RARE MOLECULAR SUBSETS AMONG LUNG TUMOURS - WHAT MAKES THEM STAND APART FROM THE COMMON?

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Abstract

While there is no universally agreed upon definition, rare malignancies are often defined as those with an incidence of \(<6\) per \(100,000\) population. However, increasingly, commonly occurring cancers are being subdivided into smaller molecular cohorts defined by the presence of driver molecular aberrations. Non-small cell lung cancer of the adenocarcinoma histological subtype is one such example, with approximately three-quarters of cases now able to be defined according to key molecular changes that drive cancer cell growth and with the potential to be targeted therapeutically. Activating mutations in the epidermal growth factor receptor and the use of specific tyrosine kinase inhibitors reflect the realisation of a personalised approach to a molecular subset in lung cancer that improves patient outcomes. However, the frequency of rare oncogenic drivers in lung adenocarcinomas is in the order of \(1-2\%\), which raises challenges in their identification and selection of the most appropriate model for clinical trial design of potential treatments. This review will highlight the potential and pitfalls of rare molecular alterations in lung adenocarcinoma.

Rare cancers, when considered together, constitute a major public health issue and pose particular challenges in diagnosis and treatment. However, their true burden on society is difficult to estimate, in part due to the lack of an international standardised definition. There is no universally agreed upon numerical cut-off and definitions vary based on either incidence or prevalence, or take into account disease severity or availability of therapy. In Europe, rare diseases are often defined as those with a prevalence of \(<50/100,000\), while the US Orphan Drug Act defines it as diseases affecting \(<200,000\) of the total US population.\(^1\) However, there are inherent limitations in using prevalence as a measure of disease rarity, particularly in the context of cancer, due to the impact of disease-related survival. The project Surveillance of Rare Cancers in Europe (RARECARE) has proposed an alternate definition based on incidence, using a threshold of \(<6/100,000\). Using this definition, rare cancers constitute \(22\%\) of all cancers diagnosed in Europe and have been shown to have inferior outcomes as compared to their more common counterparts. The discrepancy in survival between rare and common cancers becomes apparent more than one year after diagnosis, suggesting a lack of effective treatments accounts for the poorer survival observed for rare cancers.\(^2\)

Regardless of its precise definition, the landscape of what constitutes a rare cancer is changing due to the increasing identification of molecular subsets within commonly occurring cancers. These subsets are defined by the presence of specific oncogenic aberrations that drive cancer cell growth and have the potential to be targeted therapeutically. A prime example is that of non-small cell lung cancer (NSCLC), a common malignancy of the adenocarcinoma histological subtype, whose classification has evolved into distinct molecular subsets defined by the presence of actionable driver oncogenes. These molecular subsets and the development of small molecule tyrosine kinase inhibitors (TKIs) have advanced the personalised approach to lung cancer care and improved patient outcomes. However, this approach has associated challenges, in part due to the rarity of some molecularly defined subsets, resulting in issues surrounding accurate patient identification and development and validation of targeted therapeutic approaches. This review will discuss the evolution of lung cancer management and how it has been shaped by the emerging genomic classification, with a particular focus on rare molecular subtypes of lung adenocarcinoma.

Evolution of lung cancer classification and management

Historically, the most important classification of lung cancer rested upon the distinction between small cell and NSCLC, as their different biological behaviour conferred
different prognostic implications and necessitated different treatment regimens. NSCLC can be further classified histologically into adenocarcinoma, squamous cell carcinoma and large cell carcinoma subtypes. However, in the past, distinguishing between subtypes was not essential as therapy was empirical. Despite advances with the use of platinum-based chemotherapy, introduction of second-line and maintenance therapies, it was clear a therapeutic plateau had been reached with empirical chemotherapy.\textsuperscript{3, 6}

