January 2020 Edition no. 65

AMPATHCHAT

Dr Henno Olivier, Histopathologist

Molecular pathology of lung cancer

Introduction

Worldwide, primary lung cancer remains the most common malignancy after non-melanocytic skin cancer. The World Health Organization (WHO) estimates lung cancer to be responsible for 1.59 million deaths globally per year, with smoking playing a major contributory role in 70% of cases.

Non-small cell lung cancer (NSCLC) accounts for 80 to 90% of lung cancers, while small cell lung cancer (SCLC) has decreased in frequency in many countries. The incidence of the different subtypes of NSCLC has changed over the last 25 years. Adenocarcinoma has increased in both genders, while squamous cell carcinoma (SCC), formerly the predominant subtype, has decreased.

In the past, the incidence of lung cancer differed according to sex, but in the USA, the decrease in men and steady increase in women since the mid-1990s have resulted in near equal gender parity. These trends have also been documented in other parts of the world. More than 90% of patients with lung cancer are 45 years of age or older, but cases have been reported in adolescents and young children.

Approximately 10 to 15% of cases of lung carcinoma occur in people who have never smoked. Among these, 75% are women, with the majority proving to be adenocarcinoma on histology.

Most lung cancers are in a relatively advanced stage by the time of diagnosis. The overall survival rate remains low. According to a recent large cohort study, 17% of patients are expected to survive five years.

Diagnosis and classification

Minimally invasive techniques have become the preferred method of obtaining material for the diagnosis and staging of lung carcinomas. Bronchoscopy is ideally suited for large, centrally located lesions. This method can be used for bronchial washing, brushing, bronchial and transbronchial biopsy. Combined direct bronchoscopy visualisation with ultrasound guided biopsy (EBUS) provides a diagnostic yield of 75 to 85% in centrally located lesions. In cases of peripheral lesions, transthoracic percutaneous fine needle aspiration and/or core biopsy under imaging guidance is recommended. Needle biopsy is associated with a diagnostic accuracy of up to 88%. The most significant disadvantage of transthoracic needle biopsy is the risk of pneumothorax (in up to 50% of cases). If a pleural effusion is present, thoracentesis proves to be another diagnostic tool.

Histological diagnosis of NSCLC remains the cornerstone of many treatment decisions and should be based upon the criteria laid out in the WHO classification. Immunohistochemistry is an important tool during the initial diagnosis, especially in the assessment of small biopsies where specific subtyping is not possible on morphology alone. Immunohistochemistry staining should reduce the NSCLC-NOS (not otherwise specified) ratio to less than 10%.

Molecular biology

The tumour cells in lung carcinomas may harbour a variety of genetic abnormalities, but only a subgroup of these is responsible for tumour cell survival (called driver mutations). Because tumour cells are dependent on driver mutations (a phenomenon called oncogene addiction), inactivation of these mutations results in cancer cell death. Several molecular drivers for oncogene addiction represent important predictive biomarkers and excellent therapeutic targets. At present, several gene alterations have been identified as therapeutic targets in lung adenocarcinomas. They are generally mutually exclusive. Squamous cell carcinoma and small cell carcinoma exhibit a mutation spectrum different to adenocarcinoma. Mutation-targeted therapies for these tumours have not yet matured into approved treatments.

All the above tests are performed routinely at Ampath laboratories.
Please contact your local pathologist to enquire about the cost involved.

Molecular targets with approved therapeutics

- **Epidermal Growth Factor Receptor (EGFR):** This is a transmembrane protein with an intracytoplasmic tyrosine kinase domain. Mutation carriers are predominantly non-smokers, of a younger age and female. In the vast majority of cases, EGFR mutations are non-overlapping with other driver mutations found in NSCLC. Tumours with certain EGFR mutations (exon 19 and 21 mutations) are sensitive to thyrosine kinase inhibitors such as erlotinib and gefitinib. The EFGR mutation status is determined by mutation analysis, e.g. mutation-specific PCR or DNA sequencing.

