Prioritizing mutational profiling for targeted therapy of lung adenocarcinoma

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The Lung Cancer Mutation Consortium (LCMC) was launched as a cross-institutional effort to prospectively follow non-small cell lung cancer (NSCLC) patients across sixteen centers in the US, with a focus on genomic mutation profiles, in an effort to improve the selection of effective therapeutic courses (1). Optimization of personalized therapy has been essential in improving patient care, and is currently highly dependent on comprehensive tumor genome analysis and molecular classification. For instance, personalized treatment of melanoma patients harboring the V600E BRAF variant with BRAF inhibitors (e.g., sorafenib) has become standard-of-care. Yet, despite the relevant number of potential targetable alterations in NSCLC, fully translating genomic testing to the clinic heavily rests on characterizing not only driver mutations in each patient but also potential co-occurring somatic pathogenic mutations that may impact therapeutic selection and responses.

Previously, the LCMC published their first study (LCMC1) reporting on mutation data of 1,007 late-stage NSCLC patients evaluated at 14 institutions (1-3). LCMC1 performed mutational profiling of eight genes: EGFR, KRAS, ERBB2, AKT1, BRAF, MEK1, NRAS, and PIK3CA. Mutations were assessed using SNaPshot, mass spectrometry, Sanger sequencing +/- peptide nucleic acid and/or sizing assays, along with fluorescence in situ hybridization for ALK fusions and/or MET exon 14 skipping mutations. Although the resulting correlations yielded consistent results and novel clinicopathological observations, the use of such genotyping approaches remains contended with drawbacks, to name a few: incomplete coverage of certain targets due to inter-institutional variation, reduced sensitivity, the need for relatively large amounts of nucleic acids and material, as well as unsustainability of mutation analysis techniques due to an increasing number of targetable alterations. High-throughput analysis of actionable mutations with high sensitivity, and specificity, would thus require more pertinent technological platforms such as deep targeted sequencing (next-generation sequencing). Indeed, incorporation of cost-effective deep targeted sequencing technologies is now more far-reaching in the clinic compared with focused or serial testing of individual mutations. Using massively parallel sequencing (MPS), a multitude of studies were spearheaded to efficaciously divide patients into subgroups with targetable oncogenic drivers and who would thus benefit from personalized treatments.

In the present study (LCMC2), the LCMC expanded on their previous efforts now probing for somatic mutations in both targetable (including newly targetable) and non-targetable genes in a cohort of over 900 eligible lung adenocarcinoma (LUAD) patients (4). This expanded gene mutational profiling included deep sequence analysis of AKT, BRAF, EGFR, ERBB2, KRAS, MAP2K1, NRAS, PIK3CA, TP53, STK11, and PTEN, whereby 42% of eligible samples had “full” genotypes for all assessed genes. The study also statistically correlated genomic findings with clinicopathological measures including survival and response to therapy such as EGFR targeted therapy. Further, the
report investigated clinical impact of the mutations/genes in single and in combination, accounting for mutations co-occurring with drivers. Based on collected evidence, the authors show that across all core mutations investigated, patients receiving targeted therapy showed survival benefit compared to those with the same mutations but who did not receive targeted therapy. Such comprehensive and clinical deep targeted sequence analysis provides a roadmap to prioritize genomic alterations and, thus, better personalized therapy.

The authors also report a striking finding with a direct clinical translatability: an indistinguishable survival benefit to targeted therapy between smokers and never-smokers harboring specific EGFR, ALK or ROS1 alterations. Despite their known bias in never-smokers, these EGFR, ALK or ROS1 alterations were also seen among former and current smokers, whose treatment with the corresponding targeted therapy conferred a major survival benefit. Previous studies have shown that while categorization of smoking history, as never, ex-, or current smokers, is inadequate to predict the prognosis of LUAD patients with activating EGFR mutation, cumulative smoking dose classification (based on pack years, i.e., heavy versus light smokers) was a significant predictive factor for disease progression after treatment with tyrosine kinase inhibitors (5). Whether or not such stratification is possible within a cohort of interest, the LCMC2 findings highlight the pressing need to conduct systematic mutational screening regardless of smoking history. More importantly, such findings, once translated into clinically available recommendations for testing targeted alterations, will enable clinicians to update the standard-of-care procedures from diagnosis to treatment.

Mutations in TP53 are present in approximately 50% of all NSCLCs (6). Several of those mutations are reported to be due to smoking history, such as the GC to TA transversion which is strongly correlated with exposure to tobacco carcinogens (7). Indeed, TP53 mutant LUADs harboring a KRAS mutation are defined as an independent and major subset of LUAD, with distinct biology, patterns of immune-system engagement, and therapeutic vulnerabilities (8). However, due to the large number of alterations reported in tumor specimens (at both the transcriptional and post-translational levels), a widely heterogeneous array of their functional consequences has led to an overall ambiguity in the status of TP53 mutations as reliable single prognostic, predictive, or treatment response biomarkers. In LCMC2, the authors show that in LUAD patients harboring EGFR, ALK, or ROS1 mutations, co-occurring TP53 mutations are significantly associated with poorer survival. This correlation was further enhanced when considering disruptive TP53 mutations only. Although actionable gene mutation status in those patients had been identified prior to treatment, additional knowledge of concurrent mutations in individual tumors may have provided valuable insight for clinicians to direct treatment or use alternate first-line therapies. Clinical outcome of TP53 mutation status in response to therapy was also previously shown in patients harboring both KRAS and TP53 mutations, albeit with a favorable prognostic outcome (8). Such findings add TP53 mutations to the genome screening armature predicting drug sensitivities, not only in KRAS-mutant LUADs, but also in those harboring EGFR, ALK, or ROS1 alterations, which are common in never or light smokers (9). This analysis can be further extended to encompass tumors with novel driver mutations and concomitant non-targetable alterations, whose relevance to cancer, despite currently being in the grey zone, can be most informative of response to targeted therapy when investigated in combination with other mutations. For instance, recent data have identified a subset of poor-prognosis KRAS-mutant NSCLC patients enriched with RICTOR alterations. This led to an in vitro and in vivo validation of the synergistic anti-tumor effects of pharmacologic co-inhibition of mTORC1/2 and MEK1/2 (10). Therefore, a deep-sequencing approach to collect mutation evidence in lung cancer patients by MPS, is a promising proof-of-concept for the derivation of targeted agents with preclinical synergistic antitumor activity, by blocking multiple signaling pathways.