The start of personalisation of lung cancer management came in the mid-2000s, with the recognition of different efficacy and toxicity profiles of certain agents according to histological subtype. Pemetrexed demonstrated superior progression-free survival and overall survival in patients with non-squamous cell carcinoma histology, while bevacizumab, an anti-angiogenic agent, was noted to increase the risk of severe pulmonary haemorrhage in patients with squamous cell carcinoma.\textsuperscript{7, 8}

Around this time, small molecule TKIs directed against epidermal growth factor receptor (EGFR), with its signalling pathway long recognised to play an important role in cancer cell proliferation, angiogenesis and metastases.\textsuperscript{9} The early trials of the first generation EGFR TKIs showed modest benefits in unselected populations, however it was noted that a subset of patients demonstrated dramatic and durable responses. It was subsequently recognised that mutations within the EGFR gene conferred exquisite sensitivity to these agents.\textsuperscript{10} Subsequent studies have selected patients for the presence of an activating EGFR mutation and have shown the use of a targeted agent in this population to result in progression-free survival of approximately one year. Overall survival for patients with EGFR mutations receiving relevant TKI therapy at some point along their treatment course now approaches two years.\textsuperscript{11}

The targeting of EGFR in lung cancer demonstrated that a personalised approach can result in significantly improved patient outcomes. It also highlights that personalisation of lung cancer management requires the ability to identify and define molecular subsets of patients according to the presence of a ‘driver mutation’ that is responsible for the initiation and maintenance of cancer growth that can be targeted therapeutically. Through evolving molecular profiling, driven by projects such as the Cancer Genome Atlas Research Network, a deeper understanding of the molecular abnormalities in NSCLC has been acquired and a genomic classification has emerged.\textsuperscript{12} This progress over time is outlined in figure 1.

While it holds great potential, comprehensive molecular analysis is not without its challenges. NSCLC shows a high rate of somatic mutations and genomic rearrangements, which can make distinguishing passenger events from driver gene alterations difficult.\textsuperscript{13} While the proportion of tumours that lack an identifiable driver lesion continues to decline, what has emerged is that many of the more recently recognised molecular subsets occur at a frequency of approximately 1% (figure 2).\textsuperscript{12} The following discussion highlights new emerging rare molecular subsets within lung adenocarcinoma (see table 1), whose molecular profiling is more advanced than that of SCC, and addresses future challenges in the management of these rare molecular subsets.

**Figure 1: Evolution of NSCLC classification.**

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Figure 2: Driver oncogenes in lung adenocarcinoma.

Table 1: Selected rare molecular subgroups of lung adenocarcinoma

<table>
<thead>
<tr>
<th>Molecular target</th>
<th>Prevalence</th>
<th>Clinico-pathological characteristics</th>
<th>Targeted therapeutic agents</th>
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<tr>
<td>ALK rearrangements</td>
<td>2-7%</td>
<td>Younger age Non-smokers</td>
<td>Crizotinib</td>
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<td>Hsp90 inhibitors</td>
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<td>ROS1 rearrangements</td>
<td>1-2%</td>
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<td>Irreversible pan-ERBB inhibitors</td>
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<td>Former or current smokers</td>
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<td>MEK inhibitors</td>
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Rare molecular subsets of lung adenocarcinoma

**ALK**

ALK is a transmembrane receptor tyrosine kinase not normally expressed in the lung. In 2007, ALK rearrangements were reported for the first time in NSCLC and were recognised to result in constitutive ALK activity that mediates oncogenesis.1 The most common rearrangement occurs between EML4 and ALK, although other fusion partners do exist.15 ALK rearrangements are detected in approximately 2-7% of unselected NSCLC cases.16

With its identification, there was an immediate effort to screen and enrol patients onto a phase 1 trial of crizotinib, a MET, ROS1 and ALK inhibitor. In this trial, ALK positive patients were determined by a break-apart fluorescence in-situ hybridisation assay and were found to have an overall response rate of 61% and median progression-free survival of 9.7 months.17 Similar results were observed in the subsequent phase II trial.18 Crizotinib was granted accelerated US Food and Drug Administration approval in ALK-positive NSCLC based on the results of these single-arm studies.19 In subsequent phase III trials, crizotinib has been shown to be superior to second-line chemotherapy and recently presented first-line data show superior median progression-free survival and overall response rate.20,21 No overall survival benefit has been observed, which most likely reflects patient cross-over to crizotinib after progression on chemotherapy.

