We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

5,100
Open access books available

126,000
International authors and editors

145M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter

Precision Medicine in Lung Cancer: Challenges and Opportunities in Diagnostic and Therapeutic Purposes

Beatrice Aramini, Valentina Masciale, Federico Banchelli, Roberto D'Amico, Massimo Dominici and Khawaja Husnain Haider

Abstract

Lung cancer is one of the leading causes of cancer death among both men and women, making up almost 25% of all cancer deaths. Precision medicine shows promise for improving many aspects of health and healthcare, including tests, drugs, and other technologies that support innovation, with the possibility of new partnerships with scientists in a wide range of specialties. Non–small-cell lung cancer (NSCLC) has become a prominent example of the success of precision medicine in treating solid tumor malignancies. The first step in this process involves new blood-based diagnostics, which can now noninvasively provide clinically useful information. However, the identification of novel biomarkers that could be used in early diagnosis is urgently needed, especially for guiding initial therapy and predicting relapse or drug resistance following the administration of novel targeted therapies.

Keywords: precision medicine, target therapy, liquid biopsy, CTC, CSCs, miRNA, NGS, NSCLC

1. Lung cancer and the meaning of "precision medicine"

The scientific community tends to conflate the meanings of "precision medicine" and "personalized medicine" [1, 2]. In fact, the National Research Council defines "personalized medicine" with an old meaning quite similar to that of "precision medicine." However, whereas personalized medicine mainly focuses on medical actions for a single person, precision medicine explores various factors affecting that person's condition, such as diseases, the environment, etc. [3].

Precision medicine is able to provide specific genetic maps for patients with elevated cancer risks, potentially revealing gene mutations and thus calculating the likelihood of family members' developing a certain type of cancer.

Recently, the use of precision medicine has been expanded to attempt treatment of several solid tumors, including those of breast, brain, and lung cancer [4, 5]. In general, the aim of precision medicine is to find the right treatment for a specific patient at the right dose and time, which is particularly important in cancer therapy.

Finding a precise treatment for a patient could eradicate the potential problem of the variability of treatment response, including resistance. In fact, one of the main problems with cancer treatments is a nonresponse to drug therapy and the consequent metastatization of the disease.

Precision medicine is being used to treat certain cancers to help discover what tests and treatments are best. In addition, doctors could employ precision medicine to identify those at high risk for cancer, to prevent certain types of cancer, for early cancer detection, to make specific cancer diagnoses, to select the best treatment options, and to evaluate treatment efficacy [6].

The history of focused therapies to combat lung cancer began with the approval of the small molecule tyrosine kinase inhibitors (TKIs) of epidermal growth factor receptor (EGFR) [7]. This marked the beginning of the era of targeted therapies for lung cancer. On a related note, in 2004 and 2007, the first discoveries of adenocarcinoma of the lung were identified as *EGFR* mutations and *ALK*-rearrangements. These new findings paved the way for new targeted therapies – namely, tyrosine kinase inhibitors (TKIs) [8]. The responses to these inhibitors and the subsequent discoveries from numerous clinical trials (NCT00322452, NCT00932893) [9, 10], led to incorporating them into daily clinical activities. This demonstrated that TKIs are more effective than traditional treatments, such as chemotherapy. In contrast, patients with non-EGFR mutant lung cancers do not respond to EGFR TKIs, and, for this reason, chemotherapy a more effective treatment for them [9]. To complicate cancer's frequent resistance to chemotherapy, consequent threat of recurrence, and the related costs of targeted therapies, no drugs have been very effective in its treatment. Thus, the latter must be considered when introducing targeted therapies into clinical practice [11]. However, scientists have proceeded to define and characterize other oncogenic driver mutations in lung adenocarcinoma, such as KRAS. This mutation was first described in 1980 [12, 13], with a presence of 25–30% in lung adenocarcinoma and high aggression, which is even more dangerous without specific targeted therapies. Interestingly, the first recent study with promising clinical data came from a Phase I trial, in which the KRAS G12C inhibitor AMG 510 shrank lung cancer tumors harboring KRAS G12C mutations [14, 15]. This highlighted the importance of identifying new drivers' mutations therapies in lung cancer for decreasing mortality and recurrence. Other mutations have been identified in lung cancer, including ERBB2 (3%), BRAF (2%), PIK3CA (1%), MAP2K1 (1%), and NRAS (1%), [16], although these are defined as *niche* mutations. Beyond the fact that these niche mutations are infrequent, they are no less dangerous, with a high level of mortality. On this subject, a recent study by Aramini et al. examined three cohorts of mutations selected from patients with lung adenocarcinoma [17]. These mutations were 1) BRAF, c-MET, DDR2, HER2, MAP2K1, NRAS, PIK3CA, and RET; 2) K-RAS; and 3) EGFR. In this pilot study, the researchers demonstrated that niche mutations exhibited an increased risk of death when compared with EGFR mutations and a similar risk of death when compared with KRAS mutations. This aspect is key in highlighting the importance of focusing attention not only on general mutations but also on niche mutations to develop more effective cures in larger populations. In fact, a clinical trial is currently being conducted to better define the less common oncogenic driver mutations (e.g., NCT01336634).

In lung adenocarcinoma patients, the importance of testing eventual genetic mutations introduced new diagnostic perspectives. These have enhanced the treatment recommendations of the International Association for the Study of Lung Cancer (IASLC) and National Comprehensive Cancer Network (NCCN) for patients with EGFR mutation and ALK positivity. Moreover, new mutations have been studied for diagnostic purposes, including ROS, RET, MET, BRAF, and HER2, although these

are infrequent mutations [18, 19, 20]. These studies have laid crucial groundwork for creating more focused treatments tailored to each patient [18, 19, 20].

Precise molecular tests led to the correlation of EGFR mutations and sensitivity to gefitinib and erlotinib in lung adenocarcinoma, especially in non-smokers or low-smokers. The EGFR tyrosine kinase inhibitors (TKIs) are considered the baseline treatment for this cancer, although a high percentage of patients develop resistance to therapy and experience a disease recurrence within nine months [18]. However, scientists discovered new mutations, developing a more focused panel of patients' genetic characteristics. These researchers discovered that 50% of patients developing tumor dissemination showed a secondary EGFR mutation, such as T790M, which has been used for developing new target therapies, including AZD9291 and CO-1686 [21].

ALK, ROS, and RET, defined as receptor tyrosine kinase gene rearrangements, present at a frequency between 1 and 8% in lung adenocarcinoma, although patients harboring ALK fusion or ROS1 mutations have positively responded to crizotinib and to TKIs. However, these patients frequently develop recurrence, probably due to an acquired resistance and from mechanisms which must be further investigated [22, 23].

The target of mutations is particularly difficult, especially the study of the mitogen activation pathway (MAPK). This has been of recent interest for its implications regarding lung adenocarcinoma development and the subsequent results of therapeutics. Specifically, the MAPK activation mechanism has been found frequently along certain KRAS amino acids. Currently, KRAS is considered an aggressive mutation for its impact on overall survival (OS) in early-stage NSCLC. Finding specific RAS inhibitors may open the door to new target treatments that improve long-term survival and responses to therapies, even in patients with KRAS mutations. New treatments have been set against the downstream effectors of activated KRAS, such as MEK1/MEK2, PI3K, and AKT [24]. In addition, recent phase II data analyzing the inhibition of MEK1/MEK2 by selumetinib and docetaxel showed promising results in KRAS-muted patients [25].

Additional work on downstream effectors in the KRAS mutant pathway is crucial. Currently, several clinical trials employing the inhibition of PI3KCA, MEK, and PTEN are in progress [26].

Recently precision medicine is used not only in clinical practice to drive oncological decision but also in patients with rare tumors, likely due to their frequency in these patients' family histories. This aspect is important for making medical decisions, as well as for screening.

The most frequent tests used at this time are biomarker tests, chromosome tests, gene tests, and biochemical tests, all of which are derived from blood, saliva, a tissue biopsy, or body fluids. These tests are named as follows: DNA mutational analysis, genomic testing, proteomics, biomarker testing, tumor profiling, cytogenetics, next generation sequencing, or molecular testing [27, 28].

1.1 NSCLC biomarkers

The use of drugs against NSCLC in locally advanced or advanced stages may help identify targeted drugs, which are more useful and better tolerated, as well as more responsive against lung cancer. The latter remains a serious problem in the world, accounting for over 1.7 million deaths in 2018 [29], showing that therapies are still largely ineffective. In particular, EGFR and ALK are considered biomarkers that predict positive responses to specific drugs. However, not all patients with lung cancer show these mutations, and this is why not all patients respond to gefitinib, erlotinib, or afatinib, which are currently considered the most effective against EGFR mutations [30, 31].

In addition, the ALK-positive gene is rare, occurring in approximately 5% of patients with NSCLC and eliciting production of a growth-promoting enzyme [32]. Patients who are ALK-positive are usually treated with crizotinib, a tyrosine kinase inhibitor that blocks the input of the growth signals to the nucleus of the cancer cell. Immunotherapy is the last defense against cancer, and it has been developed in the last decades, including cancer vaccines, oncolytic viruses, and administration of antibodies or recombinant proteins that co-stimulate or block the immune checkpoint pathways [33]. However, there is a pressing need to identify new targets specific to a larger cohort of patients with better outcomes than those of current chemotherapeutic treatments. This need has induced the scientific community to deeply analyze other mechanisms or approaches.

Although targeted drugs and chemotherapeutic agents may be useful for weeks or months against tumors in terms of disease control, the majority of tumor relapses occur after several months of treatment.

