

# Epidermal Growth Factor Receptor (EGFR) T790M Testing in EGFR-mutated Non-Small Cell Lung Cancer: A Successful Model of Personalized Cancer Care Beyond Resistance

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**A**lthough non-small cell lung cancer (NSCLC) is the leading cause of cancer-related death worldwide [1], not all subgroups of NSCLC share the same prognosis. The understanding of the genomic alterations in different subtypes of NSCLC and the development of tyrosine kinase inhibitors (TKIs), altered the diagnosis, management, and prognosis of specific subtypes of NSCLC. The most common types are the *epidermal growth factor receptor* (EGFR) mutations and the anaplastic lymphoma kinase (ALK) rearrangement [2].

EGFR is a transmembranous receptor of the HER family of tyrosine kinase receptors [3]. Somatic activating mutations in the adenosine triphosphate (ATP)-binding region of the EGFR leads to signal-independent activation of signal transduction, promoting proliferation and development of metastases. Approximately 10–15% of the patient population from Western societies with advanced NSCLC harbor EGFR mutations. Mutations are found more frequently in adenocarcinoma histology, in women, in people from East Asia, and in those with light or no history of smoking [4].

The development of EGFR TKIs has revolutionized the management and the

prognosis of this subgroup of patients. EGFR TKIs bind effectively to lung cancer cells harboring EGFR mutations at the level of the ATP-binding site [5], which leads to better response rate and progression-free survival (PFS) compared with chemotherapy among this group of patients [6–10]. Until recently, there were three EGFR TKIs approved for first line treatment: gefitinib, erlotinib, and afatinib. These TKIs showed similar efficacy [11] and overall favorable toxicity profiles [12]. The expected overall response rate to EGFR TKI is in the range of 60–70% [7,11], compared to a response rate of 40–50% to standard chemotherapy. PFS improves with treatment with EGFR TKIs compared to chemotherapy (hazard ratio [HR] of 0.3–0.42) [13]. The prognosis of patients with NSCLC harboring the EGFR mutation is better than the patients not harboring the mutation, with median overall survival of up to 33 months [14] compared to approximately 1 year in all stage IV NSCLC.

Nevertheless, as in all cases of targeted cancer therapy, resistance to treatment is inevitable, and occurs within a median of 10–16 months [15]. The research into the EGFR TKI resistance mechanisms includes a successful model of translational research leading to development of a new efficacious therapy in a short period of time. Research of repeated biopsies from EGFR-positive NSCLC patients with disease progression after treatment with first and second generation of EGFR TKIs revealed a unique pattern of resistance. There was activation in several pathways, including MET amplification, HER-2 amplifica-

tion, and even small-cell transformation; however, approximately 60% of patients who progressed following treatment with EGFR TKI were found to have secondary resistance point mutation p.Thr790Met (T790M) in the gene encoding the EGFR [15,16]. This unique and common resistance mutation allowed for the development of new targeted therapy – osimertinib – an irreversible EGFR-TKI that is selective for both the initial EGFR mutation and T790M resistance mutation. Osimertinib demonstrated favorable response rates and a good safety profile on early phase trials of EGFR-positive NSCLC patients harboring the EGFR T790M mutation following treatment with first and second generation EGFR TKIs. The phase 3 trial of osimertinib versus the standard chemotherapy in second line for EGFR T790M positive NSCLC confirmed the results of the earlier trials, with response rates of 71% vs. 31% and PFS of 10.1 months vs. 4.4 with chemotherapy. These favorable results of the trial established the role of osimertinib as the new standard of care for second line treatment of EGFR-positive NSCLC harboring the T790M mutation [17].

Circulating tumor DNA (ctDNA) is part of cell-free DNA (cfDNA)-fragmented DNA found in the noncellular component of the blood, originating from tumor cells. Liquid biopsy refers to obtaining plasma, or any other type of body fluids containing ctDNA. The ctDNA test taken by a liquid biopsy, which is a simple blood test, offers the obvious advantage of being non-invasive, but it can also better represent the tumor heterogeneity [18]. The AURA3 trial

included preplanned analysis of the PFS of patients whose *EGFR* T790M testing was conducted by liquid biopsy and ctDNA analysis, which demonstrated comparable results, and allowed for the wide clinical use of this non-invasive method.

In this issue of the *Israel Medical Association Journal (IMAJ)*, Makarov and colleagues [19] looked retrospectively at the real-world results of osimertinib treatment following positive tissue or plasma *EGFR* T790M tests, and compared the outcomes of patients diagnosed with tissue biopsy to patients diagnosed with ctDNA. As was observed in the AURA3 trial, Makarov's group found no difference in the treatment outcomes, response rate, PFS, and overall survival, regardless of the method of *EGFR* T790M testing.

Treatment outcomes were also comparable to the prospective outcomes reported. Their study strengthens the validation of routine ctDNA testing as a method that can routinely be conducted by local laboratories. It is not limited to central laboratories as in the prospective trial.