The duration of clinical benefit observed with crizotinib is limited by the development of acquired resistance. A number of resistance mechanisms have been observed, including the acquisition of secondary ALK mutations and alternate tyrosine kinase activation, including ERBB family pathway activation, KIT amplification and KRAS mutations.22,23 A number of more potent second-generation ALK inhibitors are under evaluation and have shown promising preliminary activity in patients who are both crizotinib-naive or have developed acquired crizotinib resistance.24-27 One such agent, ceritinib, received accelerated Food and Drug Administration approval in April 2014, with confirmatory trials still in progress.28

**ROS1**

ROS1 is a tyrosine kinase receptor that shares a high degree of sequence homology with ALK.29 It can form oncogenic fusion proteins with several different partners and these gene fusions are observed in approximately 1-2% of all NSCLC patients.30,31 Crizotinib has demonstrated activity in patients harbouring ROS1 rearrangements detected by fluorescence in-situ hybridisation, with preliminary results from the expansion cohort of an ongoing phase 1 study (NCT00585195) showing an overall response rate of 56%.32 A number of clinical trials are ongoing, evaluating second and third generation ALK inhibitors in this molecular cohort (NCT01964157, NCT01449461, NCT01284192).

**RET**

RET is a receptor tyrosine kinase involved in cell proliferation, migration and differentiation. Two specific gene fusions have been described in NSCLC (CCDC6-RET, KIF5B-RET), which result in the constitutive activation of the RET kinase and are estimated to occur in approximately 1% of NSCLC.33 A number of multi-targeted kinase inhibitors have clinical activity against RET, including sunitinib, sorafenib, vandetanib and cabozantinib, however their place in the management of RET-rearranged NSCLC is yet to be elucidated.34 A phase II study evaluating cabozantinib in RET fusion-positive advanced NSCLC is ongoing (NCT01639508). An early report of the first three patients enrolled on this study describes two confirmed partial responses, with prolonged disease stabilisation in the third.35

**HER2**

HER2 is a membrane-bound tyrosine kinase of the ERBB family. The identification of EGFR mutations in lung cancer led to renewed interest in investigating activating mutations of HER2. Mutations have been identified in approximately 2-4% of patients. Most are in-frame insertions in exon 20, which result in constitutive activation of the HER2 kinase in a ligand-independent manner.36 Transgenic mouse models have confirmed the oncogenicity of HER2 mutations.37

It is hypothesised that HER2 mutations may be more relevant in lung carcinogenesis than overexpression or amplification, and can act as a predictive biomarker for the use of targeted therapies. The role of irreversible pan-ERBB TKIs has gathered the most interest in this setting.38 There have been case reports and preliminary data of objective responses to afatinib and dacomitinib in a small number of patients harbouring HER2 mutations.39,40 However, the results do not appear as robust as observed with the use of TKIs in other oncogene-addicted tumours.

**BRAF**

BRAF is downstream of KRAS in the MAP kinase pathway. BRAF mutations were first identified in 2002, with a particularly high prevalence in melanoma.41 This led to a search for BRAF mutations in NSCLC, where they have been identified in approximately 3-5% of cases. Approximately half the mutations identified are V600E.42 The BRAF inhibitor dabrafenib received “breakthrough therapy” designation from the Food and Drug Administration following preliminary efficacy data from a phase II study demonstrating an ORR of 54% in patients with BRAF V600E mutation-positive NSCLC.43
As tumours harbouring non-V600E BRAF mutations are unlikely to respond to V600E-specific inhibitors such as dabrafenib, inhibitors of downstream targets such as MEK are under evaluation.