1.2 A new kind of drug treatment: Immune checkpoint inhibitors

A new class of drugs was recently developed by Allison et al. and named *checkpoint inhibitors* [34, 35]. This group has the specific role of enhancing patients' immunity, thus increasing their chances of fighting cancer. The first one created was nivolumab, followed by pembrolizumab, which targets a receptor called programmed cell death-1 (PD-1).

However, not all patients have shown high levels of PD-1 expression in their cancer cells, revealing the major limitation of these therapies. In fact, the prognostic role of PD-L1 in solid tumors such as lung cancer, melanoma, etc. is still debated [36]. In patients with an overexpression of PD-L1, the use of antibodies able to target PD-1 and PD-L1 is one of the main points to consider for the setting of more effective therapies [37]. However, for the low immunohistochemistry accuracy based on PD-L1, the use of this biomarker as a possible predictor for satisfying immunotherapeutic results against cancer is under examination [38]. The main shortfalls of this marker are, first, the different cut-off values of positivity in different solid tumors; second, the sensitivity, which is very variable as demonstrated in several studies; and third, the potential involvement and impact of the tumor microenvironment associated with the use of other genes markers which, combined together, may be more helpful for a better-focused PD-1/PD-L1 blocking immunotherapy [39].

In particular, pembrolizumab—a humanized antibody used in cancer immunotherapy as a programmed cell death 1 (PD-1) inhibitor—seems to improve survival significantly more than standard chemotherapy in NSCLC patients with an expression of PD-1 ligand ≥50% in cancer cells [40, 41]. In addition, in nonsquamous NSCLC patients the PD-L1 positively expression of at least 1% represents a good responder against antitumor action. This aspect highlighted the importance of the presence of at least 1% PD-L1 expression for the treatment of NSCLC patients, which seem to represent two-thirds of all NSCLC population [42, 43]. In contrast, for small cell lung cancer (SCLC) which represents 15% of all types of lung cancers, there are actually few choices of cancer treatments and no molecularly targeted drug has been approved. In particular, the potential role of PD-1/PD-L1 inhibitors in SCLC has not been yet considered [44]. Recently, the first study analyzing the PD-L1 expression in SCLC has been conducted at Kyoto University Hospital, where the researchers analyzed the immunohistochemical expression of this marker in paraffin blocks from 39 patients affected by SCLC [45]. Although previous studies have been conducted—most likely for the use of different types of antibodies—the expression was arbitrary, and this represented an impediment in the elucidation of the possible

expression and role of PD-L1 in SCLC [46]. For the first time, the team from Kyoto University thought to use the standard PD-L1 antibody already tested in NSCLC with the same cut-off level (1%) as in NSCLC [45]. This approach was important to elucidate the presence of this marker, even in SCLC, although the correlation with the clinical aspects has not been yet defined.

In summary, all the aspects described would suggest that the use of PD-L1 as an exclusive biomarker in cancer may not represent a completely satisfying choice in terms of accuracy and efficacy. On the other side, at the moment, scientists cannot ignore the good responses against cancer that patients with at least 1% of positivity for PD-L1 show through the most-used checkpoint inhibitors [47]. In summary, further studies set on the combination among PD-1/PD-L1 pathways, the tumor microenvironment and other genes markers may open the way for new discoveries that are tailored to the individual patient and more effective against cancer.

2. Precision medicine and solid tumors

The development of new techniques and approaches to discovering signaling pathways to better understand tumor growth has opened to precision medicine for solid tumors [48].

In particular, the major field is to create future treatments tailored to each patient to improve their results against cancer. However, this aspect has not yet been focalized for the numerous difficulties related to the new cancer cells targets. Through current clinical trials, pharmaceutical companies are developing studies based on specific markers to find multiple options for the best treatment [49].

Recent advances regarding the biology behind these tumors have shown promising results. In several centers, patients are analyzed by RNA expression testing and protein analyses [50, 51]. These genetic analyses have already been taken into consideration, especially for hereditary tumors. Certain companies, such as Myriad Genetics Inc., have developed in the last decades several molecular diagnostic kits to test patients at risk of developing hereditary tumors [52, 53, 54]. Thus far, this aspect has been extensively analyzed for prostate cancer and breast cancer [55, 56]. It has been examined for the genes mutations that are more frequent in these diseases, as well as the development of prognostic scores related to cancer recurrence [57].

At the moment, the possibility of developing a molecular profile is limited for the presence of mutations and other genetic variations. However, scientists are planning to develop a molecular profile based on RNA expression, as described for familiar genetic diseases or by immunity profiles. There is an urgent need to develop new approaches and targeted treatments to better stratify cancer patients, to prevent recurrence, and to more effectively treat these patients.

Several clinical trials are running regarding the possibility of targeting oncologic patients. Some of these trials involve specific tumors, such as BATTLE I and II [58], and some are non-tumor specific. These studies have been designed as observational, randomized, and non-randomized [59–62].

Non-randomized trials are studying molecular profiles in the Clinical Laboratory Improvement Amendments (CLIA) certified laboratories, which were founded in 2013 in collaboration with pharmaceutical societies to identify a specific genes patent for each patient. In particular, pharmaceutical companies have been conducting independent trials of drugs in patients with specific genetic profiles [63].

However, these profiles may not be the same for patients with several solid tumors, but at this time, this aspect is not well known. The National Cancer

Institute (NCI) is preparing a study with the involvement of agents from different companies [64]. The baseline for these studies, called NCI-MATCH studies, will be the analysis by a consortium of NCI-selected CLIA-certified laboratories of the genomic profiles of several cancer patients. This process will use a new approach called next generation sequencing (NGS) for a number of selected genes.

Another interesting study, the SHIVA study, randomizes patients with specific genetic abnormalities matching generic types of cancer and patients' specific genes. It examines the possible results from standard treatments in terms of cytotoxicity and disease progression [65].

These types of combined studies involving several companies and certified laboratories may be very important to further discoveries, but the difficulty of coordinating multiple companies constitutes an effective impediment. Basic research is suggested to more deeply analyze the mechanisms and mutations involved in development and tumor progression [66, 67]. A representative panel during time of the major achievements for lung cancer therapy (**Figure 1**).

Regarding the mutations, those in the scientific community do not believe that studying a single mutation or a small panel of genes would be enough to influence future decisions or treatments for oncological patients. For this reason, the new advanced technologies require a larger panel of genes or intra- and inter-tumor heterogeneity at the protein, genetic, and epigenetic levels [68, 69]. Specifically, the genetic analysis of RNA and proteins in primary or metastatic diseases in patients with renal carcinomas have shown a large heterogeneity of cells and genes inside the tumors. This is one of the main obstacles in the battle against cancer [68, 69]. On the other hand, in colorectal and lung cancer, the panel of genes that seem to be involved is limited [70, 71]. One must be considered, such as in the case of lung cancer. Such a tumor could develop several mutations during its progression, and these would be persistent in evolving. For this reason, future patients' tumor profiles would need to be frequently updated to guarantee the best treatment options. One problem would be the impossibility of obtaining sufficient material from

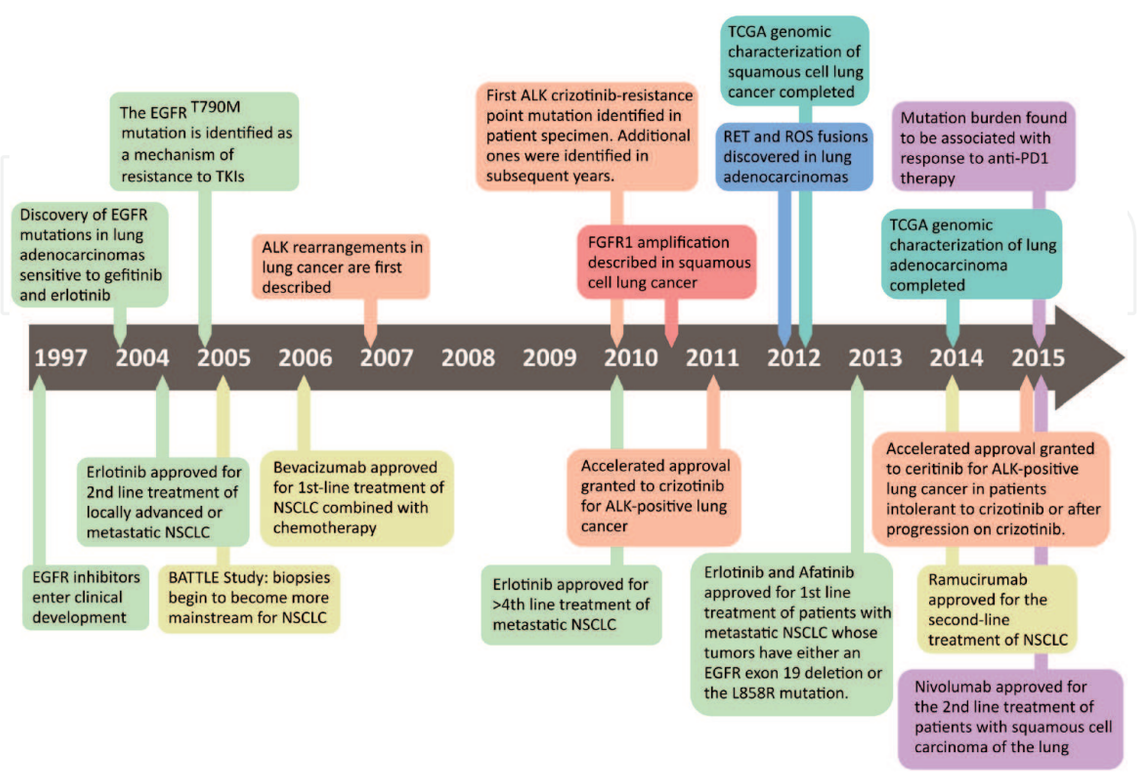

Figure 1.
Timeline of major discoveries and related therapeutic approaches in non-small cells lung cancer.

biopsies. In addition, it would be difficult to ask to these patients to perform several biopsies in order to have a more focused treatment. Thus, in order to minimize invasive procedures, scientists have attempted to develop the best approach with the least aggressiveness toward the patient. For example, the analysis of circulating free DNA (cfDNA)by liquid biopsy, widely discussed at this time, and CTCs may be considered of great value if these approaches are able to replace multiple biopsies. For the moment, the results of these techniques seem to be promising, but further investigation is needed regarding each type of solid tumor, as well as each patient [72, 73, 74].

