Editorial

Plasma-derived immune-related factors as biomarkers of osimertinib resistance in EGFR-mutant non-small cell lung cancer patients

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Non-small cell lung cancer (NSCLC) constitutes the vast majority of all lung cancer cases in the world (1). In Caucasians, up to 15% of all NSCLC tumors harbor activating mutations (most commonly deletions in exon 19, and the L858R missense mutation) in the gene encoding the epidermal growth factor receptor (EGFR) with prevalence being 3–4 times higher in NSCLC tumors in Asians (2). NSCLC patients with activating mutations in *EGFR* are amenable for targeted therapy with tyrosine kinase inhibitors (TKIs). First- and second-generation TKIs exhibit improved response rates compared to platinum-based therapy. However, inevitably, resistance to treatment emerges in all tumors. Resistance to first-generation adenosine triphosphate (ATP)-competitive EGFR TKIs, erlotinib and gefitinib, is commonly associated with an acquired gatekeeper mutation, T790M, resulting in increased affinity for ATP (3,4). This resistance mutation can be targeted by the third-generation EGFR TKI, osimertinib. Osimertinib is an irreversible and covalent EGFR TKI that targets activated EGFR receptors irrespective of presence of the T790M mutation. Osimertinib received approval by the U.S. Food and Drug Administration (FDA) as a second-line therapy in 2017 followed by approval as a first-line therapy by FDA in 2018 for NSCLC patients with activating mutations in EGFR (5,6). The benefit from treating EGFR-mutant NSCLC patients with osimertinib compared to other EGFR TKIs is apparent by documented improved progression-free survival and overall survival. Nevertheless, resistance is inevitable and accompanied by additional EGFR kinase domain mutations, e.g., C797S, and genetic lesions in other genes, including aberrations in *PIK3CA*, *MET*, *HER2* as well as aberrations in the genes encoding for cell cycle cyclins and cyclin-dependent kinases (CDKs). Intriguingly, up to 50% of all treatment resistance following osimertinib cannot be linked to specific genetic changes, arguing for a complex biological interplay involving alterations in the RNA and protein landscape of the tumor cells as well as surrounding cells in the tumor microenvironment (7). This emphasizes an urgent need for the discovery of new biomarkers of osimertinib resistance, especially biomarkers on the protein and RNA level.

Discovery of new biomarkers of osimertinib resistance requires repeated biopsy sampling in carefully supervised patient cohorts. Moreover, tumor heterogeneity will play a major role in any sampling from solid tissue biopsies. In recent years, liquid biopsies have come into play due to their non-invasive nature and feasibility for repeated sampling. In particular, repeated blood sampling is becoming a common resource for profiling of circulating DNA, RNA and/or protein. While multiple studies have mapped mutations in circulating DNA during the treatment course of osimertinib, there is a scarcity of data when it comes to longitudinal mapping of the protein and RNA landscape (8,9).

In a recent publication, Maansson *et al.* reported that seven immune-related proteins could predict overall survival of EGFR-mutant NSCLC patients following disease progression after osimertinib treatment (10). The study by Maansson *et al.* was based on repeated plasma sampling of 25 patients receiving osimertinib, either in the first-line (two patients) or second-line (23 patients) setting (10). The authors collected blood at two time-points for each patient, an osimertinib response sample and a disease progression sample. Plasma was isolated from all samples followed by protein analysis using a 92-oncology associated protein proximity extension assay panel. By comparing samples from osimertinib response with disease progression, the authors identified 7 differentially expressed proteins. Six out of seven proteins were downregulated at disease progression [CD27 antigen (CD27), CD70 antigen (CD70), C-X-C motif chemokine 13 (CXCL13), tumor necrosis factor ligand superfamily member 6 (FASLG), ICOS ligand (ICOSLG) and T-lymphocyte surface antigen Ly-9 (LY9)] while Nectin-4 (NECTIN4) was upregulated at disease progression. Patients with low NECTIN4 expression exhibited improved overall survival compared to patients with high NECTIN4 expression while none of the other proteins could independently predict survival (10). Moreover, the authors performed gene ontology (GO) enrichment analysis in relation to the differentially expressed proteins resulting in multiple GO-terms associated with the adaptive immune system. Lastly, the authors divided patients into an "immune-high" versus an "immune-low" group and compared overall survival. The authors concluded that patients with a low amount of immune-related proteins at disease progression were more likely to exhibit a prolonged survival (10).

The results by Maansson *et al.* are supported by the results from a similar recent study conducted by Alexeyenko *et al.* (11). In this study, the authors performed full transcriptomic profiling of plasma-derived exosomal RNA from 20 longitudinally sampled EGFR-mutant NSCLC patients treated with osimertinib in the second-line setting. Samples were taken at treatment baseline, instead of at osimertinib response, and at disease progression (11). Maansson *et al.* reported multiple GO-terms associated with the immune system at disease progression following osimertinib treatment (10), while Alexeyenko *et al.* reported the enrichment of 16 immune-related gene-sets at disease progression following osimertinib treatment (11). Given the differences in molecular profiling approaches between the two studies (targeted panel of 92 proteins versus systematic transcriptomics), it is remarkable that both studies conclude around factors of the immune landscape as potentially central biomarkers in osimertinib resistance. This emphasizes a future need of conducting multilayer molecular analysis of all specimens in studies aiming to uncover new biomarkers of therapy resistance. It is heavily debated in the scientific field whether expression changes in protein-coding mRNAs are functionally reflected in the protein landscape. The results from Maansson *et al.* and Alexeyenko *et al.*, derived from plasma profiling on two separate, but similar, collection of samples, argue for a positive correlation in expression between circulating mRNAs and circulating proteins. However, the 7 differentially expressed proteins highlighted by Maansson *et al.* was not individually picked up as differentially expressed on the transcript level by Alexeyenko *et al.*, possibly due to heterogeneity in combination with a limited sample size (10,11). Heterogeneity is likely to have a major negative impact in studies with limited amount of samples. In the study by Alexeyenko *et al.*, approximately 100 transcripts (out of 100,000 detected transcripts) displayed differential expression when comparing all baseline samples versus all disease progression samples. The immune-related gene sets were uncovered following a network enrichment approach taking both altered gene sets and functional gene sets into consideration (11).

The studies by Maansson *et al.* and Alexeyenko *et al.* are underpowered (25 and 20 patients, respectively) and are lacking independent validation cohorts with identical analysis approaches (10,11). Nevertheless, the results highlighted by Maansson *et al.* and Alexeyenko *et al.* calls for further biomarker investigation of the immune landscape in prospective and larger patient cohorts of EGFR-mutant NSCLC treated with osimertinib (10,11). Furthermore, future studies would benefit from systematic approaches for the mapping of both proteins and RNA, and should preferably include single-cell sequencing of solid tumor biopsies before and after osimertinib resistance. A combination of longitudinal tumor and liquid biopsies with state-of-the-art profiling techniques would be enormously powerful in determining the impact of immune-related factors as biomarkers of osimertinib resistance.

There is currently no obvious therapy choice following treatment failure to osimertinib. It is plausible that at least a subset of osimertinib refractory NSCLC would respond favorably to immunotherapy, if selected with appropriate biomarkers. However, this requires a detailed molecular assessment in larger clinical cohorts where changes in

expression of immune-related factors are correlated to osimertinib treatment and immunotherapy in patients with EGFR-mutant NSCLC.

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