Editorial

Prioritizing mutational profiling for targeted therapy of lung adenocarcinoma

Ansam Sinjab[1], Arafat Tfayli[2]

[1]Department of Biochemistry and Molecular Genetics, [2]Department of Internal Medicine, Faculty of Medicine, American University of Beirut, Beirut, Lebanon

Correspondence to: Ansam Sinjab, PhD. Department of Biochemistry and Molecular Genetics, Faculty of Medicine, American University of Beirut, Beirut, Lebanon. Email: as216@aub.edu.lb.

Comment on: Aisner DL, Sholl LM, Berry LD, *et al.* The Impact of Smoking and TP53 Mutations in Lung Adenocarcinoma Patients with Targetable Mutations-The Lung Cancer Mutation Consortium (LCMC2). Clin Cancer Res 2018;24:1038-47.

Submitted Jan 22, 2019. Accepted for publication Jan 28, 2019.
doi: 10.21037/jtd.2019.02.29
View this article at: http://dx.doi.org/10.21037/jtd.2019.02.29

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 targetable 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 (*MET*amp) 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 *MET*amp (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 *MET*amp, 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 *MET*amp 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 combinatorial 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, T-cell receptor clonality and richness, expression of PD-L1 and other immune markers, TILs, and other alterations that could potentially influence clinical outcome in response to immune-based therapies.

Altogether, the LCMC2 sets forth a valid reason to revisit existing MPS data, and integrate NGS platforms into the standard-of-care for screening newly diagnosed patients with lung cancer, irrespective of patient demographics, tumor histologic subtype, or smoking history. Furthermore, co-mutation analysis can provide survival insights valuable for selection of targeted and immune-checkpoint therapy, and prognosis for a wide spectrum of lung cancer patients. Advancing cancer patient care currently rests heavily on our understanding of immunotherapy at the genomic level, and beyond the scope of tumor mutational burden as a sole predictive marker. Similar future investigations have the power to expand the repertoire of candidate mutant genes whose testing ought to be standardized in the clinic, in an effort to improve NSCLC clinical outcome and pathogenesis. This pipeline can also be extended to investigate efficacy of targeted therapy in squamous cell carcinoma (SCC), another major subtype of NSCLC with a more aggressive phenotype. Indeed, a significant portion of SCC patients harbor actionable mutations which fall under

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.

Acknowledgements

None.

Footnote

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

References

1. Sholl LM, Aisner DL, Varella-Garcia M, et al. Multi-institutional Oncogenic Driver Mutation Analysis in Lung Adenocarcinoma: The Lung Cancer Mutation Consortium Experience. J Thorac Oncol 2015;10:768-77.
2. Kris MG, Johnson BE, Berry LD, et al. Using multiplexed assays of oncogenic drivers in lung cancers to select targeted drugs. JAMA 2014;311:1998-2006.
3. Villaruz LC, Socinski MA, Abberbock S, et al. Clinicopathologic features and outcomes of patients with lung adenocarcinomas harboring BRAF mutations in the Lung Cancer Mutation Consortium. Cancer 2015;121:448-56.
4. Aisner DL, Sholl LM, Berry LD, et al. The Impact of Smoking and TP53 Mutations in Lung Adenocarcinoma Patients with Targetable Mutations-The Lung Cancer Mutation Consortium (LCMC2). Clin Cancer Res 2018;24:1038-47.
5. Kim IA, Lee JS, Kim HJ, et al. Cumulative smoking dose affects the clinical outcomes of EGFR-mutated lung adenocarcinoma patients treated with EGFR-TKIs: a retrospective study. BMC Cancer 2018;18:768.
6. Toyooka S, Tsuda T, Gazdar AF. The TP53 gene, tobacco exposure, and lung cancer. Hum Mutat 2003;21:229-39.
7. Pfeifer GP, Denissenko MF, Olivier M, et al. Tobacco smoke carcinogens, DNA damage and p53 mutations in smoking-associated cancers. Oncogene 2002;21:7435-51.
8. Skoulidis F, Byers LA, Diao L, et al. Co-occurring genomic alterations define major subsets of KRAS-mutant lung adenocarcinoma with distinct biology, immune profiles, and therapeutic vulnerabilities. Cancer Discov 2015;5:860-77.
9. Hirsch FR, Scagliotti GV, Mulshine JL, et al. Lung cancer: current therapies and new targeted treatments. Lancet 2017;389:299-311.
10. Ruder D, Papadimitrakopoulou V, Shien K, et al. Concomitant targeting of the mTOR/MAPK pathways: novel therapeutic strategy in subsets of RICTOR/KRAS-altered non-small cell lung cancer. Oncotarget 2018;9:33995-4008.
11. Frampton GM, Ali SM, Rosenzweig M, et al. Activation of MET via diverse exon 14 splicing alterations occurs in multiple tumor types and confers clinical sensitivity to MET inhibitors. Cancer Discov 2015;5:850-9.
12. Paik PK, Drilon A, Fan PD, et al. Response to MET inhibitors in patients with stage IV lung adenocarcinomas harboring MET mutations causing exon 14 skipping. Cancer Discov 2015;5:842-9.
13. Spigel DR, Edelman MJ, O'Byrne K, et al. Results From the Phase III Randomized Trial of Onartuzumab Plus Erlotinib Versus Erlotinib in Previously Treated Stage IIIB or IV Non-Small-Cell Lung Cancer: METLung. J Clin Oncol 2017;35:412-20.
14. Kadara H, Choi M, Zhang J, et al. Whole-exome sequencing and immune profiling of early-stage lung adenocarcinoma with fully annotated clinical follow-up. Ann Oncol 2017;28:75-82.
15. Adams DR, Eng CM. Next-Generation Sequencing to Diagnose Suspected Genetic Disorders. N Engl J Med 2018;379:1353-62.

Cite this article as: Sinjab A, Tfayli A. Prioritizing mutational profiling for targeted therapy of lung adenocarcinoma. J Thorac Dis 2019;11(Suppl 3):S216-S219. doi: 10.21037/jtd.2019.02.29

