Detection of epidermal growth factor receptor mutations in circulating tumor DNA: reviewing BENEFIT clinical trial

Umberto Malapelle¹, Luis E. Raez², Maria Jose Serrano³,⁴, Christian Rolfo⁵; International Society of Liquid Biopsies (ISLB)

¹Department of Public Health, University Federico II of Naples, Italy; ²Memorial Cancer Institute/Memorial Health Care System Florida International University, Miami, Florida, USA; ³Integral Oncology Division, Clinical University Hospital, Granada, Spain; ⁴Liquid biopsy and metastasis research group, GENYO, Centre for Genomics and Oncological Research, Pfizer/University of Granada/Andalusian Regional Government PRTS, Granada, Spain; ⁵Marlene and Stewart Greenebaum Comprehensive Cancer Center, University of Maryland School of Medicine, Baltimore, Maryland, USA

Correspondence to: Prof. Dr. Christian Rolfo, MD, PhD, MBA, hc. University of Maryland Marlene and Stewart Greenebaum Comprehensive Cancer Center, University of Maryland School of Medicine, Baltimore, Maryland, USA. Email: christianrolfo@umm.edu.

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The identification of epidermal growth factor receptor (EGFR) sensitizing mutations plays a key role in the management of non-small cell lung cancer (NSCLC) patients (1-4). Tissue specimens are not always available, due to difficulty in obtaining enough material, despite the fact that more than 70% of NSCLC patients are diagnosed in the metastatic stage (5,6) due to multiple reasons. In this setting, prior to the administration of any biological or chemotherapy treatment regimen, liquid biopsy could represent a valid alternative to tissue specimens, taking in consideration the need to use appropriate molecular techniques. In addition, after treatment with first and/or second-generation tyrosine kinase inhibitors (TKIs), the arising of EGFR resistance mutations (in particular the EGFR exon 20 p.T790M) allows the treatment with third generation TKI (e.g., osimertinib) (7). Moreover, the collection of new tissue specimens after a first line treatment to detect EGFR resistance mutations and other druggable genetic alteration is challenging and time consuming. For these reasons, the European Medicines Agency (EMA) and Food and Drug Administration (FDA) approved the analysis of EGFR status on circulating tumor DNA (ctDNA) extracted from plasma samples in new NSCLC patients without tissue availability or after resistance to first and/or generation TKIs for the detection of EGFR exon 20 p.T790M (8,9).

The main challenge is represented by the low frequency of ctDNA, passively or actively released by tumor, into the bloodstream (<0.5%) respect to the total released cell free DNA (cfDNA) (6,10,11). Careful attention was paid on the technologies to adopt in order to avoid false positive (high specificity) and/or false negative (high sensitivity) results (6). In most of the clinical trials the principal methodology adopted was real-time polymerase chain reaction (RT-PCR) and the digital droplet PCR (ddPCR) (9-11). More recently, in other experiences, a next generation sequencing (NGS) approach was evaluated to achieve better results in term of sensitivity and specificity by using as a gold standard the EGFR mutational status evaluated on tissue samples (9-13).

Recently, in Lancet Respiratory Medicine journal, Wang et al. showed the results of an open-label, single arm, prospective, multicentre, phase 2 clinical trial (BENEFIT) with the aim to assess the feasibility of EGFR detection, by ddPCR, in ctDNA extracted from pre-treated NSCLC adenocarcinoma (ADC) patients’ plasma, for the administration of gefitinib as first-line treatment (14). From December 25, 2014 to January 16, 2016, 188 out
of 426 screened patients showed an \textit{EGFR} sensitizing mutation and received gefitinib. In this study the primary end point was the objective response rate (ORR), and the secondary aim was the assessment of progression free survival (PFS), disease control, overall survival (OS) and safety and tolerability of gefitinib. Of these 188 patients, 180 (95.7\%) had the \textit{EGFR} mutation both on ctDNA and tissue samples, the remaining 4.3\% showed the mutation only on ctDNA. ORR was 72.1\% (95\% CI: 65.0–78.5\%) and the median PFS was 9.5 months (95\% CI: 9.07–11.04\%).

Other studies have analyzed prospectively the ctDNA but the \textit{EGFR} mutational assessment on ctDNA was not adopted as an inclusion criterion (12,15). Another study adopted a retrospective approach: Karachaliou \textit{et al.} analyzed N=76 blood samples with a multiplex 5` nuclease RT PCR (Taqman) assay to be used in the presence of a peptide nucleic acid (PNA) clamp (12). They showed, in particular, a negative prognostic value of the \textit{EGFR} exon 21 p.L858R when detected on ctDNA respect to \textit{EGFR} exon 19 deletions (12). In the experience of Mayo-de-Las-Casas \textit{et al.}, 1,026 blood samples, without tissue specimens, of NSCLC patients at baseline were analyzed by a multiplex 5` nuclease real-time PCR (Taqman) assay to be used in the presence of a PNA clamp for wild-type (15). In this study the authors demonstrated that on a large-scale testing of an unselected population the clinical outcomes to TKIs in blood are undistinguishable from those obtained in tumour tissue.

