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Australian Recommendations for EGFR T790M Testing in Advanced Non-Small Cell Lung Cancer

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Abstract

First generation *Epidermal Growth Factor Receptor (EGFR*) tyrosine kinase inhibitors (TKIs) are used as first line therapy in patients with non-small cell lung cancer (NSCLC) harboring a sensitizing mutation in the *EGFR* gene. Unfortunately, resistance to these therapies often occurs within 10 months of commencing treatment and is mostly commonly due to the development of the *EGFR* T790M mutation. Treatment with the third generation *EGFR* TKI, osimertinib can prolong progression free survival (PFS) in patients with the T790M mutation, so it is important to determine the resistance mechanism in order to plan ongoing therapeutic strategies. Here we review the evidence and make recommendations for the timing of T790M mutation testing, the

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most appropriate specimens to test and the available testing methods in patients progressing during treatment with first line *EGFR* TKIs for NSCLC.

Key words:

T790M mutation, advanced NSCLC, *EGFR* TKI

Background

As in other jurisdictions, in Australia, *epidermal growth factor receptor* (*EGFR)* mutation testing is performed to determine eligibility for treatment with *EGFR* tyrosine kinase inhibitors (TKIs), which are recommended as first line therapy in patients with non-small cell lung cancer (NSCLC) whose tumors harbor a sensitizing mutation in the *EGFR* gene.[1] Australian Clinical Practice Guidelines for the Treatment of Lung Cancer recommend that the *EGFR* TKIs, gefitinib or erlotinib, should only be used in patients with tumors shown to have such an *EGFR* mutation.[1]

Improved response rate (RR) and prolonged progression free survival (PFS) have been demonstrated in patients with an *EGFR* sensitizing mutation treated with an *EGFR* TKI compared with chemotherapy. Despite this, several mechanisms of acquired resistance to EGFR TKI therapy have been described.[2] The commonest is the development of the T790M mutation, reported in about 60% of patients.[3, 4] Such resistance usually develops between 8 and 10 months after starting *EGFR* TKI therapy, although the range can be quite variable.[3] Recently, a third-generation *EGFR* TKI, osimertinib, that is effective in tumors harboring the T790M mutation, was approved in Australia for patients with NSCLC harboring the *EGFR* T790M mutation following progression on an *EGFR* TKI.[5] Currently, in the Asia-Pacific region, osimertinib is also approved in Japan, South Korea, Taiwan, Hong Kong, Singapore and China. With this treatment option available, it is pertinent to review the role of T790M mutation testing in patients progressing on first line *EGFR* TKIs, the potential for circulating tumor DNA (ctDNA) rather than tissue biopsy material as the preferred tumor DNA source, and propose recommendations for *EGFR* T790M testing and subsequent management of NSCLC.

While acquired resistance to *EGFR* TKI treatments is most commonly associated with the introduction of a second site mutation within the *EGFR* kinase domain (such as T790M), alternate mechanisms exist including amplification and mutation of alternative kinases (such as *MET,* HER2 and PI3 Kinase), histologic transformation from NSCLC to Small Cell Lung Carcinoma (SCLC), and epithelial to mesenchymal transition (EMT).[6] Altered pharmacokinetics or low drug levels may also lead to failure of response.[7] This review will focus primarily on T790M, the commonest resistance mechanism and detail testing strategies and management for these patients.

When is the best time to test for the T790M mutation?

The incidence of the T790M mutation in sequential biopsy samples has been reported as 52% at the first post-TKI biopsy.[8] In clinical practice, the detection rate of the secondary mutation before radiological progression is low and results are of little practical value while the patient is still deriving benefit from treatment. Additionally, first line *EGFR* TKI treatment would not currently be changed until at least radiological progression was observed. Therefore, testing to detect emerging resistance before progression remains a topic of research interest and is not yet appropriate in clinical practice. In addition, the potential for serious complications is important when considering a repeat biopsy.

Recommendations for when to test

1. T790M testing should be conducted following radiological progression on a first line *EGFR* TKI therapy (Figure 1).
2. Repeat plasma sample or tissue biopsy should be considered when initial sample quality is low, the original activating mutation is not identified or results are inconclusive.