Even the serum proteins are of interest; however, the difficulty in identifying a specific protein has made this approach very difficult to use for tumor patients. For example, PSA levels for prostate cancer patients, as well as CEA measurements, are commonly used markers, but several clinical trials and basic research are necessary to identify more markers for future cancer diagnoses [75–77].

In summary, important progress has been made in terms of molecular profiles and developing advanced genetic technologies. However, the coming years will be crucial in determining whether these new aspects will revolutionize treatments and improve prognoses in cancer patients.

3. Recent discoveries in *precision*-diagnostic and *precision*-therapeutic approaches to lung cancer

New genetic discoveries through high-throughput techniques could allow the establishment of a new era in which precision medicine could be routinely used for cancer treatment, as well as in its diagnosis and therapy [78]. Since the earlies 2000s, innovative sequencing systems called next-generation sequencing methods (NGS, Next Generation Sequencing), or massive parallel sequencing (MPS, Massive Parallel Sequencing), have been used to define high-efficiency nucleotide sequences in the simultaneous, independent analysis of millions of bp of DNA. In particular, the association of genomic data and the identification of new biomarkers may modify cancer treatments in the near future. This would require extensive knowledge of the mutational analysis of a panel of cancer genes, along with determination of copy-number variations and any other structural rearrangements. As with lung cancer, which has a high rate of recurrence after surgery independent from stages, it would be useful in treating other solid tumors to have some predictor of relapse based on genetic tests identifying the individual risks of various cancers and their consequent relapses. This chapter will discuss technical considerations for developing genomic precision diagnostic tools for clinicians to support their further use in oncological care and research trials, as represented schematically in **Figure 2**.

3.1 Single-gene assays versus next-generation sequencing

Until now, the most commonly used methods have included DNA or RNA amplification using polymerase chain reaction (PCR), followed by classical Sanger sequencing or pyrosequencing, analysis of fragments by electrophoresis after digestion with restriction enzymes, or fluorescent in situ hybridization with specific probes (FISH) [79]. Single gene analysis often has significant advantages over large-scale genomic sequencing due to the lower cost and reduced complexity in test development, execution, and interpretation. In molecular oncology, for example, there is frequent identification of BCR-ABL1 translocation by FISH in patients with chronic myeloid leukemia. Single gene analysis is a useful approach when the genetic alterations are well known. On the other hand, high-throughput screening,

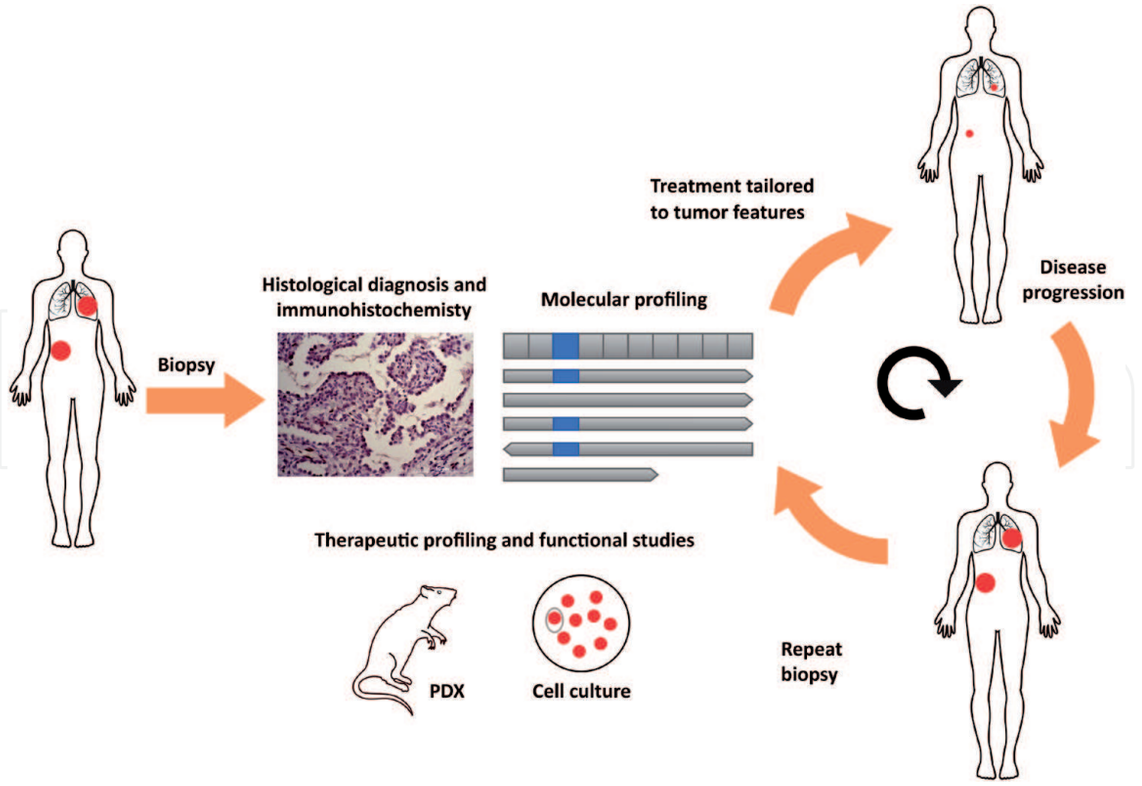

Figure 2.
Future perspectives in molecular profiling and diagnostic approaches in lung cancer.

such as NGS, is more sensitive than many monogenic methodologies, such as Sanger sequencing. As a consequence of the discovery of more relevant genes in a clinical context, NGS has become an increasingly attractive approach. Molecular testing of advanced non-small cell lung cancer (NSCLC) provides a good example of the rapidly growing need for the molecular profile of several genes, especially cancer. Initially, the only knowledge about the genetics of lung cancer was the deletion of exon19 in the EGFR gene and the mutation of the L858R gene, which could lead to the first targeted therapy with tyrosine kinase inhibitor (TKI) [80–88]. However, within a few years, effective targeted therapies approved by the U.S. Food and Drug Administration (FDA) have been developed and are now effective in treating lung cancer with other EGFR and BRAF mutations [83, 84], as well as ALK and ROS1 rearrangements [84–88]. Other solid tumors have been associated with target therapies involving other molecular alterations, such as exon 14 MET skip mutations [89–91], RET rearrangements [92–94], and ERBB2 (HER2) mutations [95], which have led to a new setting for therapeutic recommendations from the National Comprehensive Cancer Network (NCCN) [96].

3.2 Gene panels versus unbiased genomic and transcriptomic analyses

Given their high speed of execution, NGS techniques have been used for the identification of disease genes by whole genome sequencing (WGS) or whole exome sequencing (WES), as well as target gene panels [97]. The potential advantage of these techniques is the possibility of detecting essentially any genomic alteration, including novel or rare alterations. However, certain critical points must be considered. To begin, WGS is far too expensive and generates a huge amount of raw data requiring complex bioinformatics analyses to extract useful information. As a consequence, analysis may be performed only on selected cases. In NSCLC, for instance, whole-genome studies have demonstrated a median of 888 and 15,659 mutations

in NSCLC samples from, respectively, nonsmokers and smokers [98]. The major part of these variants lacks any relevant pathogenic significance. Nevertheless, the comparison between tumor and normal DNA is mandatory, distinguishing somatic mutations, due to cancer, from germline polymorphisms, which will be inherited by patients' offspring. However, WES is an unbiased approach that has also found utility in certain laboratories as a tool for unraveling cancers. WES limits sequencing to the ~1.5% of the genome that lies in the exons of genes. Nevertheless, this approach also generates a large number of potential variants, the vast majority of which even in this case currently do not have annotated clinical implications. Exome sequencing would also fail to detect pathogenic variants, such as structural rearrangements with intronic breakpoints. DNA quality requirements are lower than those of WGS, so the drawbacks of this approach include the fact that the depth of sequencing obtained through WES is much lower than that obtained from targeted panels. For diagnostic purposes, it has been argued that a high sensitivity is needed to reduce the number of false negatives. Although a genetic variant of uncertain significance can be detected, it would be better to be cautious even if there were no clinical treatment for the alteration. Another crucial element that may be investigated with WGS is the copy number alteration (CNA), which is a parameter that takes into account the number of repeated alterations in the DNA. These hallmarks in cancer often lead to the activation of oncogenes and inactivation of tumor suppressor [99]. The WES is primarily used to discover all of the variations in the DNA sequence, but the RNA-Seq is specifically used for the measurement of gene expression, gene fusion detection, and identification of splicing events, since it is based on direct sequencing of cDNA. One of the most important applications of the RNA-Seq is for cancer. For example, a large-scale RNA-Seq has been useful for the detection of several cancer driver genes in adenocarcinoma of the lungs [100, 101]. That study compared the transcriptome of lung cancers between smokers and nonsmokers and found a significant difference in the number of point mutations between the two groups. In summary, the amount of smoking (packs/year) was positively correlated with the number of somatic point mutations in the cancer genome. As for the study described, a complete molecular analysis conducted on the transcriptome or the entire genome or exosome through higher coverage of the genomic regions allowed the detection of lower-level molecular alterations. Moreover, the principle difference between targeted genetic panels and unbiased, extensive genomic and transcriptomic analysis is not necessary in the last case to know a priori the molecular alterations to be detected.

