Diagnostics for Targeted NSCLC Therapy

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Abstract: Despite an increasing number of molecular biomarkers identified in non-small cell lung cancer (NSCLC), the number of approved therapy options targeting these biomarkers remains limited. Although some biomarkers may influence the therapy outcome of a distinct drug and have been shown to be useful in phase 2 or 3 clinical studies, diagnostics of biomarkers without an approved drug available or a possible off-label use is currently too expensive for routine diagnostics in non-academic institutions. For this reason, the present review is intended to summarize the current state of the art of molecular diagnostics that is both available and could lead to therapy guidance in NSCLC courses. Thereby, economic aspects are taken into account in order to take up the cudgels for a more comprehensive, even if more expensive, diagnostic scheme that in turn may save enormous costs by reducing therapy costs.

Keywords: lung cancer; NSCLC; diagnostics

1. Introduction

In Germany, lung carcinomas are the most frequent cancer entity in male patients and are among the top 5 cancer entities in female patients (Deutsches Krebsforschungszentrum, Häufigste Krebserkrankungen, available online: https://www.dkfz.de/de/krebsatlas/gesamt/mort_6.html, accessed November 2017), which is consistent with the worldwide cancer mortality statistics (http://www-dep.iarc.fr/WHOdb/WHOdb.htm). The knowledge on this medical and economic burden led to the development of an increasing number of targeted therapies—most of them being tyrosine-kinase inhibitor therapies—that in contrast to chemotherapies have been shown to be more specific and more efficient in distinct tumors, while showing fewer side effects. These approaches linking diagnostic and targeted treatment belong to personalized or precision medicine and require more sophisticated and complex procedures to evaluate the molecular situation in the patient’s tumor. The established therapy approaches are frequently restricted to small patient groups that may benefit from the respective treatments, as genetic patterns of a tumor may predict therapy success or failure.

It is important to know in this context to keep in mind the basic concepts that explain the stepwise development of a tumor. Tumors are characterized by an unrestricted growth that is frequently based on a single gene that is falsely expressed, the so-called oncogene. This phenomenon is known as oncogene addition. In addition, also not being truly defined, genes that may contribute to the development of cancers or are pathologically regulated in or associated with cancers are called driver genes. Within these genes, or also in other genetic or epigenetic factors, there are the so-called driver mutations, i.e., changes in the DNA and/or protein sequences ranging from copy number variation via point mutations/single nucleotide variations to insertions and deletions, i.e., any change in the DNA that may be associated with a loss of function, pathological effects, therapy resistance, or any other relevant clinical or molecular entity.
This increasing fragmentation of tumor classes into molecular subgroups independent of the previously determined histo-morphological subtype complicates the diagnostic algorithms and result interpretation. This is due to the fact that besides pathogenic mutations also benign mutations without or with a hitherto unknown impact may occur. Thereby, pathogenic mutations are those mutations that to our present knowledge have an effect on the disease and thus could be also named as driver mutations, whereas the benign mutations are silent to date and have not yet been associated with any effect on the disease today. For this reason, it remains to be investigated to what extent mutations influence each other by synergistic or compensatory mechanisms, as observed in some viral infections (HIV and HBV) [1–6].

On the other hand, the economic burden of precision medicine starts to explode with therapy costs of up to 10,000 € per patient and month exclusively for a single drug, other costs not yet included. Consequently, it is required to identify patients eligible for an available therapy more precisely while for the non-eligible patient alternative therapies or optimized palliative care should be implemented.

2. Proposal

2.1. Prerequisites

The start of the precision medicine era in cancer appears to be accompanied by an explosion of costs. These do not only result from increasing challenges in direct patient care but rather from development expenses for novel drug compounds and the pricing policy for novel drugs, from the respective obligate companion diagnostics, as well as from increasing costs for bio-informatics’ analyses of therapy relevant data. Thereby, the companion diagnostics induce solely a fraction amount of the overall costs, although they are most accused cost factors e.g., by health insurance systems.