Which samples should we use to test for EGFR Mutation Testing?

Tissue Biopsy

The probability of detecting a T790M mutation in a tumor biopsy is dependent on the site and burden of the disease, intra- and inter-tumor heterogeneity, and previous exposure to chemo-radiation. Identifying the most active and accessible lesions for biopsy using computed tomography (CT) or CT-PET (positron emission tomography) scans can improve the likelihood of suitable biopsy sites and of therefore confidently identifying mutations.

Tumor tissue is the preferred source for testing but at the time of progression, only about 50% of patients are well enough and have an accessible site for biopsy.[9] The rate of pneumothorax with percutaneous needle biopsy is between 0% and 35%, though for endobronchial ultrasound (EBUS) guided peripheral biopsy is less than 2%.[10] Bleeding is rare with EBUS and uncommon with percutaneous FNA (<1%), although can be catastrophic should this occur.[11] Despite these risks, tumor sampling remains the current "gold standard" for detection of resistance mutations, but also in documenting histological type. Rarely SCLC transformation results in resistance, but can only be diagnosed on tissue biopsy.

Cytology Samples

According to IASLC guidelines, EGFR mutation testing can be performed on fine needle aspirate (FNA) samples, core needle biopsies and resection specimens.[12] Endobronchial Ultrasound (EBUS) allows concurrent diagnosis and staging of lung cancer in a single procedure, is minimally invasive, and accurate.[13]

In a study by IASLC of 702 patients with diagnosed NSCLC, the yield of cells for definitive cellular subtype classification and EGFR mutation analysis was compared between surgical (resection) and non-surgical procedures. These included histologic biopsies (endobronchial and transthoracic cores), cytology samples from radiologically guided transthoracic needle aspiration (TTNA) and conventional transbronchial needle aspiration (TBNA).[14] Unsatisfactory results for EGFR analysis (insufficient cells or failed mutation assay) were found in 1.8% of surgical resections, 10% (95% CI 2.2 – 27.4%) of TTNA and 18% (95% CI 8.6 – 31.4) of core biopsies. The yield from non-guided TBNA was relatively low with unsatisfactory molecular results in 30% (95% CI 18.5 – 42.6%). The yield from other cytology procedures (bronchoalveolar lavage and pleural fluid) were comparable in cellular subtype diagnosis and mutation yield not discussed. *EGFR* mutation testing of either primary tumor or lymph nodes also showed high concordance suggesting that lymph node samples can be effectively used for *EGFR* mutation testing.[14]

FNA and core biopsies have equivalent success for diagnosis of lung cancer, although the quantity of tissue obtained is often greater from core biopsy.[10] In terms of mutational testing, concordance rates of between 93.1% and 96.6% have been reported for cytology samples from bronchofiberscopic brushing and formalin-fixed, paraffin embedded (FFPE) samples across five different *EGFR* mutation assays.[15] Mutations have been detected in cytology samples with low DNA concentrations where matching FFPE samples were assessed as mutation negative.[15]

The choice of sampling method may be dictated by the lesion presentation and location, however well sampled, radiographically guided cytology samples are suitable for diagnosis and molecular analysis if sufficient cellular material is obtained.

Bone Lesions

Bone biopsy samples are generally the least preferred site given the low yield of tumor cells and poor DNA quality. The use of strong acids to decalcify specimens degrades DNA often rendering the sample unsuitable for subsequent mutational testing. However, this can be improved by taking the biopsy from the soft tissue components of bony metastases and gentle decalcification using ethylenediaminetetraacetic acid (EDTA). Discussion with the laboratory at the time of specimen collection may help prioritize the sample for molecular analysis.