Survival benefit in the LCMC2 cohort was also shown in two patients who received targeted therapy for MET amplifications (METamp) in comparison to those who had the same modification but did not receive targeted therapy. Indeed, novel MET inhibitors (crizotinib, cabozantinib, and more recently, capmatinib) are currently being investigated in advanced stage lung cancers, targeting a number of MET genetic lesions such as exon 14 skipping mutations and METamp (11,12). Despite promising preliminary results, NSCLC patient selection for MET targeted therapy is still controversial, particularly in the light of co-occurring driver mutations and combinatorial targeted therapy (e.g., conferring resistance to erlotinib in the presence of a co-occurring EGFR mutation). Of note, survival advantage to targeting the MET pathway is better interpreted when investigating correlations with MET mutation (splice-site mutations in exon 14) or a high level of MET amplification, compared to MET overexpression,
a rather late event consecutive to the transformed phenotypes identified by MPS. This might explain the lack of improved overall survival seen in patients who were selected to receive erlotinib and onartuzumab, the MET-targeting monoclonal antibody, based on positivity of MET immunohistochemistry (IHC) (13). MPS platforms have the potential to fill this void when investigating biomarkers for targeted therapy, particularly since LCMC2 reported that METamp, but not MET positivity by IHC, showed prolonged survival in response to targeted therapy (although with a modest n=2). The need for improved stratification of patients with MET alterations is further supported by Aisner et al.’s observation that METamp presented as a concurrent oncogenic driver in a number of BRAF p.V600E, KRAS, and EGFR-mutated cases in LCMC2. Therefore, deep sequencing data from samples with targetable MET alterations and prior therapy warrant a re-investigation of hitherto underappreciated associations with other co-occurring mutations, such as EGFR, and response to combinatory therapy arms or prior therapy (such as EGFR-targeting therapies, e.g., erlotinib), due to crosstalk between MET signaling and other pathways (EGFR signaling). In fact, the MET cohort is one of many examples where treatment history can be a determinative factor, all the more reason to initiate MPS on patient samples prior to selection of targeted therapy.

The impact of the findings highlighted in this study extend beyond drawing a relationship between the presence of a particular set of mutations and survival in response to targeted therapy in advanced or late stage lung cancer. The need for integrating measures of the immune response (e.g., tumor mutation burden, PD-L1 protein expression) cannot be over-emphasized. Indeed, shortly before writing this editorial, Dr. Jim Allison and Dr. Tasuku Honjo were awarded the 2018 Nobel Prize in Physiology/Medicine for their seminal discoveries of immune checkpoints. It is reasonable to surmise, that personalization of immunotherapy may be augmented by orthogonal studies, such as mutational profiling. NSCLCs, particularly those in former or current smokers, harbor relatively high mutation rates and burdens. Our knowledge of such profiles can inform of tumor-specific antigens and tumor immunogenicity. For instance, TP53 mutant LUADs have been shown to harbor an overall increased immune response including significantly elevated expression of PD-L1 and presence of tumor-infiltrating lymphocytes (TILs) which correlated with improved response to anti-PD-1 antibody pembrolizumab. Whereas STK11 mutations in LUADs alone or co-occurring with PIK3CA or KRAS mutations, were shown to exhibit a muted immune response evidenced by low TILs (8,14). Skoulidis and colleagues also recently showed that STK11-mutant LUADs were in fact relatively resistant to immune checkpoint blockade (8). With the rapid evolution of clinical deep sequencing technologies, the importance of identifying key mutations extends beyond the need to find corresponding targeted therapies or assess eligibility for clinical trial enrollment (15). The utility of mutation testing as a predictor of clinical benefit became plausible and was widely investigated owing to its superior practicality to, for instance, assessment of tumor mutational burden, the downstream mechanistic mediator of immune-evasion. Although patients with prior immune-therapy were not incorporated into LCMC2, possibly due to the novelty of the approach and lack of follow-up data, these reports constitute a paradigm shift in our search for reliable early detection and prediction markers. It is therefore empirical that understanding oncogenic processes should go hand-in-hand with investigating the anti-tumor (or pro-tumor) immune response. Thus, future MPS studies ought to incorporate additional features, such as mutations that impact the interaction between LUADs and the host immune system, tumor mutational burden, the downstream mechanistic mediator of immune-evasion, beyond the need to find corresponding targeted therapies or assess eligibility for clinical trial enrollment (15). The rapid evolution of clinical deep sequencing technologies, the importance of identifying key mutations extends beyond the need to find corresponding targeted therapies or assess eligibility for clinical trial enrollment (15). The utility of mutation testing as a predictor of clinical benefit became plausible and was widely investigated owing to its superior practicality to, for instance, assessment of tumor mutational burden, the downstream mechanistic mediator of immune-evasion. 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a narrower scope of subtypes compared to LUAD patients. This lays the groundwork to investigate the mutational landscape of SCC to determine eligibility for targeted therapies and clinical trials.

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None.

**Footnote**

*Conflicts of Interest:* The authors have no conflicts of interest to declare.

**References**


