Preface

Lung cancer (LC) is the leading cause of oncological morbidity and mortality, accounting for approximately 1.8 million new cases and 1.6 million cancer deaths every year worldwide (http://globocan.iarc.fr/old/FactSheets/cancers/lung-new.asp). LC is used to be the disease of smokers and therefore was believed to be a largely preventable malignancy. Recent studies demonstrated an alarming increase of LC incidence among non-smokers (1). Tumours arising in smokers and non-smokers show clearly distinct mutation profiles, indicating that these two categories of LC may require distinct avenues for research and medical intervention (2-4).

Lung cancer is usually classified for non-small cell LC (NSCLC) and small-cell LC (SCLC). SCLC is a highly aggressive category of LC, which is rarely treated by surgery but demonstrates substantial sensitivity to cytotoxic therapy. In contrast to NSCLC, gross amounts of primary SCLC tissue are rarely available for investigation. Recent technological advances provided a handful of methods, which allow comprehensive molecular profiling of minimal amounts of tumour cells. Focus on SCLC revealed a number of intriguing observations, which are discussed in this book. In particular, SCLCs are almost always characterized by inactivation of two most known suppressor genes, p53 and RB1. SCLC may develop directly from lung epithelial cell precursors, or, alternatively, evolve from NSCLC, especially if the latter is treated by tyrosine kinase inhibitors (TKI). SCLCs share some similarities with large-cell neuroendocrine lung carcinomas (LCNECs). Recent studies led to identification of molecular events distinguishing SCLC and LCNEC (5). Furthermore, next-generation sequencing analysis of LCNEC revealed a number of potentially druggable targets in this category of tumours (6). Treatment options for SCLC and LCNEC are currently limited to cytotoxic therapy, which usually produces only short-term effects. Recent clinical trial of rovalpituzumab tesirine, a conjugate of DNA cross-linking agent and antibody recognizing a member of Notch receptor ligand family, DLL3, showed promising results in this difficult-to-treat category of patients (7). Various aspects of SCLC and LCNEC biology and treatment are comprehensively discussed in this book.

Notch signalling pathway is implicated in the regulation of cell-cell communication and plays a role in cellular differentiation. Alterations of Notch cascade are involved in pathogenesis of various human malignancies. Recent studies revealed that Notch pathway may be a promising target for the management of KRAS-mutated tumours. RAS-driven cancers account for approximately 15-30% of LCs. Activating RAS mutations are mutually exclusive with the mutations in druggable kinases (EGFR, ALK, ROS1, BRAF, HER2, MET), with no specific RAS-targeted therapy available at the moment. Ambrogio *et al*., 2016 investigated early stages of KRAS-driven lung cancer transformation and revealed a critical role of DDR1 kinase activation. Combined inhibition of DDR1 and Notch signalling resulted in the regression of KRAS-mutated tumours in mice (8).

Cancer progression was long believed to be a relatively slow gradual process involving multiple consecutive genetic events and more or less time-consuming transition from curable to incurable disease. Therefore, extraordinary efforts have been invested in the development of tools permitting to identify cancer disease at early, yet curable stages. These activities led to some success, for example, to the reduction of mortality from cervical, prostate and some other cancers (9,10). However, there are some unexpected findings. For example, while lung cancer can be relatively easy visualized through the use of various types of X-ray imaging, positive impact on LC mortality has been obtained only with the use of highly sophisticated diagnostic method, low-dose computed tomography (CT) (11). Furthermore, while the reduction of mortality approaches to only 20%, the frequency of false-positive findings and consequent unnecessary medical interventions is unacceptably high. For above reasons, LC low-dose CT screening is recommended only for current or recent heavy smokers aged between 55 and 80 years, and the optimal interval for examinations is believed to be around 2 years. Further details on LC screening are summarized in this book.