Liquid Biopsies

Liquid biopsies can utilize tumor DNA from bodily fluids such as blood, cerebrospinal fluid or urine to test for mutations.[16, 17] Whole blood contains circulating tumor DNA (ctDNA) released into the circulation from necrotic or apoptotic tumor cells in the primary tumor, metastatic sites or circulating tumor cells (CTCs). ctDNA accounts for a small but variable proportion of the circulating cell free DNA (cfDNA), which is derived from normal tissues, where increasing tumor burden can be correlated with increases in the cfDNA fraction detected in the plasma. Thus, the plasma fraction of blood can be used as a surrogate for tissue in for the detection of the T790M mutation. Recent data from the LUX-Lung studies, suggested that plasma DNA may be an alternative to lung biopsy for *EGFR* testing, however detectable mutations were only identified

in plasma in 60.5% of cases, and the ability to detect these was greatest in those with more advanced disease and poorer prognosis.[18]

In addition to obtaining plasma samples for cfDNA testing, urine and cerebrospinal fluid (CSF) have been shown to be a potential source of ctDNA for molecular testing.[17, 19] Concordance between plasma and both FFPE tumor specimens and urine specimens was higher than between FFPE tumor specimens and urine specimens. Patients with evidence of central nervous system (CNS) disease are more likely to demonstrate mutations in the CSF whereas their plasma may be negative for the tumor related mutations.[17]

Currently, the NCCN guidelines recommend the use of liquid biopsy as an alternative to tissue biopsy for initial T790M mutation testing.[6] If the plasma biopsy is negative then tissue biopsy is recommended if feasible. Limitations of using blood-based testing include taking blood using a larger gauge needle instead of a butterfly to reduce hemolysis and ensuring the sample is processed by the laboratory within four hours. Stabilized tubes such as Streck can be used to prolong the time to processing to 14 days by avoiding white blood cell lysis and associated DNA contamination.

Detection of T790M in plasma has been reported up to 15 to 344 days before disease progression based on RECIST criteria. Regular sampling to detect resistance could enable clinicians to institute more effective treatment options earlier.[20] But it remains unclear whether new therapy affects response rates if radiological disease progression has not been seen and further studies are required to answer this question.

Recommendations for type of tissue to test

1. Plasma testing for resistance mutations if available. If negative or indeterminate result, continue to step 2.
2. Radiologically or EBUS guided tumor biopsy samples or FNA.

- Bone biopsy samples are the least preferred method for T790M testing.

Which is the most effective testing method for the T790M Mutation on ctDNA?

Various technologies are being used and further refined to test for *EGFR* resistance mutations, including T790M. Some are similar technologies to those used for initial detection of *EGFR* mutations in tissue, although modified to improve sensitivity.[21] The processing of samples however is of critical importance given the low level of ctDNA in plasma, special stabilized blood tubes are preferred and higher sensitivity testing methods are required. These methods are technically more challenging and no standardized methods are currently available. Likewise,

there are no clear guidelines for validation, cut- off thresholds, implementation and interpretation of ctDNA testing.. Platforms available include real-time quantitative polymerase chain reaction (RT-qPCR), mass array, massive parallel sequencing (MPS), digital droplet PCR (ddPCR), and beads, emulsion, amplification and magnetics (BEAMing).[22] There are advantages and disadvantages for each of these techniques in their sensitivity, specificity, their ability to multiplex, cost, turnaround times and whether they are quantitative or semi-quantitative. The choice of any assay is dependent on local circumstances, technical expertise and instrument availability.

The RT-qPCR methods include the ARMS, cobas® and Idylla *EGFR* tests which are standardized, relatively inexpensive and technically straightforward; and have been used in a number of trials for T790M detection, with cutoff and interpretation set within the systems. The mass array PCR Ultraseek Panel (Agena) has been developed also with sensitivity down to 0.1% and includes multiple mutations in multiple genes analyzed in one test. Various commercial next generation sequencing or MPS gene panels are available and in development, however these are generally complex since they focus on clinically relevant mutations in multiple genes sequenced thousands of times to increase sensitivity. MPS generally require a higher input of DNA to gain the same sensitivity as BEAMing or digital PCR. Digital PCR offers faster turnaround times than MPS with high sensitivity, around 0.1%, for T790M detection in ctDNA and FFPE tissue, but this technology generally analyses only for single mutations per test, such as the T790M or the known driver mutation.[21]

Nevertheless, both real-time qPCR cobas® *EGFR* Mutation Test v2 and BEAMing dPCR have demonstrated high sensitivity (82 -87%)for T790M mutation detection.[23] The US Food and Drug Administration (FDA) have approved the former for *EGFR* mutation testing of liquid biopsies as a companion diagnostic to osimertinib in the detection of T790M mutations.[24]

Although sensitivity needs to be improved, next generation sequencing methods allow detection of other potential gene mutation variants responsible for the resistance mechanisms, including point mutations in other genes, rearrangements and copy number variants. Advances in this area are progressing with improved methodological developments.