3.3 Applications of molecular oncology

3.3.1 Diagnosis

For different tumors, molecular diagnostic tests, as for example, BCR-ABL1 in chronic myeloid leukemia or other data, may be very helpful in influencing the decisions of oncologists or pathologists. That is, they could develop more detailed diagnoses, as well as more appropriate approaches, although molecular analyses would need to be correlated with clinicopathological patients' characteristics.

In particular, certain mutations detected in malignant tumors have also been found in healthy individuals [102, 103, 104]. However, the new technologies related to advanced molecular analysis are now able to distinguish between cancer mutations and normal tissue mutations. One of the most important aspects of this precision medicine tailored to the patient is the possibility of stratifying the prognosis. Several studies are examining this aspect in several solid tumors [105–108].

3.3.2 Therapy

Several clinical trials are currently being conducted regarding specific target alterations in different cancer types. The Molecular Analysis for Therapy Choice (MATCH; http://www.cancer.gov/aboutcancer/treatment/clinical-trials/nci-supported/nci-match) trial and the Targeted Agent and Profiling Utilization Registry (TAPUR) trial were designed to identify particular molecular targets able to determine a specific therapy against cancer. The main difficulty arises from the fact that each tumor shows a specific mutation that may be different in each patient. This genetic heterogeneity has led to targeting specific drivers in each tumor. Furthermore, the identification through the NGS technique introduced new possibilities for finding specific oncogenic drivers that could maximize the possibility of receiving the benefit of a very focused, tailored therapy. The use of NGS is intended to guide treatment decisions. In fact, this technique can identify oncogenic alterations, which may be target inhibitors or monoclonal antibodies. For example, the BRAF V600E mutation can be cured by BRAF inhibitors and MEK inhibitors approved by the FDA. For instance, patients with colorectal cancer and KRAS and NRAS mutations showed a therapeutic resistance to EGFR antibody therapy [109, 110].

The integration of genomic results into reports and the clinical decision supported by NGS are a powerful tool that enables the simultaneous interrogation of many regions of the human genome [111]. However, as the volume of data from NGS testing grows, so does the challenge of distinguishing the findings that are clinically meaningful and prioritizing their clinical utility. Given the large number of genetic variants that occur in cancer genomes and the many low-frequency or nonrecurring mutations detected using NGS, a systematic approach to prioritizing variants is necessary to effectively implement NGS-based precision diagnostics in routine clinical contexts [112]. Molecular pathologists, in collaboration with their oncology colleagues, have been tasked with evaluating this abundance of data, distilling it to what is clinically relevant, and communicating this information in the most cogent, manageable manner possible. Several components are required to properly integrate genomic results into clinical reports, among which is the understanding of the clinical evolution of the genomic variant in patients.

4. Future perspectives on lung cancer treatments

The role of cfDNA has been extensively analyzed in terms of the definition of new-targeted therapies, and the interpretation of this role in driving immunotherapy has just begun [113]. The mutation in a cancer patient can be studied from cfDNA by NGS [114]. Only one study has found conflicting results from the blood tumor mutation burden (TMB) [115]. It has been found that a high blood sample, TMB, is correlated with the reaction to inhibitors of programmed cell death (PD)1 and its ligand (PD-L1) [115, 116], as in NSCLC with atezolizumab in POPLAR and OAK trials [117]. The TMB is more correlated with advanced disease, and it expresses a high value of circulating tumor DNA (ctDNA) concentrations [118]. Different studies have shown that there is a correlation between ctDNA kinetics and clinical course in terms of possibility of predicting the prognosis [119]. In particular, it has been demonstrated that the variation of circulating the tumor DNA burden is able to distinguish a real and unreal tumor progression. Another interesting application of cfDNA, which scientists are studying, is the possibility of detecting the minimal residual disease (MRD) for the setting of immunotherapy or the

possibility of finding the drug resistance as JAK1/2 or B2M mutations [120]. With regard to the early stages' NSCLC, the prospect of setting screening tests is very challenging. The National Lung Screening Trial [121] and the NELSON trial have shown that to test asymptomatic men with high risks factors by chest CT reduced the deaths in men to 26% and in women to 41% [122]. However, the problem of false positives is still one of the most difficult factors to eliminate [123]. These trials showed that the combination of the high sensitivity of CT scans and liquid biopsy may have an important effect in driving clinical decisions, as well as therapeutic approaches. One limitation is the fact that ctDNA quantities may be low or absent in the early stages of disease [124]. Another important value of cfDNA assay may be the opportunity to identify recurrent mutations. This aspect is important in terms of prognosis and developing new targeted treatments. For example, the Cancer SEEK assay can combine the genomic analysis of 16 genes in ctDNA and eight biomarkers detectable for eight non metastatic diseases [125–127]. Nevertheless, certain limitations remain regarding sensitivity to early-stage detection. For instance, lung cancer does not currently have a specific circulating protein marker. The most promising test at the moment is the multi-region exome sequencing of a tumor, but this technique is limited by the costs and the excessive time required, which make this approach currently unavailable to the patients. However, the most discussed approaches developed for circulating tumor cells (CTC) isolation are based on the following: 1) antigen expression and 2) biophysical characteristics [128–130].

In summary, the microfluidic technologies have probably been the most common approach to CTC isolation since 2007, with the "CTC-ship" [131]. However, several limitations are ongoing, and further studies must better stratify this approach not only in the early stages of NSCLC but also for other solid tumors. The world of exosomes is complex because of their vast numbers and various roles. In particular, they were found to contain microRNA (miRNA) that could be exchanged via horizontal intercellular transfer with the possibility of activating an oncogene or a tumor suppressor gene. In 60–75% NSCLC, miRNAs play crucial roles. Moreover, recent studies have provided evidence that exosomes may mediate interactions among different types of cells to enhance cell–cell communication within the tumor microenvironment. In particular, exosome signaling may provide new insights into how cancer stem cells (CSCs) confer drug resistance between drug-resistant and drug-sensitive cells [132]. In fact, CSCs exhibit self-renewal, proliferation, tumor initiation, and propagation, and the "stemness" of cancer cells seems to be supported by the release of exosomes [133, 134, 135]. Cancer stem cells are thought to secrete microvesicles and exosomes that interact with neighboring stromal cells. For instance, experimental evidence has shown that breast cancer stem cells secrete exosomes with characteristics of cancer cell-derived exosomes [135, 136]. Exosomes released by cancer stem cells mediate tumor growth in different cancer types. For example, in a renal cancer model, microvesicles released from human renal cancer stem cells were described to stimulate angiogenesis and the formation of a pre-metastatic niche in the lungs [137]. Elsewhere, a study on glioma stem cells reported that glioma-associated stem cells increased the biological aggressiveness of glioma-initiating cells through the release of exosomes. However, both exosomes and cancer stem cells targeted against tumors must be thoroughly analyzed in the future. This is important because there is no clear identification of a specific target against NSCLC [138, 139] or tumors in general, and it is difficult to characterize cancer stem cells and necessary to optimize the roles and definitions of specific exosomes for each type of cancer. Such research would be a milestone in developing new therapies and new approaches to screening oncologic patients.

IntechOpen

Author details

Beatrice Aramini[1*], Valentina Masciale[1], Federico Banchelli[2], Roberto D'Amico[2], Massimo Dominici[3] and Khawaja Husnain Haider[4]

1 Division of Thoracic Surgery, University of Modena and Reggio Emilia, Modena, Italy

2 Center of Statistic, Department of Medical and Surgical Sciences, University of Modena and Reggio Emilia, Modena, Italy

3 Division of Oncology, Department of Medical and Surgical Sciences, University of Modena and Reggio Emilia, Modena, Italy

4 Department of Basic Sciences, Sulaiman AlRajhi Medical School, Sulaiman Alrajhi University, Kingdom of Saudi Arabia

*Address all correspondence to: beatrice.aramini@unimore.it

IntechOpen

© 2020 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. CC BY

References

[1] Marson FAL, Bertuzzo CS, Ribeiro JD. (2017) Personalized or Precision Medicine? The Example of Cystic Fibrosis. Front Pharmacol.;8:390. Published 2017 Jun 20. doi:10.3389/fphar.2017.00390

[2] Ho D, Quake SR, McCabe ERB, Chng WJ, Chow EK, et al. (2020) Enabling Technologies for Personalized and Precision Medicine. Trends Biotechnol. 38(5):497-518. doi: 10.1016/j.tibtech.2019.12.021.

[3] Goetz LH, Schork NJ. (2018) Personalized medicine: motivation, challenges, and progress. Fertil Steril. 109(6):952-963. doi:10.1016/j.fertnstert.2018.05.006.

[4] Malone ER, Oliva M, Sabatini PJB, Stockley TL, Siu LL. (2020) Molecular profiling for precision cancer therapies. Genome Med. 12(1):8. doi:10.1186/s13073-019-0703-1.

[5] Davis AA, McKee AE, Warren AK, and Villaflor VM. (2018) Complexity of Delivering Precision Medicine: Opportunities and Challenges. American Society of Clinical Oncology Educational Book 38, 998-1007.

