Advances in targeted therapy and immunotherapy for treatment of lung cancer

Motonobu Saito¹,², Seiichi Takenoshita², Takashi Kohno¹

¹) Division of Genome Biology, National Cancer Center Research Institute
²) Department of Organ Regulatory Surgery, Fukushima Medical University School of Medicine

Abstract

Personalized therapy based on targetable genetic aberrations has become a standard therapy for cases of lung adenocarcinoma (LADC) harboring EGFR mutations and ALK fusions. The effects of such personalized therapy are significantly positive with a higher response rate and longer survival compared to conventional chemotherapy. Therefore, further identification of druggable genetic aberrations and the development of molecular targeting drugs for them are required. For LADC, several new targeted drugs against driver mutations in EGFR, KRAS, HER2, and BRAF; and driver fusions involving ALK, RET, and ROS1 have been developed, and clinical trials of these new targeted drugs are currently being conducted. On the other hand, personalized therapy against driver mutations has not well progressed in squamous cell lung cancer and small cell lung cancer. For those subtypes, immunotherapy might be an effective treatment strategy.

Key Words: next-generation sequencing, driver gene aberration, personalized medicine, clinical sequencing

(Molecular targeted therapy using specific inhibitors for activated oncogene products has started and replaced conventional chemotherapy in LADC due to high efficacy⁶,¹⁰. To further expand the personalized therapy, it is important to find druggable targets as well as to develop diagnostic methods that are able to identify gene mutations and rearrangements. After identification of such targetable genes, patients can be treated by existing targeted therapies or by participating in appropriate clinical trials. For further facilitating personalized therapy, clinical sequencing of cancer genome in each lung cancer patient will help decision-making for treatment selection.

In this review, we discuss the current topics of targeted therapies in lung cancer based on driver gene aberrations and clinical sequencing to expand those therapies. In addition, we also discuss the current topic of immunotherapy, which is expected to be a next personalized therapy in lung cancer. Immune checkpoint blockade of the programmed death-1 (PD-1) and its ligand PD-L1 pathway showed marked effectiveness in patients with lung cancer¹¹,¹³. While the efficacy of anti-PD-1 treatment is varies among patients, a predictive biomarker has not yet been determined. Although candidate responders for PD-1/PD-L1 blockade therapy seem to be patients with higher PD-L1 expression by immunohistochemistry (IHC) or a higher number of nonsynonymous mutations, further discussion is needed¹¹,¹⁴.)
Identification, diagnosis and targeted therapy of driver genes in lung cancer

During the development of cancer, a number of genetic alterations occur. Among a huge number of mutated genes, several oncogene activations have a pivotal role in the development of cancer, and such genes are called driver genes. The driver gene aberrations induce the constitutive activation of intracellular signaling, making cancer cells oncogene-addicted (i.e., living strongly dependent on the oncogene activations)\(^1\). In fact, molecular targeted therapy that inhibits driver gene-induced signaling demonstrates higher specificity and higher cytotoxic rate than conventional cytotoxic anticancer agents.

To date, several (candidate) driver genes have been identified in lung cancer by whole genome sequencing, RNA sequencing, or whole exon sequencing (Table 1). The effectiveness of targeted therapy against driver genes is dependent on the accurate diagnosis of these genomic aberrations in each tumor. For the diagnosis of driver gene mutations, DNA extracted from surgical specimens is subjected to PCR-based assays. On the other hand, gene mutations, DNA extracted from surgical specimens is subjected to PCR-based assays. On the other hand, driver genes aberrations in each tumor. For the diagnosis of driver gene aberrations, among a huge number of mutated genes, several oncogene activations have a pivotal role in the development of cancer, and such genes are called driver genes. The driver gene aberrations induce the constitutive activation of intracellular signaling, making cancer cells oncogene-addicted (i.e., living strongly dependent on the oncogene activations)\(^1\). In fact, molecular targeted therapy that inhibits driver gene-induced signaling demonstrates higher specificity and higher cytotoxic rate than conventional cytotoxic anticancer agents.

Table 1 Large scale genomic sequencing results in major histological types of lung cancer