Also, Kimura \textit{et al.} showed on 42 paired blood and tissue samples, by using Scorpion Amplification Refractory Mutation System (ARMS), that it is feasible to use DNA extracted from serum to assess \textit{EGFR} mutational status on ctDNA is suitable for the administration of TKIs. The main limitation of the discussed study is based on the use of molecular techniques with a limited multiplexing power: in fact, also if the modified RT PCR (such as PNA-RT PCR) reaching a good level of sensitivity, showed a poor level reference range, not being able to identify all the possible clinically relevant gene alterations (13).

In a retrospective experience by Malapelle \textit{et al.} showed that a narrow NGS panel (SiRe®) is a feasible tool for ctDNA analysis in clinical practice, and by adopting ctDNA extracted from both plasma and serum they obtained a sensitivity of 90.5\% and a specificity of 100\% (13). As shown by Wang \textit{et al.} by using digital droplet PCR approach, also Pisapia \textit{et al.} by using ultra—deep NGS, in a prospective series showed a key role of the analysis of ctDNA for \textit{EGFR} molecular assessment and subsequently treatment decision for NSCLC patients in the basal setting (patients at diagnosis without tissue availability), but in addition to Wang \textit{et al.}, in the experience of Pisapia \textit{et al.}, in addition to the \textit{EGFR} mutational status is also possible the simultaneous evaluation of other clinical relevant gene alteration in NSCLC patients, such as \textit{KRAS}, \textit{NRAS} and \textit{BRAF} mutations (9). Raez \textit{et al.} showed that in real life even doing NGS we not always get all the genetic aberrations in tissue (17). Comparing tissue with liquid biopsies (Guardant 360) in NSCLC patients, this group showed that we were able to get molecular markers in tissue in only 78\% of the cases mainly due to insufficient tissue, and we needed liquid biopsies to complement the other 22\% of the patients, moreover up to 1/3 of all the actionable mutations were found only in plasma NGS but not in tissue NGS (17).

Beyond the role of specific molecular techniques (Figure 1), considering all together (Table 1) the data obtained from
the experiences analyzed in this editorial and taking in to account the recent evidences obtained from the FLAURA clinical trial, a phase 3 trial that confirmed in new patients with a sensitizing EGFR mutation (ex19del or p.L858R), that osimertinib has a high efficacy respect to gefitinib or erlotinib as first-line treatment in advanced NSCLCs, the role of EGFR mutation assessment on ctDNA in basal setting became even more important in clinical setting respect to the role that today have to define the second line treatment after an EGFR TKI first generation administration (18-20).

In conclusion, as shown in the analyzed studies, the identification of EGFR mutations in ctDNA plays a crucial role to better identify patients who might benefit from TKIs treatment option comparably with the data obtained starting from tissue extracted DNA. In particular, following the approval of osimertinib as first line treatment, the analysis of EGFR mutational status on ctDNA recovered from basal setting NSCLC patients may represent a key weapon in patient population without tissue specimens form molecular testing, taking into account the implementation of more sensitive techniques, such digital PCR and ultradeep NGS.

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**Footnote**

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

**References**


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### Table 1 Different clinical trials evaluating the role of EGFR mutational assessment on ctDNA to select NSCLC patients for TKIs treatment administration

<table>
<thead>
<tr>
<th>Reference</th>
<th>Patients enrolled (patients with paired tissue samples)</th>
<th>Drug</th>
<th>Blood collection</th>
<th>Methodology</th>
<th>Median OR (%)</th>
<th>Median PFS (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wang et al. (14)</td>
<td>188 [188]</td>
<td>Gefitinib</td>
<td>Streck cfDNA blood collection tubes (Streck, Omaha, NE, USA)</td>
<td>ddPCR</td>
<td>72.1</td>
<td>9.5</td>
</tr>
<tr>
<td>Karachaliou et al. (12)</td>
<td>76 [76]</td>
<td>Erlotinib</td>
<td>Vacutainer tubes (BD, Plymouth, UK)</td>
<td>Multiplex 5´ nuclease RT-PCR (Taqman) assay to be used in the presence of a PNA clamp for wild-type</td>
<td>65.3</td>
<td>12.3</td>
</tr>
<tr>
<td>Mayo-de-Las-Casas et al. (15)</td>
<td>1,026 [0]</td>
<td>Different TKIs</td>
<td>Vacutainer tubes (BD, Plymouth, UK)</td>
<td>Multiplex 5´ nuclease RT-PCR (Taqman) assay to be used in the presence of a PNA clamp for wild-type</td>
<td>72</td>
<td>11</td>
</tr>
<tr>
<td>Kimura et al. (16)</td>
<td>42 [42]</td>
<td>Gefitinib</td>
<td>NR</td>
<td>Scorpion ARMS</td>
<td>NR</td>
<td>NR</td>
</tr>
</tbody>
</table>

OR, objective response; PFS, progression free survival; cfDNA, cell free DNA; ddPCR, digital droplet polymerase chain reaction; RT-PCR, real-time PCR; PNA, peptide nucleic acid; TKIs, tyrosine kinase inhibitors; ARMS, Amplification Refractory Mutation System; NR, not reported.