[6] Morgensztern D, Campo MJ, Dahlberg SE, Doebele RC, Garon E. et al. (2014) Molecularly targeted therapies in non-small-cell lung cancer annual update 2014. J Thorac Oncol. 2015;10(1 Suppl 1):S1-S63. doi:10.1097/JTO.0000000000000405.

[7] Mambetsariev I, Wang Y, Chen C, Nadaf S, Pharaon R, et al. (2020) Precision medicine and actionable alterations in lung cancer: A single institution experience. PLoS One. 15(2):e0228188. doi: 10.1371/journal.pone.0228188.

[8] Politi K, Herbst RS. Lung cancer in the era of precision medicine. (2015) Clin Cancer Res. 21(10):2213-2220. doi:10.1158/1078-0432.CCR-14-2748.

[9] Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, et al. (2009) Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med361:947-57. doi: 10.1056/NEJMoa0810699.

[10] Shaw AT, Kim DW, Nakagawa K, Seto T, Crino L, et al. (2013) Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. N Engl J Med. 368:2385-94. doi: 10.1056/NEJMoa1214886.

[11] Djalalov S, Beca J, Hoch JS, Krahn M, Tsao MS, et al. (2014) Cost effectiveness of EML4-ALK fusion testing and first-line crizotinib treatment for patients with advanced ALK-positive non-small-cell lung cancer. J Clin Oncol. 32:1012-9. doi: 10.1200/JCO.2013.53.1186.

[12] Santos E, Martin-Zanca D, Reddy EP, Pierotti MA, Della Porta G, et al. (1984) Malignant activation of a K-ras oncogene in lung carcinoma but not in normal tissue of the same patient. Science. 223:661-4. doi: 10.1126/science.6695174.

[13] Rodenhuis S, van de Wetering ML, Mooi WJ, Evers SG, van Zandwijk N, et al. (1987) Mutational activation of the K-ras oncogene. A possible pathogenetic factor in adenocarcinoma of the lung. N Engl J Med. 317:929-35. doi: 10.1056/NEJM198710083171504.

[14] Yang H, Liang SQ, Schmid RA, Peng RW. (2019) New Horizons in KRAS-Mutant Lung Cancer: Dawn After Darkness. Front Oncol. 9:953. doi:10.3389/fonc.2019.00953.

[15] Hallin J, Engstrom LD, Hargis L, Calinisan A, Aranda R, et al. (2020) The KRASG12C Inhibitor MRTX849

Provides Insight toward Therapeutic Susceptibility of KRAS-Mutant Cancers in Mouse Models and Patients. Cancer Discov. 10(1):54-71. doi: 10.1158/2159-8290.CD-19-1167.

[16] Pao W, Girard N. New driver mutations in non-small-cell lung cancer. (2011) Lancet Oncol. 12:175-80. doi: 10.1016/S1470-2045(10)70087-5.

[17] Aramini B, Banchelli F, Bettelli S, Manfredini S, D'Amico R. et al. (2020) Overall survival in patients with lung adenocarcinoma harboring "niche" mutations: an observational study. Oncotarget.11(5):550-559. Published 2020 Feb 4. doi:10.18632/oncotarget.27472.

[18] Lindeman NI, Cagle PT, Beasley MB, Chitale DA, Dacic S. et al. (2013) Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology [published correction appears in J Thorac Oncol. 8(10):1343]. J Thorac Oncol. 2013;8(7):823-859. doi:10.1097/JTO.0b013e318290868f.

[19] Cardarella S, Johnson BE. (2013) The impact of genomic changes on treatment of lung cancer. Am J Respir Crit Care Med. 188(7):770-775. doi:10.1164/rccm.201305-0843PP.

[20] Pennell NA, Arcila ME, Gandara DR, and West H. (2019) Biomarker Testing for Patients With Advanced Non–Small Cell Lung Cancer: Real-World Issues and Tough Choices. American Society of Clinical Oncology Educational Book 39, 531-542.

[21] Ma C, Wei S, Song Y. (2011) T790M and acquired resistance of EGFR TKI: a literature review of clinical reports. J Thorac Dis. 3(1):10-18. doi:10.3978/j.issn.2072-1439.2010.12.02

[22] Shaw AT, Kim DW, Nakagawa K, Seto T, Crinó L. et al. (2013) Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. The New England journal of medicine. 368:2385-2394. doi: 10.1056/NEJMoa1214886.

[23] Kwak EL, Bang YJ, Camidge DR, Shaw AT, Solomon Bet al. (2010) Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. The New England journal of medicine. 363:1693-1703. doi: 10.1056/NEJMoa1006448.

[24] Ghimessy, A., Radeczky, P., Laszlo, V. Hegedus B, Renyi-Vamos F et al. (2020) Current therapy of KRAS-mutant lung cancer. Cancer Metastasis Rev . https://doi.org/10.1007/s10555-020-09903-9.

[25] Janne PA, Shaw AT, Pereira JR, Jeannin G, Vansteenkiste J. et al. (2013) Selumetinib plus docetaxel for KRAS-mutant advanced non-small-cell lung cancer: a randomised, multicentre, placebo-controlled, phase 2 study. The lancet oncology 14:38-47. doi: 10.1016/S1470-2045(12)70489-8.

[26] Temraz S, Mukherji D, Shamseddine A. (2015) Dual Inhibition of MEK and PI3K Pathway in KRAS and BRAF Mutated Colorectal Cancers. Int J Mol Sci. 2015;16(9):22976-22988. doi:10.3390/ijms160922976.

[27] Tainsky MA. Genomic and proteomic biomarkers for cancer: a multitude of opportunities. (2009) Biochim Biophys Acta. 1796(2):176-193. doi:10.1016/j.bbcan.2009.04.004.

[28] Chen ZQ, Huang LS, Zhu B. (2018) Assessment of Seven Clinical Tumor Markers in Diagnosis of Non-Small-Cell Lung Cancer. Dis Markers. 9845123. Published 2018 Dec 11. doi:10.1155/2018/9845123.

[29] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA et al. Global cancer statistics 2018: GLOBOCAN estimates

of incidence and mortality worldwide for 36 cancers in 185 countries. A CANCER J CLIN 2018;68:394-424. doi: 10.3322/caac.21492.

[30] Chang LC, Lim CK, Chang LY, Chen KY, Shih JY, et al. (2019) Non-small cell lung cancer harbouring non-resistant uncommon EGFR mutations: Mutation patterns, effectiveness of epidermal growth factor receptor-tyrosine kinase inhibitors and prognostic factors. Eur J Cancer. 119:77-86. doi: 10.1016/j.ejca.2019.06.025.

[31] Crosbie PA, Shah R, Summers Y, Dive C, Blackhall F. (2013) Prognostic and predictive biomarkers in early stage NSCLC: CTCs and serum/plasma markers. Transl Lung Cancer Res. 2(5):382-397. doi:10.3978/j.issn.2218-6751.2013.09.02.

[32] Golding B, Luu A, Jones R, Viloria-Petit AM. (2018) The function and therapeutic targeting of anaplastic lymphoma kinase (ALK) in non-small cell lung cancer (NSCLC). Mol Cancer.;17(1):52. doi:10.1186/s12943-018-0810-4.

[33] Voena C, Menotti M, Mastini C, Di Giacomo F et al. Efficacy of a Cancer Vaccine against ALK-Rearranged Lung Tumors. (2015) Cancer Immunol Res. 3(12):1333-1343. doi:10.1158/2326-6066.CIR-15-0089.

[34] Sharma P, Allison JP. (2015) Immune checkpoint targeting in cancer therapy: toward combination strategies with curative potential. Cell.161(2):205-214. doi:10.1016/j.cell.2015.03.030.

[35] Farkona S, Diamandis EP, Blasutig IM. (2016) Cancer immunotherapy: the beginning of the end of cancer? BMC Med.;14:73. doi:10.1186/s12916-016-0623-5.

[36] Cottrell TR, Taube JM. (2018) PD-L1 and Emerging Biomarkers in Immune Checkpoint Blockade Therapy. CancerJ.;24(1):41-46. doi:10.1097/PPO.0000000000000301.

[37] Yi M, Jiao D, Xu H, Liu Q, Zhao W. et al. (2018) Biomarkers for predicting efficacy of PD-1/PD-L1 inhibitors. Mol Cancer.;17(1):129. Published 2018 Aug 23. doi:10.1186/s12943-018-0864-3.

[38] Chakravarti N, Prieto VG. (2015) Predictive factors of activity of anti-programmed death-1/programmed death ligand-1 drugs: immunohistochemistry analysis. Transl Lung Cancer Res. 4(6):743-751. doi:10.3978/j.issn.2218-6751.2015.12.10.

[39] Li H, Xu Y, Wan B, Song Y, Zha P. et al. (2019) The clinicopathological and prognostic significance of PD-L1 expression assessed by immunohistochemistry in lung cancer: a meta-analysis of 50 studies with 11,383 patients. Transl Lung Cancer Res.;8(4):429-449. doi:10.21037/tlcr.2019.08.04

[40] Dang TO, Ogunniyi A, Barbee MS, Drilon A. (2016) Pembrolizumab for the treatment of PD-L1 positive advanced or metastatic non-small cell lung cancer. Expert Rev Anticancer Ther. 16(1):13-20. doi:10.1586/14737140.2016.1123626.

[41] Incorvaia L, Fanale D, Badalamenti G, Barraco N, Bono M. et al. (2019) Programmed Death Ligand 1 (PD-L1) as a Predictive Biomarker for Pembrolizumab Therapy in Patients with Advanced Non-Small-Cell Lung Cancer (NSCLC). Adv Ther.;36(10):2600-2617. doi:10.1007/s12325-019-01057-7.