Israel is one of the few countries in which all patients with NSCLC harboring the *EGFR* mutation have access to both plasma and tissue testing for *EGFR* T790M at disease progression on first and second generation *EGFR* TKIs, with validated next generation sequencing or ddPCR testing routinely conducted by clinical molecular pathology laboratories. With the good access to testing since 2016, it is not surprising that Makarov's study reported a shift in the trend of the testing, in which two-thirds of the *EGFR* T790M tests were conducted by liquid biopsy. It is the current standard of care to refer the patient to the less invasive liquid biopsy first.

However, ctDNA testing has a 30% false negative rate, and sometimes there are not sufficient ctDNA levels to allow for mutational profiling from plasma. In addition, the level of ctDNA is expected to be low when disease burden is mostly in the brain, a common site of metastases in *EGFR*-positive NSCLC [18]. When the ctDNA is negative, a confirmatory tissue biopsy is required. The study by Makarov

et al. does not indicate the percentage of positive tests from all *EGFR* T790M tests conducted on tissue and plasma samples, nor is the false negative rate for patients who underwent more than one test for diagnosis of the *EGFR* T790M mutation compared, although the researchers noted that all 33 patients who tested positive underwent only one diagnostic test.

Osimertinib is the current standard of care for second line treatment of *EGFR*-positive NSCLC harboring the *EGFR* T790M mutation, although the FLAURA trial studied osimertinib in the first line setting of *EGFR*-mutated NSCLC versus first generation *EGFR* TKIs. That study reports longer PFS of 18.9 versus 10.2 months.

Osimertinib has been reimbursed by health funds in Israel since January 2019 as the first-line setting for all *EGFR*-positive NSCLC patients. For patients treated with osimertinib as the first-line setting, no *EGFR* T790M testing is required on resistance, although treatment with osimertinib creates a new landscape of resistance, with data currently emerging and indicating new mutations and resistance pathways, detected by tumor and ctDNA biopsies, including *EGFR* C797S mutation, acquired *KRAS* mutation, *RET* fusion, and *MET* amplification, all of which are still not targetable today other than for clinical trials [20].

The *EGFR*-positive NSCLC subgroup is a successful model of clinical and translational research tracking for ongoing changes in the molecular landscape of the tumor and allows for treatment development to target the resistance. Ongoing trials are trying to find the next generation of targeted therapy in osimertinib resistance (NCT03539536). Other molecular subgroups of NSCLC, such as *ALK* and *ROS1*, did not demonstrate a specific targetable resistance pattern as the common T790M mutation, although ongoing clinical and translational research is trying to better characterize the resistance pathways and find appropriate therapeutic strategy to prolong the patient's life.

#### Conflict of interest

Mor Moskovitz reported personal fees for consulting, honorarium, or advisory services

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### Capsule

#### In the RELM $\alpha$ realm

Alternatively activated macrophages (AAMs) are critical to many different immune responses. **Krljanac** and co-authors developed a tool to track and characterize AAMs based on expression of the immunomodulatory protein resistin-like molecule a (RELM $\alpha$ ). They generated RELM $\alpha$  reporter/deleter mice and observed that RELM $\alpha$ + macrophages were enriched in white adipose tissue, gut, and peritoneum at steady state. Primary infection with the helminth *Nippostrongylus brasiliensis* induced expansion of RELM $\alpha$ + lung interstitial

macrophages but not alveolar macrophages in a signal transducer and activator of transcription 6-dependent manner. The presence of RELM $\alpha$ + macrophages was required for protection from fatal primary infection and resistance against secondary infection. Thus, RELM $\alpha$  provides a marker of AAMs and plays a role in defense against helminth infection in the lung.

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### Capsule

#### IRF2 induces gasdermin D for pyroptosis

Inflammasome activation triggers the cleavage of the protein gasdermin D. The N-terminal fragments oligomerize and form pores in the plasma membrane, leading to the release of the inflammatory cytokine interleukin-1 $\beta$  (IL-1 $\beta$ ) and cell death by pyroptosis. **Kayagaki** et al. found that loss of the transcriptional regulator IRF2 reduced gasdermin D levels in mice and in human cells, resulting in decreases in IL-1 $\beta$

secretion and pyroptosis in response to inflammasome activation. Thus, like gasdermin D, IRF2 might also be a therapeutic target for the treatment of sepsis and other inflammasome-mediated diseases.

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### Capsule

#### Liquid crystal immune sensing

Systemic sclerosis (SSc) is an autoimmune disease of the connective tissue thought to be the result of aberrant innate immune signaling. CXCL4, a platelet-derived chemoattractant for neutrophils, monocytes, and fibroblasts, can serve as a biomarker for this disease. **Lande** et al. reported that high CXCL4 levels found in SSc patients correlates with plasmacytoid dendritic cell (pDC) activation and interferon- $\alpha$  production. They show that CXCL4 organizes both self and

foreign DNA into liquid-crystalline supramolecular complexes in a DNA size-dependent manner. These complexes chaperone nucleic acids into pDCs and allow for optimal clustering and activation of the innate immune sensor Toll-like receptor 9. Beyond SSc pathogenesis, these findings may illuminate the role of platelets in wound healing and tissue repair.

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**“Fear is a disease that eats away at logic and makes man inhuman”**

Marian Anderson (1897–1993), singer