It appears that conventional imaging technologies are unlikely to resolve the issue of reliable and early LC detection in the near future, therefore alternative diagnostic approaches are being intensively studied. Use of molecular diagnostic tools is considered to be the most promising, given that somatic genetic alterations are highly specific for transformed cells and that PCR-based technologies are capable to detect single copies of altered genes. None of well-known mutations (KRAS, EGFR, ALK, p53 etc.) occurs in all LC, therefore the perspectives for mutation-based screening may look limited. It has been established that the majority of LC are characterized by hypermethylation of some regulatory gene regions. Furthermore, the pattern of methylated DNA sequences is more or less conservative, so a relatively limited set of methylation DNA markers may theoretically distinguish between cancerous and non-cancerous tissues (12).

Almost all next-generation laboratory diagnostic tools rely on so-called liquid biopsy. Presence of residual tumour cells and/or their fragments in body fluids of cancer patients was acknowledged a long time ago. There is a number of serum protein markers, e.g., PSA, CA-125, CEA, which demonstrate reasonable tissue specificity and are often elevated in patients with prostate, ovarian, or gastrointestinal cancers, respectively. DNA-based diagnostic tools are likely to have advantages as compared to protein markers. As already mentioned above, presence of cancer-related mutations is relatively specific for transformed cells, therefore some common

pathological processes, such as inflammation or hyperplasia, are unlikely to result in false-positive signals. Liquid biopsy may be applied to various body liquids, including blood, urine, saliva etc. Analytical methods may be targeted to circulating tumour cells (CTCs), cell-free DNA, microRNA etc.

There are three main avenues for the use of liquid biopsy. First, it is projected to become a screening method, aimed to replace or assist various imaging techniques. As example, one may refer to already existing PSA screening for prostate cancer. For lung cancer, the main challenge is to compose sufficiently specific panel of markers, which would reflect molecular alterations in the majority of LC. Secondly, there are studies demonstrating that molecular alterations in primary LC may be reliably detected using blood tests. The need for blood-based molecular profiling for already diagnosed LC is limited: in modern oncology the mere diagnosis of cancer disease is almost always based on tumour biopsy, so the cancer tissue is available anyway; furthermore, treatment-naïve neoplasms usually do not show intratumoural heterogeneity with respect to driver mutations. Thirdly, liquid biopsy may serve as a tool for monitoring and analysis of the tumour burden during the treatment. When tailored to molecular markers detected in primary malignancy, it may estimate an overall cancer volume during the treatment. Furthermore, novel drugs, like osimertinib, are tailored to secondary mutations acquired during earlier lines of therapy, e.g. EGFR T790M. The diagnosis of these mutations in tumour tissue may not be feasible, as it requires multiple re-biopsies and ignores possible heterogeneity of treatment resistance pathways in distinct metastases obtained from the same patients. Accordingly, liquid biopsy is clearly a method of choice in these clinical situations. This book offers a comprehensive discussion on various aspects of liquid biopsy.

The discovery of TKI-sensitizing mutations in lung cancer may be regarded as the most impressive achievement of clinical oncology in the last decade. Patients with EGFR, ALK and ROS1 mutations have drastically improved survival if treated with appropriate targeted drugs (13). Testing for druggable mutations has been incorporated in LC management routine. It is of high interest to see the reports of French Cooperative Thoracic Intergroup (IFCT) initiative, which summarized the first results of LC molecular profiling in a nationwide scale (14,15). There are several other nationwide studies carried out in China, Taiwan, Korea, Russia etc., which allowed to investigate some LC molecular markers with an unprecedented level of comprehension (16-20).

This book provides a valuable update on recent LC clinical trials. In particular, extensive efforts are being invested to further optimize the treatment of EGFR-mutated LC. LUX-7 trial performed the direct comparison of gefitinib and afatinib. While the efficacy of gefitinib towards LC carrying EGFR mutations was discovered by retrospective analysis of the results of lung cancer clinical trials, afatinib was intentionally designed to target EGFR mutation-driven LC. In addition, afatinib exerts activity towards other kinases of HER family. LUX-7 trial involved 319 patients. The investigators acknowledged that afatinib demonstrated statistically longer progression-free survival (11.0 *vs.* 10.9 months, P=0.017) and time to treatment failure (13.7 *vs.* 10.5 months, P=0.007) (21). It is of question whether these small advantages, although being clearly statistically significant, are of high medical relevance (22,23), especially given that no differences in overall survival was observed in this trial (24).