T790M mutation testing methods are technically challenging. Further research is needed to develop clear testing guidelines and cutoff sensitivities for assays. It is unclear at what level in different assays a T790M mutation is likely to predict response to osimertinib. For example, for ddPCR the current accepted positive is the demonstration of a certain number of positive droplets over wildtype control droplets. However, would fewer droplets also predict for benefit? Currently the cut-offs for the assays are determined around the inherent error of each test and not the predictability of response to a T790M inhibitor.

The value in testing for the driver mutations in addition to the T790M resistance mutation is important as it represents an internal sensitivity control for the assay. If the driver is not present, then it is possible that the amount of any ctDNA present in the plasma is below the level of detection for the assay and the result for T790M is indeterminate. This does not exclude a

T790M mutation causing progression and a tissue biopsy would be indicated in this instance. Further, it is important to consider a repeat biopsy or cytology sample when T790M mutation testing is negative. This is the only method that can confirm small cell transformation, which will still retains the original driver mutation. [25] The repeat biopsy sample can also be used to retest for T790M mutations in addition to other resistance mutations.

Third Generation EGFR TKI treatment for Patients with T790M mutation

Patients with NSCLC who have acquired resistance, not due to T790M mutation may continue to receive erlotinib, gefitinib or afatinib.[6] In the case of resistance due to *MET* amplification, an additional inhibitor may be added to the existing *EGFR* inhibitor, although data supporting the effectiveness of such an intervention are limited. Treatment with *EGFR* TKIs beyond progression has been shown to be beneficial in selected patients after the development of resistance whereas discontinuation can lead to rapid progression. It is important therefore to continue the initial TKI until the next treatment strategy can be determined.

Osimertinib (AZD9291) was approved in Australia in August 2016 for the treatment of patients with locally advanced or metastatic *EGFR* T790M mutation-positive NSCLC.[5] The approval of osimertinib in the USA and subsequently in Australia was based on the AURA trials. [26, 27]

In the initial AURA phase I trial, osimertinib at doses of 20mg, 40mg, 80mg, 160mg or 240mg were studied.[26] Heavily pretreated patients with the T790M mutation had a median PFS of 9.6 months (95% CI, 8.3 to not reached) and response rate of 61% (95% CI, 52 to 70). In patients negative for the T790M mutation the median PFS was 2.8 months (95% CI, 2.1 to 4.3) and response rate was 21% (95% CI, 12 to 34). It is notable that most of the patients who responded to osimertinib but were T790M negative, were later shown to be T790M positive using an alternative testing method. The most common adverse events were diarrhea (47%), rash (40%), nausea (22%), and reduced appetite (21%). The frequency of diarrhea and rash was dose-dependent.

The AURA3 randomized controlled trial compared osimertinib to platinum-pemetrexed chemotherapy in T790M positive patients who had progressed on first line *EGFR* TKI therapy.[27] The study showed significantly longer PFS with osimertinib (10.1 vs. 4.4 months; HR 0.30; 95% CI, 0.23 to 0.41; P<0.001). This improvement in PFS included patients with central nervous system (CNS) metastases.

Osimertinib is also recommended for patients with T790M mutation who have progressed with symptomatic brain metastases.[6]

Recommendations for therapy in patients with *EGFR* T790M mutation

Patients with *EGFR* T790M mutation positive NSCLC, who have demonstrated progression after prior *EGFR* TKI therapy, should initiate treatment with osimertinib.

Immunotherapy

Given the excitement surrounding immunotherapy, it is important to clarify the role of resistance mutation testing for patients progressing on first generation TKIs. PD-1 / PD-L1 (checkpoint) inhibitors are recommended by the NCCN as subsequent therapy after second disease progression in patients with advanced NSCLC and performance status score of between zero and two who have PD-L1 expression level ≥1%.[6] In Australia, nivolumab is approved in both adenocarcinoma and squamous cell NSCLC irrespective of PD-L1 expression.

