Circulating tumour DNA and resistance mechanisms during EGFR inhibitor therapy in lung cancer

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The lung cancer patient had tolerated well the first few cycles of her treatment. Her breathlessness improved but the pain in the ribcage was still difficult to control. The computer tomography scan showed enlargement of the bone lesions and regression of the primary tumour. Would you continue this treatment or would you change it?

Tumour heterogeneity has been recognized in oncological practice since its outset. It influences treatment and follow up decisions, but that is one of the challenges of the oncologist work: to travel through the grey scale of cancer's variegation. Tumour heterogeneity and the genomic instability that maintains it are thus major challenges in cancer management with potential influence on the length of treatment response and patient prognosis.

The biological evidence of cancer's intrinsic heterogeneity and clonal evolution was shown in 1978 by Fidler (1), using a xenograft model with melanoma cell lines, and in recent years it was elegantly ratified in lung cancer and other cancer types, using gene sequencing of multiple tissue biopsies (2,3). In spite of this, pathologists still have to work with one single portion of the heterogeneous cancer tissue to generate key data ahead of patient management. But technology is catching up and this is now bound to change.

Plasma and other bodily fluids carry in healthy and diseased individuals free RNA and DNA. Already in 1948, Mandel and Metais (4) reported the presence of highly fragmented DNA, known now as circulating free DNA (cfDNA). The presence of this cfDNA was later attributed to pathologies promoting tissue breakdown (5), and in 1977, Leon *et al.* reported high levels of cfDNA in cancer patients (6).

This circulating tumour DNA (ctDNA) is influenced by the tumour volume and cancer cell turnover, but overall it can reflect all relevant genetic alterations of the tumor and the various degrees of prevalence at different time-points. To implement this in standard practice there are only a few (but significant) obstacles to overcome.

The technological approach is not standardized. The broad range of ctDNA isolation techniques, DNA analysis and quantification (7) creates its own issues when comparing with different studies. Most of these options are now relatively cheaper than for example, a CT scan, and results are available within a few days after blood collection. Unfortunately, the fast paced technological progress leads to confusion as to which technique should be adopted in clinical practice. A standard in technological options, quality control validation and result interpretation is required.

Another challenge is the need to specify the markers for each clinical setting. The role of ctDNA can embrace a broad range of clinical scenarios:

- Early cancer diagnosis;
- Patient monitoring after radical treatment;
- Identification of predictive biomarkers of response to aid treatment selection;
- Early detection of markers of resistance to aid treatment discontinuation while the therapeutic window is still open for the next treatment line.

The latter is exciting to both clinicians and scientists in that given the right parameters and evidence it may be useful during patient treatment follow up, perhaps contributing to guide the need for radiological assessments,

reducing the costs of patient follow up and aid treatment discontinuation decisions. Besides, these samples may prove a valuable source of information to identify novel resistance mechanisms and their evolution over time; personalized medicine requires regular biological observations and parallel translational research.

We now have evidence that drug resistance mechanisms may be more complex than previously anticipated. Chabon *et al.* (8) analyzed plasma samples of a highly selected and pre-treated group of 43 patients before and after the novel EGFR inhibitor rociletinib. All these patients had progressed after first line TKI treatment and carried a T790M mutation. This is a population that would currently be amenable to treatment with osimertinib, a specific EGFR T790M mutation targeted treatment. Anyhow, rociletinib is currently tested within the TIGER-3 phase III study in EGFR mutated patients regardless of T790M status.

The method of choice was cancer personalized profiling by deep sequencing (CAPP-Seq) described in 2013 by Newman *et al.* (9). CAPP-Seq uses information stored in public gene databases such as COSMIC and TCGA to identify and specifically target multiple genomic regions recurrently mutated in a given cancer at once, lowering costs and increasing sensitivity and specificity with only minimal amounts of input DNA. Chabon *et al.* selected 252 target genes and postulate they may have a biological role in rociletinib resistance.

The authors highlight a high concordance in EGFR mutation results between tissue and blood samples: 95% for activating EGFR mutations and 91% for T790M mutations, which confirms the strength of CAPP-Seq. The authors reference previous tissue-based studies where new genetic alterations on recurrence were observed in 5–10% of patients. Here the prevalence of gene modification rose to 46% in ctDNA. The authors use this to highlight the importance of ctDNA above single biopsies, but this conclusion requires further evidence: the contrast could have been influenced by patient selection variability, disparity in the effects of each drug, differences in target gene selection, diverse sequencing techniques and non-identical data analysis. Nevertheless, as described by Newman *et al.* (9), this confirms the existence of a method to detect a high number of genetic alterations in plasma of lung cancer patients.

Putative resistance gene modifications were identified in 68% of patients. Their data shows that copy number gains in MET, ERBB2 and EGFR correlate with innate resistance, whilst acquired resistance to rociletinib

correlates mainly with emergent or increasing single nucleotide variants (SNVs). However, somatic copy number alterations (SCNAs), alone or in combination with SNVs, also appeared during patient treatment in lower numbers. Interestingly, one of these SNVs, the EGFR C797S mutation, “the most common acquired resistance to osimertinib”, was only present here in 2% of patients progressing on rociletinib, suggesting different drug-dependent resistance mechanisms.

The authors then proceed to perform *in vitro* and *in vivo* experiments to assert MET overexpression as a putative negative predictive biomarker of response to rociletinib and a potential positive biomarker of response to combination therapy using rociletinib and crizotinib.

It is understandable that the authors did not explore the evolution of SNVs further in their paper, as the plasma samples were obtained mainly at two different time points on 43 patients, and to discern a pattern of evolution a large number of sequential samples would be required.

These SNVs could just represent markers of a clonal population shift. Some could be directly or indirectly related to mechanisms of drug resistance to rociletinib or to EGFR inhibition therapy in general. In any case, the pattern of emergence of these mutations during treatment could also bring some light to the biological evolution of cancers during treatment, and contribute to inform clinicians on the right timing for treatment discontinuation. In view of the low incidence of EGFR mutated lung cancers in non-Asian populations, this could only be achieved by building a standardized infrastructure for consistent and systematic plasma, data collection and analysis parallel to daily oncological practice.

As oncological therapeutic options are now increasing in lung cancer, it would be unfortunate if the novel biological advances were ignored and quantifiable patient follow up continues to rely exclusively on radiological assessment. Novel advances in personalized medicine require an urgent adaptation of standard pathological testing, but this can only happen if we embrace the potential of utilising ctDNA in clinical practice and strengthen the links between clinicians, pathologists and translational scientists.

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Footnote

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