<table>
<thead>
<tr>
<th>Histology</th>
<th>Area</th>
<th>Cases</th>
<th>Sequencing</th>
<th>Gene aberrations detected</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>LADC</td>
<td>Japan</td>
<td>30</td>
<td>RNA sequencing</td>
<td>RET fusion.</td>
<td>Kohno, 2012</td>
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<td>USA and Japan</td>
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<td>RET fusion.</td>
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<td></td>
<td>Korea</td>
<td>200</td>
<td>RNA sequencing</td>
<td>Mutations in ARID1A and SMARCA4. fusions in ALK, RET, ROS1, FGFR2, AXL, and PDGFR. RET exon skipping.</td>
<td>Seo, 2012</td>
</tr>
<tr>
<td></td>
<td>USA</td>
<td>183</td>
<td>WGS, WES</td>
<td>Mutations in EGFR, fusion.</td>
<td>Inmielinski, 2012</td>
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<tr>
<td></td>
<td>USA</td>
<td>36</td>
<td>Target sequencing</td>
<td>NTRK1 fusion.</td>
<td>Vaishnavi, 2013</td>
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<td></td>
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<td>97</td>
<td>WES</td>
<td>Mutations in EGFR and KRAS.</td>
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<td></td>
<td>Germany and Japan</td>
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<td>Japan</td>
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<td>Fusions in NRG1, ERBB4. RAF, and RET (invasive mucinous adenocarcinoma).</td>
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<td>USA</td>
<td>230</td>
<td>WES, RNA sequencing</td>
<td>Mutations in NF1, MET, ERBB2, and RIT1.</td>
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<td>Japan</td>
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<td>WES</td>
<td>Distinct profile according to driver gene aberrations.</td>
<td>Saito, 2015</td>
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<td>SCLC</td>
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<td>WGS, WES</td>
<td>Mutations in TP53, NFE2L2, KEAP1, and PHK pathway genes.</td>
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<td>Korea</td>
<td>104</td>
<td>WES</td>
<td>Mutations in TP53, NFE2L2, and KEAP1. FGFR3 fusion.</td>
<td>Kim, 2014</td>
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<td>SCLC</td>
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<td>WGS, WES, RNA sequencing</td>
<td>Mutations in CREBBP, EP300, and MLL. FGFR1 amplification.</td>
<td>Peifer, 2012</td>
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<td></td>
<td>USA</td>
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<td>WGS, WES, RNA sequencing</td>
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<td>Japan</td>
<td>44</td>
<td>WES</td>
<td>Mutations in TP53, RBL, PIN.</td>
<td>Iwakawa, 2015</td>
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<td></td>
<td>Germany, Japan and 14 other countries</td>
<td>110</td>
<td>WGS</td>
<td>Activating TP73 rearrangements, CREBBP, EP300 and NOTCH1-J inactivations.</td>
<td>George, 2015</td>
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</tbody>
</table>

Abbreviations: LADC, lung adenocarcinoma; SCLC, squamous cell lung cancer; WGS, whole genome sequencing; WES, whole exome sequencing.
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Table 2  Approved or conducted clinical trials of anti-PD-1/PD-L1 antibodies

<table>
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<tr>
<th>Drug name</th>
<th>Approved (as of 2015 December)</th>
<th>Representative clinical trials for lung cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PD-1 antibodies</strong></td>
<td></td>
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</tr>
</tbody>
</table>
| Nivolumab | Treatment-refractory unresectable melanoma and NSCLC (USA and Japan), and renal cell carcinoma (USA) | Phase III NSCLC (advanced or metastatic)  
Phase II NSCLC (after with Azacitidine and Entinostat)  
Phase I NSCLC (with or without chemotherapy or targeted therapy)  
Phase I ALK-positive NSCLC (with Ceritinib)  
Phase III SCLC (Nivolumab or chemotherapy after platinum-based first line chemotherapy) |
| Pembrolizumab | Treatment-refractory unresectable melanoma and NSCLC (USA) | Phase III NSCLC (Pembrolizumab or platinum-based chemotherapy)  
Phase III NSCLC (PD-L1 positive)  
Phase II NSCLC (brain metastasis)  
Phase I/II NSCLC (with chemotherapy or targeted therapy)  
Phase I ALK-positive NSCLC (with Crizotinib)  
Phase II SCLC (after chemotherapy) |
| **PD-L1 antibodies** |
| MPDL3280A | | Phase II NSCLC (PD-L1 positive, locally advanced or metastatic)  
Phase I NSCLC (locally advanced or metastatic)  
Phase I NSCLC (with Bevacizumab or Bevacizumab and chemotherapy) |
| MEDI4736 | | Phase II/III NSCLC (recurrent stage IIIIB-IV)  
Phase II NSCLC (locally advanced or metastatic)  
Phase I NSCLC (with Gefitinib) |

Fig. 1  The proportion of driver genes in Japanese lung adenocarcinoma (LADC) and approved (in blue) or candidate (in black) targeted drugs against driver gene aberrations. Driver mutations in \(\text{EGFR}, \text{KRAS}, \text{HER2}, \text{and BRAF}\), and driver fusions involving \(\text{ALK}, \text{RET}, \text{and} \text{ROS1}\) were examined in 608 LADC patients who had undergone surgical resection at the National Cancer Center. This figure was modified from our previous report[3].