Besides the development of drug resistances, the increasing number of novel drugs related to new companion diagnostic assays complicates routine diagnostics, as different methods not directly comparable are approved for one biomarker. Although there is still a limited number of therapy relevant mutations including point mutations, copy number variations, translocations and fusions, insertions and deletions [7,8], several studies test for virtually all mutations known to be associated with cancers. This includes for example the tumor mutational burden (TMB), which was shown to be associated with a better outcome of immunotherapies, although the biological mechanism is not yet known [9]. As long as the underlying biological significance of the TMB is not assessed, it is rather a parameter for statistical correlations than a diagnostic biomarker. And even with regard to the established markers relevant for therapy decisions recent investigations in European NSCLC non-clinical study cohorts revealed that data differs from clinical studies [10–12]. This leads to an imbalance in patient care and suggests hope for re-convalescence even in those cancers that are solely eligible for palliative care yet [13].

Finally, it has to be kept in mind that tumors are not homogenous clones of a single cell origin with identical genetic background, but instead are constantly evolving [14]. Consequently, the tumor most often is a melting pot of several cell lines with different mutation patterns, which in turn are subjected to the Darwinian evolutionary theory in case of cancer treatments. NSCLC molecular diagnostics is not an emergency diagnostics in the majority of cases. The doubling time of NSCLC cells takes significantly longer than 24–72 h [15,16], thus it would be desirable to invest some more time in a more expensive laboratory diagnostic approach. In turn that may reduce therapy costs in a remarkable manner by more reliable and predictive test results. In any case, molecular subtyping of tumors has to be performed in all cases of recurrence, progression, or development of resistances and these data have also be included in future prediction models.

2.2. Current Diagnostic Algorithm

Today it is standard to characterize the NSCLC by histopathological methods including macroscopy, classical stainings, immunohistochemistry, and microscopic investigation. This basic
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approach is and in future will also be essential to initially define a tumor tissue. Following the microscopic investigation, molecular analyses are requested in order to make therapy decisions, as besides chemotherapeutic regimens also personalized therapies such as tyrosine kinase inhibitors and immunotherapies can be used to treat NSCLC, meanwhile both in first and second line treatments [17–23]. Thereby it is important to note that, e.g., ALK and PD-L1 immunostainings also belong to the companion diagnostic assays. In addition, making therapeutic decisions for molecular targeted drugs do not totally depend on molecular analyses: For example, lung adenocarcinoma with a specific genetic alteration shows characteristic histology; i.e., genetic alteration-histology association; some EGFR-mutated adenocarcinoma are histologically characterized by lepidic component a micropapillary pattern; ALK-rearranged adenocarcinoma are sometimes characterized by acinar structure with mucin as well as young onset and never/light smoking history. To sum this up, although in principle every molecular subtype is possible in each histological type, the histology vice versa may predict the most likely alteration and thus characteristic histologies as well as clinical features may also determine possible candidates for molecular targeted therapies.

The molecular analyses are based on either qPCRs, Sanger-sequencing, pyrosequencing, hybridization methods, or next generation sequencing approaches that are performed either in parallel or stepwise according to the probability to which extent and on which level genes related to cancer pathways are most frequently mutated. Thereby it is important to know that all these methods have different sensitivities depending on the respective pathogenic mutation with limits of detection between 25% (Sanger sequencing) and less than 0.1% (ultra-deep sequencing). In this respect, it should be noted that there is limited information on the clinical relevance of low level mutations with a frequency nearby the detection limit. In addition to these nucleic acid analysis based methods, further markers such as ALK, ROS, RET, cMET and others are analyzed by fluorescence in situ hybridization and/or immunohistochemistry, mostly if the previous analyses revealed no therapy relevant mutations. However, the latter is also country specific and an international harmonization with consent guidelines for diagnostics and therapy would be desirable.

2.3. Future Diagnostic Algorithm

Meanwhile, next generation sequencing has become an economically acceptable tool in molecular pathology laboratories, and several assays have been developed and launched by commercial providers, thus ensuring a broad spread of the technology. For example, the novel GeneReader platform from Qiagen (Hilden, Germany) enables the simultaneous diagnostics of different therapy relevant genes now, and will be able to detect all-embracing SNVs, CNV, InDels, and fusions/translocations relevant in NSCLC. This technology as well as comparable assays from other companies such as Illumina or IonTorrent as well as further NGS platforms could deliver the datasets for a machine learning database. Such a database should be ideally implemented in a multicenter, worldwide, international manner and should also include ethnical aspects, as ethnical differences in the tumor biology are likely to occur, but no true borders exist for patients, e.g., Asian patients are rare in Europe but occur, and vice versa.

