical laboratory. Additional tissue biopsy molecular profiling was done with the same NGS panel for a subset of 36 patients. Concordance between methods and sample types were assessed. This study is registered under NCT04254497. **Results:** Among confirmed NSCLC cases (n = 54), diagnostic molecular EGFR test results were available for 38 patients. Of the 16 NSCLC cases without EGFR test results, 10 were biopsied but informative results could not be obtained. Two patients above 80 years of age were not biopsied and remaining 4 passed away within 1 to 4 weeks of initial diagnosis by cytology or histology. Plasma testing was successful in 100% of patients versus 70.4% by routine diagnostic testing. Of the NSCLC cases with routine EGFR tissue test results, 38% (13/38) were positive for EGFR sensitizing mutations. Compared to standard testing, overall sensitivity of EGFR detection by plasma NGS testing was 77% (10/13) and specificity was 100% (25/25). Among EGFR-negative cases, plasma NGS testing identified additional actionable mutations in 7 cases, including KRAS G12D, MET exon 14 skipping, BRAF V600E, EGFR exon 20 insertion mutations. This amounted to an additional diagnostic yield of 28% (7/25) by plasma testing of NSCLC cases. The finding of additional actionable mutations was verified by the concomitant testing of tissue/pleural effusion samples with the same NGS panel for 5 cases. Two of 16 cases lacking standard EGFR testing results, were positive for EGFR mutations by NGS in both plasma and tissue. Cases with no EGFR mutations also harbored non-targetable mutations in nearly half (12 of 25 = 48%) of cases. Overall, the concordance of actionable mutations observed between plasma and tumor testing by NGS for NSCLC cases (n = 30) was 91.7%. Among cases confirmed to be not cancer (n = 9), only one case harboured a tissue and plasma-matched ALK frameshift mutation of unknown significance and all other cases had no mutations detected in both plasma and tissue. **Conclusions:** A high concordance of actionable mutations between NSCLC tissue and plasma was observed in the first-line setting. Additional actionable mutations were observed using plasma, underlining the potential of the ‘plasma-first’ approach for complementing the comprehensive diagnostic profiling of lung cancer patients.

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