Pan-negative patients, whose tumors are negative for known driver gene aberrations, account for approximately 30% of all LADC cases. Among such cases, several new driver genes have been identified, such as \(\text{NTRK1}\) fusions[20] and \(\text{NRG1}\) fusions[24, 25]. \(\text{NRG1}\) fusions were preferentially detected in invasive mucinous lung adenocarcinoma. In addition, amplification and aberrant splicing of \(\text{MET}, \text{RIT1}\) mutations, and \(\text{MEK1}\) (\(\text{MAP2K1}\)) mutations were also mutually exclusively detected and
are considered to be new driver genes\(^1,26\). In addition, inactivating mutations in chromatin remodeling genes, such as SMARCA4/BRG1 and ARID1A, are a promising target for synthetic lethal therapy, since several synthetic lethal targets for those mutations have been reported by us and others\(^27,28\).

**SCLC**

SCLC is still a disease for which targeted therapy has not progressed due to the low frequency of activating mutation in oncogenes encoding tyrosine kinases. Because the FGFR (fibroblast growth factor receptor) genes are genetically activated in several cancers, their specific TKIs have been developed to treat the cases with those alterations\(^2\). Amplifications of FGFR1 and activating mutations of FGFR2 and FGFR3 have been identified in SCLC. Furthermore, FGFR3-TACC3 fusion was also identified in a small subset of SCLC cases and is expected to be a therapeutic target\(^29\). Even though these mutations, which drive SCLC development, are not frequently detected, they have a potential to be a new therapeutic target. In fact, several clinical trials have been conducted to examine the efficacy of TKIs for SCLC with FGFR amplifications or mutations\(^30\).

Immunotherapy is one of the major breakthroughs of cancer therapy\(^31,32\). Inhibition of interactions between PD-1 and PD-1 ligands, PD-L1 and PD-L2, overcomes immune resistance and has shown durable clinical responses in various cancers, including NSCLC\(^11,12,33\). Anti-PD-1 drugs were approved for the treatment-refractory unresectable melanoma; nivolumab in the US and pembrolizumab in the US. Nivolumab and pembrolizumab were also approved for chemotherapy-refractory SQCL in the US\(^34,35\) (Table 2). PD-1 blockade therapy has not become a personalized therapy yet due to a lack of biomarkers of responses to anti-PD-1 drugs. However, after the establishment of specific predictors, immunotherapy will be a personalized therapy for SCLC, which has few druggable targets.

**SCLC**

SCLC, which has few druggable targets. However, activating mutation in kinase genes have rarely been identified in SCLC, and no kinase inhibitors have been used in personalized therapy for SCLC.

Blockade of the PD-1/PD-L1 pathway is also another potential therapeutic target in SCLC. A small subset of SCLC cases demonstrated that CD274/PD-L1, which is located on chromosome 9p24.1, showed arm-level or focal amplifications\(^40\). Although it is still unknown whether PD-L1 expression is associated with the response of anti-PD-1 treatment, immune checkpoint blockade therapy is also expected to be a promising targeted therapy for SCLC.

**Clinical genome sequencing for the entry to clinical trials**

A large-scale trial has been started with the aim of finding patients harboring rare driver genes and trying to adapt molecular targeted therapy accordingly. LC-SCRUM-Japan (Lung Cancer Genomic Screening Project for Individualized Medicine in Japan) is one of the successful consortiums that has screened driver genes in advanced NSCLC without EGFR mutations, in over 190 Japanese institutions (http://epoc.ncc.go.jp/scrum/lc_scrum/). After the diagnosis of driver genes, patients with NSCLC harboring RET fusions, ROS1 fusions, ALK fusions or BRAF mutations participated in appropriate clinical trials. A phase II clinical trial, which was designed to evaluate the therapeutic response of vandetanib for advanced RET fusions patient, was named LURET (lung cancer with RET rearrangement study) trial. Because vandetanib was approved by the US Food and Drug Administration (FDA) for the treatment of advanced medullary thyroid cancer that was frequently activated by RET mutations, therapeutic effect against RET fusions is also expected in LADC. In fact, a RET-fusion positive LADC responded to vandetanib in the USA\(^42\).

The nationwide cancer genome screening project by NGS-based was renewed and started as SCRUM-Japan from March 2015. The purpose of this consortium is to find rare targetable genetic aberrations in lung cancer by using a multiplex genetic diagnosis panel (Oncomine® Cancer Research Panel), which investigate aberrations in >100 genes\(^43\). Genomic screenings of SQCL and SCLC have also started.

**Conclusion**

Personalized therapy against driver gene aberrations has been significantly developed for LADC, but not for other types of lung cancer. The need of this therapy will be increasing in all cancer subtypes. A genome screening project has just been started to find patients with driver gene aberrations, and is expected to lead to effective cancer therapy.
Acknowledgment

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References