It will take a two-step approach to implement an optimized diagnostic algorithm based on optimal patient care with broad access to appropriate diagnostic methods also beyond some specialized academic centers. At first it is crucial that as much data as possible (age of the patient, tumor type, histologic pattern, pathogenic and benign mutations of the NSCLC, treatment, data on disease progress) from all qualified diagnostic laboratories is systematically collected in order to build a database which will allow to identify molecular subtypes of tumors/tumor mutation patterns and their likely response to a distinct treatment. Such approaches have been successfully performed in other disciplines. The Geno2pheno project, which aims to develop tools for the prediction of the outcome of antiviral therapies, is based on a machine learning database that is able to predict drug resistances based on sequence information. Meanwhile the system has learned which mutations and combinations of mutation lead to drug resistances and which mutation patterns may be compensatory or act synergistically on the treatment outcome [1–6]. The database originates from
the learning process derived from clinical observation correlated with sequencing data and phenotypic analyses. Such an approach could and should also be performed for NSCLC as well as for other cancer entities, and should be done on a broad basis that goes beyond a simple multicenter study.

However, because of the data needed for machine learning, this approach means that the individual patient will be over-diagnosed, because besides the detection of well-known driver or resistance mutations further data is generated by NGS that up to now due to lack of data does not play any role for therapy decisions. Currently, this kind of over-diagnoses is avoided as strictly as possible, as there is no standard re-imbursement through the health care system. But focusing on ALK and the TKI Crizotinib as an example, it could be shown, that the over-diagnoses of today result in optimized therapies in future, which will enable us to identify those tumors/patients that will indeed be eligible for a distinct therapy [24].

In a second step towards an optimized diagnostic approach in the future specialized academic institutions have to perform the according phenotyping assays by generating cell lines with detected mutations and mutation patterns and to test these cell lines for susceptibilities and resistances against available drugs. As some tumor cell types have a suboptimal growth characteristics, the cell culture approach should be complemented by cancer xenograft approaches in mice. This procedure is the essential step to a full understanding of the clinical relevance of mutation patterns in NSCLC and other tumor entities. In addition, the TMB should also be subjected to phenotypic analyses before it is hyped as a novel biomarker especially against the background of the ethnic diversity of patients [25]. These challenges should have the same priority as the implementation and improvement of liquid biopsies as a reimbursed diagnostic tool for those cases in which a biopsy is not possible.

In conclusion, this comprehensive diagnostic approach of generating and collecting NGS data accompanied by clinical data and the respective phenotypic datasets to develop machine learning systems, will lead to a tool that will be able to predict the therapy outcome more precisely. This, in turn, will save costs for expensive drugs, which could have been ineffective in a given tumor, and will provide a more realistic assessment of the patient’s situation.

3. Discussion

Currently, it seems that molecular diagnostics of NSCLC becomes an increasing and cost expensive issue in routine diagnostic. But although the costs for molecular diagnostics have increased and still rise, it has to be taken into account that molecular approaches are a prerequisite for an also increasing number of available therapies, and that a proper and sophisticated diagnostic approach is required to avoid unsuitable and expensive therapies. In order to achieve this goal, it is required to adept reimbursement systems and to enable the broad basis of Pathology Institutes to include “real-life” data into machine learning prediction algorithms. It is further required to support academic institutions that confirm clinically relevant data from patient cohorts by controlled in vitro systems. This approach may improve considerably the patients’ situation as it also may facilitate the specific treatment of distinct molecular NSCLC subtypes. However, this proposal may not sound attractive for pharmaceutical companies, as it may lead to a decline in sales of novel drugs; it will also be unattractive for health insurance/reimbursement systems, as the cost-saving opportunities appear vague and are not feasible timely; in contrast, the approach will lead to a long-term effect by supporting patient care and treatment outcome, and will serve as the basis for any future development in the field.

4. Conclusions

In summary, we propose a novel tool that as a long-term challenge could contribute to a simplified and optimized prediction of the outcome of personalized cancer therapies. The tool could help to improve therapy decision while saving health-economic resources.
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References


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