There are also trials attempting the simultaneous use of chemotherapy and gefitinib in patients with EGFR-mutated lung cancer (25,26). Development of osimertinib, a potent EGFR inhibitor specific both for gefitinib-sensitizing mutations (ex19del and L858R) and for gefitinib-resistant substitution (T790M) represents a major breakthrough in the LC treatment. Osimertinib demonstrated remarkable activity in LC patients, who progressed on gefitinib via an acquisition of EGFR T790M mutation (27,28). It also produced unprecedentedly long progression-free survival when given as an upfront therapy (29).

Crizotinib was the first drug approved for the treatment of ALK and ROS1-rearranged lung cancer. It demonstrates significant advantage over chemotherapy with regard to response rate, progression-free survival and control of brain metastases (30-32). Focus on ALK-rearranged cancers led to development of next-generation ALK inhibitors, e.g., ceritinib, which is more potent than crizotinib, capable to penetrate blood-brain barrier and shows activity towards crizotinib-resistant disease. Ceritinib demonstrated pronounced and durable responses both in crizotinib-treated and TKI-naïve LC patients with ALK translocations, including subjects with intracranial metastases (33,34). Similar results were reported for ROS1-rearranged LC (35). Another novel ALK inhibitor, brigatinib, also showed promising activity towards lung carcinomas carrying ALK fusions (36).

Administration of ALK inhibitors usually relies on FISH analysis, which demonstrates the mere fact of the presence of ALK translocation but is unable to identify the type of ALK rearrangement among the diversity of existing fusion variants. This practice may not be supported by the clinical and laboratory evidences, which suggest that the type of ALK translocation may influence tumour responsiveness to crizotinib (37-39).

Crizotinib was initially developed and clinically assessed as a MET inhibitor, however subsequent discovery of ALK and ROS1 translocations led to the change of its indications for tumours carrying rearrangements in the above genes. However, recent studies

revealed that approximately 3% of lung carcinomas carry activating mutations in MET gene, and these tumours are generally responsive to this drug (40). Thus, if one considers frequencies of all crizotinib-sensitizing mutations (ALK: 4-8%; ROS1: 1-2%; MET: 3%), the total fraction of non-squamous non-small LCs amenable to crizotinib treatment would approach to approximately 10%.

Approximately 2% lung carcinomas carry activating mutations in the codon 600 of BRAF oncogene. Treatment strategies for this category of tumours were initially established in melanoma, as this type of skin tumours is generally not responsive to conventional cytotoxic treatment and BRAF V600E mutations are detected in approximately a half of these neoplasms. Similarly, good responses were detected in lung cancer patients, where pronounced and durable reduction of tumour size was observed in the majority of subjects receiving BRAF mutation-specific inhibitor alone or in combination with MEK inhibitor (41-43).

Failure of immune system was long considered to be an essential component of cancer progression. Extensive efforts have been invested to find the signs of systemic immune suppression in cancer patients, however these studies were largely unsuccessful. Recent investigations resolved this apparent paradox. It was revealed, that immune deficiency indeed contributes to cancer development, however its extent is limited to a peritumoural space, as immune suppressors are produced locally either by tumour cells or tumour-infiltrating immune cells (44,45). This decade may be regarded as a triumph of immune checkpoint inhibitors, which demonstrated substantial clinical activity towards many cancer types. Noteworthy, targeted immune drugs led to a breakthrough in the treatment of lung cancer, especially of LC arising in smokers and lacking druggable mutations. Unfortunately, immune checkpoint inhibitors result in clinical responses only in a subset of patients, therefore there is continuing search for better drugs and their combinations. In addition, studies aimed to identify predictive markers for immune therapy are currently underway (29,46-48).

This book is likely to be of high interest for medical oncologists, translational researchers and cancer biologists.

Acknowledgement

This work has been supported by the Russian Foundation for Basic Research (grant 17-54-12007).

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