Impact and challenges of the molecular characterisation of NSCLC

The evolving characterisation of molecular subsets of NSCLC has resulted in a paradigm shift in lung cancer management. Many clinical practice guidelines now recommend biomarker testing for EGFR mutations and ALK rearrangements for all tumours with an adenocarcinoma component. The increasing awareness of treatment-by-histology interactions and different observed frequencies of driver mutations according to histological subtype has resulted in a change to the histological classification of NSCLC. There is new emphasis on the importance of distinguishing between histological subtypes and strategic use of samples to preserve sufficient tissue for subsequent molecular studies.

Despite its merits, the genomic classification of lung cancer still poses many challenges, including issues of access at a public health level, tissue availability, data interpretation and clinical trial design. These are discussed below.

Access to molecular testing

It is critical for molecular profiling to be available to all NSCLC patients in order to personalise treatment decisions by molecular subgroups. For example, France has introduced a program that offers free molecular diagnostic testing for all patients with solid tumours. However, the results of molecular testing are highly dependent upon the quality and quantity of tumour tissue available and the technology platform utilised.

As an increasing number of genomic subgroups are identified, gene-based molecular tests that focus on a single biomarker are no longer adequate. The advent of multiplex testing has enabled the evaluation of mutation status or expression of several genes simultaneously, thus maximising diagnostic information from limited tumour tissue and avoiding unnecessary time delays from sequential biomarker testing. However, such approaches require a significant investment in bioinformatics in order to aid a clinician’s decision-making as to which genomic data is relevant to an individual patient’s treatment.

With the increasing awareness of inter and intra-tumoural heterogeneity, and as mechanisms of acquired resistance continue to be elucidated, it is apparent that a single genomic profile from a single tumour site at one time-point is insufficient. Serial biopsies over the course of a disease, particularly at times and sites of disease progression, may provide a more accurate genomic analysis and insights into appropriate strategies to overcome the emergence of acquired resistance. The need for serial biopsies has led to interest in the potential role of minimally invasive techniques, such as molecular analysis of circulating tumour cells and free DNA.

Clinical trial design

It is increasingly unlikely that large randomised trials in unselected lung cancer patients will yield clinically significant results. In this evolving molecular era, smaller trials selecting patients defined by molecular aberrations are necessary for therapeutic development. However, there is also the risk that molecularly stratified trials may miss other targets for drug development. Furthermore, the small size of newly identified molecular subsets decreases the relative cost-effectiveness of developing novel agents and thus reduces the appeal to the pharmaceutical industry.

The appropriateness of the traditional phases of drug development is less certain for targeted therapies. This is in part reflected by the Food and Drug Administration’s accelerated approval program, which is designed to facilitate patient access to new therapies while post-marketing studies are conducted to confirm efficacy and safety. However, foregoing randomised phase III trials altogether has its disadvantages, including less definitive efficacy and toxicity data. There is an urgent need for novel clinical trial designs to improve the efficiency of the drug-development process, enable testing of multiple molecular targets and increase patient access to investigational agents.

For instance, the BATTLE (biomarker-integrated approaches of targeted therapy for lung cancer elimination) trial demonstrated the feasibility of identifying subsets of NSCLC patients more likely to benefit from a specific agent and the incorporation of an adaptive design to guide treatment selection for subsequently enrolled patients.

Conclusion

It is becoming increasingly clear that common tumours such as NSCLC are composed of multiple rarer subgroups defined by the presence of an oncogenic alteration. The identification and targeting of driver mutations has enabled a paradigm shift from empirical to personalised care and resulted in improved patient outcomes. However, important challenges still need to be overcome, including the issues of acquired treatment resistance, rational clinical trial design and treatment selection and support for ongoing research and development. Identifying relevant molecular subtypes and matching patients with appropriate targeted therapies is crucial for the progress of cancer management.

References

ALK inhibitor, ASP3026, observed in a phase I dose escalation trial. ASCO suppl):8031.