However, it is becoming clearer that immunotherapy appears to be less efficacious in patients whose tumors harbor an *EGFR* mutation. In subgroup analyses of three large second line NSCLC immunotherapy trials, *EGFR* mutation rates in the POPLAR, Checkmate-057, and Keynote-010 studies were reported as 11%, 14%, and 8.3% respectively.[28-30]

In Keynote-010, which included only PD-L1 positive patients, the median PFS was significantly increased with pembrolizumab alone compared with platinum-based chemotherapy (10.3 months vs. 6.0 months; hazard ratio (HR) for disease progression or death, 0.50; (95% CI, 0.37 to 0.68; P<0.001)).[31] In the POPLAR study, atezolizumab treatment resulted in statistically significant and clinically relevant improvement in OS (HR 0.73; P = 0.0003) compared with docetaxel and regardless of PD-L1 expression levels.[32]

When the *EGFR* mutant subgroup was investigated, overall survival did not improve with the use of immunotherapy with either pembrolizumab (PFS HR 0.88; 95% CI 0.45-1.70) or nivolumab (HR 1.18; 95% CI 0.69-2.00) compared with docetaxel.[28, 29] Resistance inhibitors such as osimertinib should therefore be considered ahead of immunotherapy.

Barriers to implementation

Although there are now impressive data for the use of ctDNA for detection of the T790M mutation, tumor biopsy analysis remains the standard of care, with ctDNA testing considered only as an alternative. Tumor biopsy testing has the advantage of allowing the detection of small cell change as well as assessment of other mutational changes causing for resistance. However, heterogeneity within the tumor and rates of T790M positivity at different metastatic sites can vary widely.

Moreover, data from both the AURA and TIGER studies, which investigated rociletinib, a now discontinued T790M specific inhibitor, suggest that patients with a positive result from either blood or tissue are likely to respond to third generation TKI therapy.[19, 27] However, ctDNA results would be available more quickly and at a lower cost, allowing faster access to treatment without the need for an invasive biopsy. Indeed, access to radiology services varies between hospitals and states, with typical wait times ranging from two to six weeks. Differences have also been observed between private and public sector services, where waiting times may be longer in public hospitals which have resource constraints such as availability of day procedure beds, access to interventional radiology services, and access to bronchoscopy. The increased

number of biopsies required on progression may cause concern within individual hospitals, with the need for additional resource and subsequent increased waiting times. A turnaround time of less than seven days which is possible with ctDNA testing would allow oncologists to manage patient visits and expedite initiation of appropriate treatment.

Although there are centers with ctDNA testing capability in Australia, more widespread availability of T790M testing needs to be developed with validation and quality assurance processes established. Nevertheless, the ability to use Streck tubes that stabilize ctDNA for up to 14 days enable samples to be sent nationally and internationally to centers of excellence, a process that is being utilized especially in rural and remote areas.

Lastly, the ability to quantify ctDNA could be used for ongoing monitoring of tumor response to treatment in selected patients. Should this be shown to be beneficial, ctDNA could act as a specific tumor marker which may thereby minimize the need for radiological imaging. Further research into the clinical applicability for ctDNA is ongoing, however these assays promise to deliver better personalization of therapy at relatively minimal cost.

Next Steps

Education for oncologists about resistance to first generation TKIs and next generation therapies is required. The third generation EGFR TKI osimertinib is well tolerated and effective and importantly prolongs median PFS by nearly six months compared to platinum-pemetrexed chemotherapy. [27] There are currently no guidelines for T790M testing available globally; although ASCO guidelines are expected shortly for public consultation. Guidelines for biopsies include the International Association for the Study of Lung Cancer (IASLC) and the Thoracic Society; the Cancer Council are looking at developing local guidelines for Australia.

Regular communication between the medical oncologist, respiratory physician or radiologist and the pathology department can optimize the speed and accuracy of reporting. Providing the patient's clinical background and the reason for the test on the request form avoids wasting tissue on unnecessary tests, leaving adequate tissue for the tests of most clinical relevance.

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Figure 1 Summary of Recommendations