[42] Santini FC, Hellmann MD. (2018) PD-1/PD-L1 Axis in Lung Cancer. Cancer J.;24(1):15-19. doi:10.1097/PPO.0000000000000300.

[43] Davis, A.A., Patel, V.G. (2019) The role of PD-L1 expression as a predictive biomarker: an analysis of

all US Food and Drug Administration (FDA) approvals of immune checkpoint inhibitors. J. Immunotherapy Cancer 7, 278. https://doi.org/10.1186/s40425-019-0768-9.

[44] Onoi K, Chihara Y, Uchino J, Shimamoto T, Morimoto Y. et al. (2020) Immune Checkpoint Inhibitors for Lung Cancer Treatment: A Review. J Clin Med.;9(5):1362. Published 2020 May 6. doi:10.3390/jcm9051362.

[45] Nakajima N, Yoshizawa A, Moriyoshi K, Sonobe M, Menju T. et al. (2019) P40 expression in small cell lung cancer: The presence of p40-positive cells does not always indicate squamous differentiation. Thorac Cancer.;10(5):1188-1192. doi:10.1111/1759-7714.13062.

[46] McLaughlin J, Han G, Schalper KA, Carvajal-Hausdorf D, Pelekanou V. et al. (2016) Quantitative Assessment of the Heterogeneity of PD-L1 Expression in Non-Small-Cell Lung Cancer [published correction appears in JAMA Oncol. Jan;2(1):146]. JAMA Oncol. 2016;2(1):46-54. doi:10.1001/jamaoncol.2015.3638.

[47] Ancevski Hunter K, Socinski MA, Villaruz LC. (2018) PD-L1 Testing in Guiding Patient Selection for PD-1/PD-L1 Inhibitor Therapy in Lung Cancer. Mol Diagn Ther.;22(1):1-10. doi:10.1007/s40291-017-0308-6.

[48] Jürgensmeier JM, Eder JP, Herbst RS. (2014) New strategies in personalized medicine for solid tumors: molecular markers and clinical trial designs. Clin Cancer Res. 20(17):4425-4435. doi:10.1158/1078-0432.CCR-13-0753.

[49] Fogel DB. (2018) Factors associated with clinical trials that fail and opportunities for improving the likelihood of success: A review. Contemp Clin Trials Commun. 11:156-164. doi:10.1016/j.conctc.2018.08.001.

[50] Von Hoff DD, Stephenson JJ, Jr, Rosen P, Loesch DM, Borad MJ et al. Pilot study using molecular profiling of patients' tumors to find potential targets and select treatments for their refractory cancers. J Clin Oncol. 2010;28:4877-4883. doi: 10.1200/JCO.2009.26.5983.

[51] Meric-Bernstam F, Farhangfar C, Mendelsohn J, Mills GB. (2013) Building a personalized medicine infrastructure at a major cancer center. J Clin Oncol. 31:1849-1857. doi: 10.1200/JCO.2012.45.3043.

[52] Wang Y, Wang Y, Wang Y, Zhang Y. (2020) Identification of prognostic signature of non–small cell lung cancer based on TCGA methylation data. Scientific Reports 10:8575. doi: 10.1038/s41598-020-65479-y.

[53] Wistuba II, Behrens C, Lombardi F, Wagner S, Fujimoto J, et al. (2013) Validation of a Proliferation-Based Expression Signature as Prognostic Marker in Early Stage Lung Adenocarcinoma. Clin Cancer Res 19(22); 6261-71. doi: 10.1158/1078-0432.CCR-13-0596.

[54] Bueno R, Hughes E, Wagner S, Gutin AS, Lanchbury JS et al. (2015) Validation of a Molecular and Pathological Model for Five-Year Mortality Risk in Patients with Early Stage Lung Adenocarcinoma. J. Thorac Oncol. 10: 67-73. doi: 10.1097/JTO.0000000000000365.

[55] Cuzick J, Swanson GP, Fisher G, Brothman AR, Berney DM et al. (2011) Prognostic value of an RNA expression signature derived from cell cycle proliferation genes for recurrence and death from prostate cancer: A retrospective study in two cohorts. Lancet Oncol. 12:245-55. doi: 10.1016/S1470-2045(10)70295-3.

[56] Wirapati P, Sotiriou C, Kunkel S, Farmer P, Pradervand Set al. (2008)

Meta-analysis of gene expression profiles in breast cancer: toward a unified understanding of breast cancer subtyping and prognosis signatures. Breast Cancer Res 10, R65. doi: 10.1186/bcr2124.

[57] Aramini B, Casali C, Stefani A, Bettelli S, Wagner S et al. (2016) Prediction of distant recurrence in resected stage I and II lung adenocarcinoma. Lung Cancer 101 82-87. doi: 10.1016/j.lungcan.2016.09.005.

[58] Kim ES, Herbst RS, Wistuba II, Lee JJ, Blumenschein GR et al. (2011) The BATTLE trial: personalizing therapy for lung cancer. Cancer Discov. 1:44-53. doi: 10.1158/2159-8274.CD-10-0010.

[59] Tran B, Dancey JE, Kamel-Reid S, McPherson JD, Bedard PL et al. Cancer genomics: technology, discovery, and translation. J Clin Oncol. 2012;30:647-660. doi: 10.1200/JCO.2011.39.2316.

[60] Le Tourneau C, Kamal M, Tredan O, Delord JP, Campone M et al. (2012) Designs and challenges for personalized medicine studies in oncology: focus on the SHIVA trial. Target Oncol. 7:253-265. doi: 10.1007/s11523-012-0237-6.

[61] Tsimberidou AM, Iskander NG, Hong DS, Wheler JJ, Falchook GS et al. (2012) Personalized medicine in a phase I clinical trials program: the MD Anderson Cancer Center initiative. Clin Cancer Res. 18:6373-6383. doi: 10.1158/1078-0432.CCR-12-1627.

[62] Arnedos M, Andre F, Farace F, Lacroix L, Besse B et al. (2012) The challenge to bring personalized cancer medicine from clinical trials into routine clinical practice: the case of the Institut Gustave Roussy. Mol Oncol. 6:204-210. doi: 10.1016/j.molonc.2012.02.008.

[63] ClinicalTrials.gov. Novartis Pharmaceuticals, NCT01831726, NCT 01833169, NCT 01885195, NCT 01981187, NCT 02002689. ClinicalTrials.gov.

[64] Conley BA. Precision Cancer Medicine; Exceptional Responders; NCI-MATCH. http://deainfo.nci.nih.gov/advisory/ctac/1113/PrecisionCancerMedicine.pdf.

[65] Belin L, Kamal M, Mauborgne C, Plancher C, Mulot F, et al. (2017) Randomized phase II trial comparing molecularly targeted therapy based on tumor molecular profiling versus conventional therapy in patients with refractory cancer: cross-over analysis from the SHIVA trial. Ann Oncol. 28(3):590-596. doi: 10.1093/annonc/mdw666.

[66] Burrell RA, McGranahan N, Bartek J, Swanton C. (2013) The causes and consequences of genetic heterogeneity in cancer evolution. Nature.501:338-345. doi: 10.1038/nature12625.

[67] Awada A, Aftimos PG. (2013) Targeted therapies of solid cancers: new options, new challenges. Curr Opin Oncol.25:296-304. doi: 10.1097/CCO.0b013e32835ff318.

[68] Gerlinger M, Rowan AJ, Horswell S, Math M, Larkin J et al. (2012) Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med. 366:883-892. doi: 10.1056/NEJMoa1113205.

[69] Yachida S, Jones S, Bozic I, Antal T, Leary R et al. (2010) Distant metastasis occurs late during the genetic evolution of pancreatic cancer. Nature. 467:1114-1117. doi: 10.1038/nature09515.

[70] Vakiani E, Janakiraman M, Shen R, Sinha R, Zeng Z et al. (2012) Comparative genomic analysis of primary versus metastatic colorectal carcinomas. J Clin Oncol. 30:2956-2962. doi: 10.1200/JCO.2011.38.2994.

[71] Jakobsen JN, Sorensen JB. (2012) Intratumor heterogeneity and chemotherapy-induced changes in EGFR status in non-small cell lung cancer. Cancer Chemother Pharmacol. 69:289-299. doi: 10.1007/s00280-011-1791-9.

[72] Narayan A, Carriero NJ, Gettinger SN, Kluytenaar J, Kozak KR et al. (2012) Ultrasensitive measurement of hotspot mutations in tumor DNA in blood using error-suppressed multiplexed deep sequencing. Cancer Res. 72:3492-3498. doi: 10.1158/0008-5472.CAN-11-4037.

[73] Perkins G, Yap TA, Pope L, Cassidy AM, Dukes JP et al. (2012) Multi-purpose utility of circulating plasma DNA testing in patients with advanced cancers. PLoS One. 7:e47020. doi: 10.1371/journal.pone.0047020.

[74] Yu M, Stott S, Toner M, Maheswaran S, Haber DA . Circulating tumor cells: approaches to isolation and characterization. (2011) J Cell Biol. 2011;192:373-382. doi: 10.1083/jcb.201010021.

[75] Ebeling FG, Stieber P, Untch M, Nagel D, Konecny GE et al. (2002) Serum CEA and CA 15-3 as prognostic factors in primary breast cancer. Br J Cancer. 86:1217-1222. doi: 10.1038/sj.bjc.6600248.