- **BRAF gene:** Mutations of the BRAF gene are present in approximately 3% of patients with lung adenocarcinomas and are more likely to be found in current or former smokers. The BRAF protein belongs to the family of serine-threonine kinases that are mediators in the MAP kinase signalling cascade. Mutations of BRAF are also non-overlapping with other common oncogene mutations found in adenocarcinomas. Half of the mutations are a V600E substitution. The BRAF inhibitors include vemurafenib and debrafenib.

- **Anaplastic Lymphoma Kinase (ALK):** Activation rearrangements of the ALK gene by fusion with the EML4 gene are seen in 3 to 7% of patients with lung adenocarcinoma. The resulting EML4-ALK fusion gene encodes for a chimeric EML4-ALK protein with constitutive tyrosine kinase activity. Patients with ALK rearrangements are usually younger and non- or light smokers. Patients whose tumours harbour these translocations respond well to ALK/MET/ROS1 inhibitors such as crizotinib. FISH testing is the mainstream method for diagnosing ALK gene rearrangements.

- **ROS1:** ROS1 is a member of the insulin receptor family. It is a type 1 integral membrane protein with tyrosine kinase activity. Gene rearrangements create fusion proteins with different partners, which results in activation of the kinase domain of ROS1. The ROS1 fusion genes are present in 1% of lung adenocarcinomas and are associated with younger patient age and patients who have never smoked. Crizotinib is effective in patients with ROS1 fusions.

Molecular targets with potential therapeutics

The field of precision ("personalised") medicine is evolving rapidly. Recent clinical trials demonstrated multiple novel potential molecular targets, including ERBB2(Her2), MET, RET, Neurotrophic Receptor Tyrosine Kinase 1 and Fibroblast Growth Factor Receptor. More data is required before any of these newer targets can be routinely incorporated into NSCLC biomarker testing.

Liquid biopsy/Cell-free DNA

Tumour cells can release small fragments of DNA into the circulation (cfDNA). Various PCR-based and next-generation sequencing techniques can be utilised to detect cfDNA. The information obtained can be used to determine eligibility for target therapy, monitor treatment efficacy and for the detection of resistance mutations. A recent study demonstrated a concordance rate of 89.1% between tissue analysis and cfDNA for EGFR mutations.

Cancer immunotherapy

Tumours may avoid the immune system by making use of several mechanisms, including a selective outgrowth of antigen negative variants, loss or reduced expression of histocompatibility antigens and immunosuppression mediated by the expression of certain factors (e.g. TGF-B and PD-1 ligand). Preclinical evidence suggests that activation of the PD-1/PD-L1 signal serves as an important mechanism for tumours to evade an antigen-specific T-cell immunologic response. Consequently, PD-1 and PD-L1 inhibitors were devolved for the treatment of advanced solid tumours. Pembrolizumab is the first agent to be approved for the treatment of advanced or metastatic PD-L1-positive non-small cell lung cancers. Confirmation of PDL-1 expression by immunohistological staining is necessary to determine eligibility for anti-PDL-1 treatment.

Conclusion

The diagnosis and care of lung carcinoma patients continue to improve as technology, scientific understanding and clinical practice progress. The treatment of NSCLC has changed tremendously in the past 10 years with the development of treatment options targeting specific molecular alterations within the tumour cells. Molecular diagnostic techniques play an integral role in identifying those patients who will benefit from these targeted treatments.

References

Oberndorfer, F and Mullauer, L. 2018. Molecular pathology of lung cancer: current status and perspectives. *Current Opinion in Oncology* 30(2):69–76.

Lindeman, NI, Cagle, PT, Aisner, DL, Arcila, ME, Beasley, MB, Bernicker, EH, Colasacco, C, Dacic, S, Hirsch, FR, Kerr, K, Kwiatkowski, DJ, Ladanyi, M, Nowak, JA, Sholl, L, Temple-Smolkin, R, Solomon, B, Souter, LH, Thunnissen, E, Tsao, MS, Ventura, CB, Wynes, MW and Yatabe, Y. 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. Archives of Pathology and Laboratory Medicine 142(3):321–346

Travis, WD, Brambilla, E, Burke, AP, Marx, A and Nicholson, AG (Eds). 2015. WHO classifications of tumors 7(4):9–22. IARC: Lyon.