[76] Okamoto T, Nakamura T, Ikeda J, Maruyama R, Shoji F, et al. (2005) Serum carcinoembryonic antigen as a predictive marker for sensitivity to gefitinib in advanced non-small cell lung cancer. Eur J Cancer 41:1286-1290. doi: 10.1016/j.ejca.2005.03.011.

[77] Jürgensmeier JM, Schmoll HJ, Robertson JD, Brooks L, Taboada M et al. (2013) Prognostic and predictive value of VEGF, sVEGFR-2 and CEA in mCRC studies comparing cediranib, bevacizumab and chemotherapy. Br J Cancer108:1316-1323. doi: 10.1038/bjc.2013.79.

[78] Politi K, Herbst RS. (2015) Lung cancer in the era of precision medicine. Clin Cancer Res. 21(10):2213-2220. doi:10.1158/1078-0432.CCR-14-2748.

[79] Yeung CC, Egan D, & Radich JP. (2016) Molecular monitoring of chronic myeloid leukemia: present and future. Expert review of molecular diagnostics, 16(10), 1083-1091. doi: 10.1080/14737159.2016.1227243.

[80] Principale- Gregg JP, Li T, & Yoneda KY. (2019). Molecular testing strategies in non-small cell lung cancer: optimizing the diagnostic journey. Translational lung cancer research, 8(3), 286-301. doi: 10.21037/tlcr.2019.04.14.

[81] Lindeman NI, Cagle PT, Aisner DL, Arcila ME, Beasley MB et al. (2018) Updated molecular testing guideline for the selection of lung cancer patients for treatment with targeted tyrosine kinase inhibitors: guideline from the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology. Arch Pathol Lab Med 142:321-46.

[82] Cooper W, Fox S, O'Toole S, Morey A, Frances G et al. (2014) National Working Group Meeting on ALK diagnostics in lung cancer. Asia Pac J Clin Oncol. 10(suppl):211-217. doi: 10.1111/ajco.12190. doi: 10.1111/ajco.12190.

[83] Dietel M, Bubendorf L, Dingemans AM, Dooms C, Elmberger G et al. (2016) Diagnostic procedures for non-small-cell lung cancer (NSCLC): recommendations of the European Expert Group. Thorax. 71(2):177-184. doi: 10.1136/thoraxjnl-2014-206677.

[84] Duffy MJ, Sturgeon CM, Soletormos G, Barak V, Molina R et al. (2015) Validation of new cancer biomarkers: a position statement from the European group on tumor markers.

Clin Chem. 61(6):809-820. doi: 10.1373/clinchem.2015.239863.

[85] Garcia-Campelo R, Bernabe R, Cobo M, Corral J, Coves J et al. (2015) SEOM clinical guidelines for the treatment of non-small cell lung cancer (NSCLC) 2015. Clin Transl Oncol.;17(12):1020-1029. doi: 10.1007/s12094-015-1455-z.

[86] Gridelli C, Balducci L, Ciardiello F, Di Maio M, Felip E et al. (2015) Treatment of elderly patients with non-small-cell lung cancer: results of an International Expert Panel Meeting of the Italian Association of Thoracic Oncology. Clin Lung Cancer. 16(5):325-333. doi: 10.1016/j.cllc.2015.02.006.

[87] Joseph L, Cankovic M, Caughron S, Chandra P, Emmadi R et al. (2016) The spectrum of clinical utilities in molecular pathology testing procedures for inherited conditions and cancer: a report of the Association for Molecular Pathology. J Mol Diagn. 18(5):605-619. doi: 10.1016/j.jmoldx.2016.05.007.

[88] Kerr KM, Bubendorf L, Edelman MJ, Marchetti A, Mok T et al. (2014) Second ESMO consensus conference on lung cancer: pathology and molecular biomarkers for non-smallcell lung cancer. Ann Oncol. 25(9):1681-1690. doi: 10.1093/annonc/mdu145.

[89] Monso E, Montuenga LM, Sanchez de Cos J, Villena C; Lung Cancer CIBERES-RTICC-SEPAR-Plataforma Biobanco Pulmonar. Biological marker analysis as part of the CIBERES-RTIC Cancer-SEPAR Strategic Project on Lung Cancer. Arch Bronconeumol. 2015;51(9):462-467. doi: 10.1016/j.arbres.2014.11.010

[90] Popper HH, Gruber-Mosenbacher U, Hutarew G, Luka Brcic L, Malgorzata Szolkowska M et al. (2016) Recommendations of the Austrian Working Group on Pulmonary Pathology and Oncology for predictive molecular and immunohistochemical testing in non-small cell lung cancer. Memo. 9(4):191-200. doi: 10.21037/tlcr.2020.04.07.

[91] Sepulveda AR, Hamilton SR, Allegra CJ, Grody W, Cushman-Vokoun AM et al. (2017) Molecular biomarkers for the evaluation of colorectal cancer: guideline from the American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology, and the American Society of Clinical Oncology. J Clin Oncol. 35(13):1453-1486. doi: 10.1200/JCO.2016.71.9807.

[92] van der Heijden EH, Casal RF, Trisolini R, Steinfort DP, Hwangbo B, et al. (2014) Guideline for the acquisition and preparation of conventional and endobronchial ultrasoundguided transbronchial needle aspiration specimens for the diagnosis and molecular testing of patients with known or suspected lung cancer. Respiration. 88(6):500-517. doi: 10.1159/000368857.

[93] Villar Alvarez F, Muguruza Trueba I, Belda Sanchis J, Rodríguez Suárez PM, de Cos Escuín JS et al. (2016) Executive summary of the SEPAR recommendations for the diagnosis and treatment of nonsmall cell lung cancer. Arch Bronconeumol. 52(7):378-388. doi: 10.1016/j.arbr.2016.02.020

[94] von Laffert M, Schirmacher P, Warth A, Büttner R, Huber RM et al. (2017) ALK-testing in non-small cell lung cancer (NSCLC): immunohistochemistry (IHC) and/or fluorescence in-situ hybridisation (FISH)?: statement of the Germany Society for Pathology (DGP) and the Working Group Thoracic Oncology (AIO) of the German Cancer Society e.V. (Stellungnahme der Deutschen Gesellschaft fur Pathologie und der AG Thorakale Onkologie der

Arbeitsgemeinschaft Onkologie/ Deutsche Krebsgesellschaft e.V.). Lung Cancer103:1-5. doi: 10.1016/j.lungcan.2016.11.008.

[95] Scottish Intercollegiate Guidelines Network (SIGN). Management of lung cancer. Edinburgh: SIGN; 2014. SIGN publication no. 137. http://www.sign.ac.uk. Accessed June 12, 2017.

[96] Yale University School of Medicine. GuideLines Into DEcision Support (GLIDES). http://medicine.yale.edu/cmi/glides/index.aspx. Accessed June 21, 2017.

[97] van El CG, Cornel MC, Borry P, Hastings RJ, Fellmann F, et al., & ESHG Public and Professional Policy Committee (2013). Whole-genome sequencing in health care. Recommendations of the European Society of Human Genetics. European journal of human genetics : EJHG, 21 Suppl 1(Suppl 1), S1–S5. doi: 10.1038/ejhg.2013.46.

[98] Nakagawa H, & Fujita M. (2018). Whole genome sequencing analysis for cancer genomics and precision medicine. Cancer science, 109(3), 513-522. doi: 10.1111/cas.13505.

[99] O'Brien TD, Jia P, Xia J, Saxena U, Jin H, et al. (2015) Inconsistency and features of single nucleotide variants detected in whole exome sequencing versus transcriptome sequencing: A case study in lung cancer. Methods. 2015 Jul 15;83:118-27. doi: 10.1016/j.ymeth.2015.04.016.

[100] Seo JS, Ju YS, Lee WC, Shin JY, Lee JK, et al. (2012) The transcriptional landscape and mutational profile of lung adenocarcinoma. Genome Res. 22:2109-2119. doi: 10.1101/gr.145144.112.

[101] Horak P, Fröhling S, & Glimm H. (2016). Integrating next-generation sequencing into clinical oncology: strategies, promises and pitfalls. ESMO open, 1(5), e000094. doi: 10.1136/esmoopen-2016-000094.

[102] Jeromin S, Haferlach T, Weissmann S, Meggendorfer M, Eder C et al. (2015) Refractory anemia with ring sideroblasts and marked thrombocytosis cases harbor mutations in SF3B1 or other spliceosome genes accompanied by JAK2V617F and ASXL1 mutations. Haematologica. 100:e125-127. doi: 10.3324/haematol.2014.119032.

[103] Malcovati L, Karimi M, Papaemmanuil E, Ambaglio I, Jädersten M et al. (2015) SF3B1 mutation identifies a distinct subset of myelodysplastic syndrome with ring sideroblasts. Blood. 126:233-241. doi: 10.1182/blood-2015-03-633537.

[104] Ding L, Ley TJ , Larson DE, Miller CA, Koboldt DC, et al. (2012) Clonal evolution in relapsed acute myeloid leukaemia revealed by whole-genome sequencing. Nature. 481:506-510. doi: 10.1038/nature10738.

[105] Garg M, Nagata Y, Deepika Kanojia D, Mayakonda A, Yoshida K et al. (2015) Profiling of somatic mutations in acute myeloid leukemia with FLT3-ITD at diagnosis and relapse. Blood. 126:2491-2501. doi: 10.1182/blood-2015-05-646240.

[106] Jan M, Snyder TM, Corces-Zimmerman MR, Vyas P, Weissman IL, et al. (2012) Clonal evolution of preleukemic hematopoietic stem cells precedes human acute myeloid leukemia. Science translational medicine. 4:149ra118. doi: 10.1126/scitranslmed.3004315.

[107] Shlush LI, Zandi S, Mitchell A, Chen WC, Brandwein JM, et al. (2014) Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. Nature 506:328-333. doi: 10.1038/nature13038.

[108] Klco JM, Miller CA, Griffith M, Petti A, Spencer DH et al. (2015) Association Between Mutation Clearance After Induction Therapy and Outcomes in Acute Myeloid Leukemia. Jama. 314:811-822. doi: 10.1001/jama.2015.9643.

[109] Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SA, Behjati S et al. (2013) Signatures of mutational processes in human cancer. Nature. 500:415-421. doi: 10.1038/nature12477.

[110] Greenman C, Stephens P, Smith R, Dalgliesh GL, Hunter C, et al. (2007) Patterns of somatic mutation in human cancer genomes. Nature. 446:153-158. doi: 10.1038/nature05610.

[111] Conway JR, Warner JL, Rubinstein WS, and Miller RS (2019). Next-Generation Sequencing and the Clinical Oncology Workflow: Data Challenges, Proposed Solutions, and a Call to Action. JCO Precision Oncology 3, 1-10. doi: 10.1200/PO.19.00232.

[112] Shyr, D., & Liu, Q. (2013). Next generation sequencing in cancer research and clinical application. Biological procedures online, 15(1), 4. doi: 10.1186/1480-9222-15-4.

[113] Fabrizio D, Lieber D, Malboeuf C, Silterra J , White E. et al. (2018) Abstract 5706: a blood-based next-generation sequencing assay to determine tumor mutational burden (bTMB) is associated with benefit to an anti-PD-L1 inhibitor, atezolizumab. Cancer Res 78: 13 Suppl., 5706-5717. doi: 10.1158/1538-7445.AM2018-5706.

[114] Koeppel F, Blanchard S, Jovelet C, Genin B, Marcaillou C et al. (2017) Whole exome sequencing for determination of tumor mutation load in liquid biopsy from advanced cancer patients. PLoS One 12: e0188174. doi:10.1371/journal.pone.0188174.

[115] Khagi Y, Goodman AM, Daniels GA, Patel SP, Sacco AG et al. (2017) Hypermutated circulating tumor DNA: correlation with response to checkpoint inhibitor-based immunotherapy. Clin Cancer Res 23: 5729-5736. doi:10.1158/1078-0432.CCR-17-1439.

[116] Chan TA, Yarchoan M, Jaffee E, Swanton C, Quezada SA et al. (2019) Development of tumor mutation burden as an immunotherapy biomarker: utility for the oncology clinic. Ann Oncol. 30(1):44-56. doi:10.1093/annonc/mdy495.

[117] Chalabi M, Cardona A, Nagarkar DR, Dhawahir Scala A, Gandara DR, et al. (2020) imCORE working group of early career investigators. Efficacy of chemotherapy and atezolizumab in patients with non-small-cell lung cancer receiving antibiotics and proton pump inhibitors: pooled post hoc analyses of the OAK and POPLAR trials. Ann Oncol.31(4):525-531. doi: 10.1016/j.annonc.2020.01.006.

[118] Yang N, Li Y, Liu Z Du D, Cao Xet al. (2018). The characteristics of ctDNA reveal the high complexity in matching the corresponding tumor tissues. BMC Cancer 18, 319. doi: 10.1186/s12885-018-4199-7.

[119] Guibert N, Mazieres J, Delaunay M, Casanova A, Farella M et al. (2017) Monitoring of KRAS-mutated ctDNA to discriminate pseudo-progression from true progression during anti-PD-1 treatment of lung adenocarcinoma. Oncotarget 8: 38056-38060. doi: 10.18632/oncotarget.16935.

[120] Cabel L, Proudhon C, Romano E, Girard N, Lantz O et al. (2018) Clinical potential of circulating tumour DNA in patients receiving anticancer immunotherapy. Nat Rev Clin Oncol 15: 639-650. doi: 10.1038/s41571-018-0074-3.

[121] Aberle DR, Adams AM, Berg CD, Black WC, Clapp JD et al. (2011) Reduced lung-cancer mortality with low-dose computed tomographic screening. National Lung Screening Trial Research Team, Aberle DR, Adams AM, et al. N Engl J Med 365: 395-409. doi: 10.1056/NEJMoa1102873.

[122] De Koning H, Van Der Aalst C, Ten Haaf K, M. Oudkerk (2018) PL02.05 Effects of volume CT lung cancer screening: mortality results of the NELSON randomised-controlled population-based trial. J Thorac Oncol ; 13: Suppl., S185. dOI:https://doi.org/10.1016/j.jtho.2018.08.012.

[123] Guibert N, Mazieres J, Delaunay M, Casanova A, Farella M et al. (2017) Monitoring of KRAS-mutated ctDNA to discriminate pseudo-progression from true progression during anti-PD-1 treatment of lung adenocarcinoma. Oncotarget 8: 38056-38060. doi:10.18632/oncotarget.16935.

[124] Cohen JD, Li L, Wang Y, Thoburn C, Afsari B, et al. (2018) Detection and localization of surgically resectable cancers with a multi-analyte blood test. Science 359: 926-930. doi:10.1126/science.aar3247.

[125] Jamal-Hanjani M, Wilson GA, McGranahan N, Birkbak NJ, Watkins TBK et al. (2017) Tracking the evolution of non-small-cell lung cancer. N Engl J Med 376: 2109-2121. doi:10.1056/NEJMoa1616288.

[126] Abbosh C, Birkbak NJ, Wilson GA, Jamal-Hanjani M, Constantin T et al. (2017) Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution. Nature 545: 446-451. doi:10.1038/nature22364.

[127] Ulrich BC, Guibert N. (2017) Towards a comprehensive framework for cell-free DNA analysis: lessons from TRACERx. Ann Transl Med 5: 428. doi:10.21037/atm.2017.08.12.

[128] Oxnard GR, Paweletz CP, Kuang Y, Mach SL, O'Connell A, et al. (2014) Noninvasive detection of response and resistance in EGFR-mutant lung cancer using quantitative next-generation genotyping of cell-free plasma DNA. Clin Cancer Res 20: 1698-1705. doi:10.1158/1078-0432.CCR-13-2482

[129] Sacher AG, Alden RS, Oxnard GR. (2016) Early intervention in lung cancers with rapid plasma genotyping for EGFR and KRAS mutations-reply. JAMA Oncol. 2: 1096-1097. doi:10.1001/jamaoncol.2016.1963

[130] Yanagita M, Redig AJ, Paweletz CP, Dahlberg SE, O'Connell A, et al. (2016) A prospective evaluation of circulating tumor cells and cell-free DNA in EGFR-mutant non-small cell lung cancer patients treated with erlotinib on a phase II trial. Clin Cancer Res 22: 6010-6020. doi:10.1158/1078-0432.CCR-16-0909.

[131] Nagrath S, Sequist LV, Maheswaran S, Bell DW, Irimia D et al. (2007) Isolation of rare circulating tumour cells in cancer patients by microchip technology. Nature 450: 1235-1239. doi:10.1038/nature06385.

[132] Yang Z, Zhao N, Cui J, Wu H, Xiong J, et al. (2020) Exosomes derived from cancer stem cells of gemcitabine-resistant pancreatic cancer cells enhance drug resistance by delivering miR-210. Cell Oncol (Dordr). 43(1):123-136. doi: 10.1007/s13402-019-00476-6.

[133] Adorno-Cruz V, Kibria G, Liu X, Doherty M, Junk DJ, et al. (2015) Cancer stem cells: targeting the roots of cancer, seeds of metastasis, and sources of therapy resistance. Cancer Res. 15;75(6):924-929. doi: 10.1158/0008-5472.CAN-14-3225.

[134] Gernapudi R, Yao Y, Zhang Y, Wolfson B, Roy S, et al. (2015) Targeting exosomes from preadipocytes inhibits preadipocyte to cancer stem

cell signaling in early-stage breast cancer. Breast Cancer Res Treat. 2015 Apr;150(3):685-95. doi: 10.1007/s10549-015-3326-2.

[135] Aramini B, Masciale V, Haider KH. (2020) Defining lung cancer stem cells exosomal payload of miRNAs in clinical perspective. World J Stem Cells.;12(6):406-421. doi:10.4252/wjsc.v12.i6.406.

[136] Kumar D, Gupta D, Shankar S, Srivastava RK. (2015) Biomolecular characterization of exosomes released from cancer stem cells: Possible implications for biomarker and treatment of cancer. Oncotarget. 20;6(5):3280-91. doi: 10.18632/oncotarget.2462.

[137] Grange C, Tapparo M, Collino F, Vitillo L, Damasco C, et al. (2011) Microvesicles released from human renal cancer stem cells stimulate angiogenesis and formation of lung premetastatic niche. Cancer Res. 71(15):5346-56. doi: 10.1158/0008-5472.CAN-11-0241.

[138] Masciale V, Grisendi G, Banchelli F, D'Amico R, Maiorana A et al. (2019) Isolation and Identification of Cancer Stem-Like Cells in Adenocarcinoma and Squamous Cell Carcinoma of the Lung: A Pilot Study. Front Oncol. 9:1394. Published 2019 Dec 18. doi:10.3389/fonc.2019.01394.

[139] Masciale V, Grisendi G, Banchelli F, D'Amico R, Maiorana A et al. (2020) CD44+/EPCAM+ cells detect a subpopulation of ALDHhigh cells in human non-small cell lung cancer: A chance for targeting cancer stem cells? Oncotarget 11(17):1545-1555. Published 2020 Apr 28. doi:10.18632/oncotarget.27568.