Best practices in epidermal growth factor receptor T790M testing for advanced non–small-cell lung cancer in Hong Kong

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ABSTRACT

The T790M mutation in the epidermal growth factor receptor gene causes most acquired resistance to first- or second-line epidermal growth factor receptor–tyrosine kinase inhibitors in advanced non–small-cell lung cancer. The results of T790M testing can guide subsequent treatment. Despite the availability of guidelines from international organisations, T790M testing practices in Hong Kong must be streamlined and adapted to the Hospital Authority setting. To address this issue, a panel of experts in oncology and pathology met for discussion of key topics regarding T790M testing practices in Hong Kong, including the appropriate timing of testing and re-testing, as well as optimal testing methods. All panel members voted on the results of the discussion to achieve consensus. Items supported by a majority vote were adopted as consensus statements regarding current best practices for T790M testing in Hong Kong. Among the topics discussed, the panel agreed that T790M testing should be initiated upon radiological progression, including symptomatic disease progression or central nervous system–only progression. The experts also preferred initial testing with liquid biopsy, using the widely available digital polymerase chain reaction platform. This document provides the final consensus statements, as well as a testing and treatment workflow, for clinicians in Hong Kong to use as guidance in T790M testing.

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Introduction

Epidermal growth factor receptor (EGFR)–directed tyrosine kinase inhibitors (TKIs) are recommended as first-line therapy for non–small-cell lung cancer (NSCLC) carrying a sensitising mutation in the *EGFR* gene. Compared with platinum-based chemotherapy, first- and second-generation EGFR-TKIs have shown superior efficacy; they are regarded as the standard of care for advanced NSCLC.[1,2] However, acquired resistance to EGFR-TKIs eventually occurs, leading to disease progression.[3] The T790M substitution mutation in exon 20, present in 50% to 60% of cases, is the most frequent cause of resistance to first- and second-generation EGFR-TKIs.[4-6] According to a laboratory report in Hong Kong (Sanomics, unpublished data presented in a meeting on 27 June 2019), the proportion of T790M-positive NSCLC cases across seven hospitals under the Hospital Authority from 2017 to 2020 (n=3398) ranged from 19.0% to 32.8%.

In cases of resistance to first- and second-generation TKIs, next-line therapeutic options were limited prior to the introduction of osimertinib, a TKI selective for *EGFR*-sensitising mutations and the T790M resistance mutation. Osimertinib received accelerated Food and Drug Administration approval in the United States in November 2015, along with a companion diagnostic test for the T790M mutation; it received full approval in March 2017 based on the results of the AURA3 study.[7] The AURA trials were included in a clinical investigation of osimertinib as second-line therapy in T790M-positive NSCLC.[7] AURA3 was a randomised, open-label phase 3 trial that enrolled patients with T790M-positive advanced NSCLC refractory to first-generation TKIs (n=419). The study showed that progression-free survival

was significantly longer and the response rate was significantly higher in patients receiving osimertinib than in patients receiving platinum-pemetrexed chemotherapy.[8] Osimertinib is now regarded as the standard of care for patients with T790M-positive tumours and acquired TKI resistance.[1,2,9]

Molecular analysis of T790M mutation status should be performed upon progression of *EGFR*-mutated NSCLC to identify patients for whom osimertinib would be an appropriate next-line option. The DNA used for testing can be obtained via repeated biopsy of tissue, or by analysis of circulating tumour DNA (ctDNA) circulating in blood or other body fluids (ie, liquid biopsy).[10] Technologies available for T790M detection in tissue and body fluids include real-time polymerase chain reaction (PCR)–based methods such as the Cobas and Therascreen tests, and digital PCR (dPCR) platforms such as droplet digital PCR (ddPCR) and BEAMing (beads, emulsions, amplification, magnetics).[11] Next-generation sequencing (NGS) is a high-throughput sequencing method that can simultaneously analyse variable regions of the genome and detect somatic mutations (eg, single-nucleotide variations, copy number variations, and insertion/deletions or gene fusions); the method can also be used to detect the T790M mutation as well as other genomic alterations that cause EGFR-TKI resistance.[9]

Guidelines published in the past several years have outlined recommendations for T790M testing within the context of a region's reimbursement policy, hospital system, and laboratory infrastructure.[12,13] To provide guidance to local hospitals under the Hong Kong Hospital Authority, a panel of Hong Kong experts was convened to discuss current practices in T790M testing and adaptations to promote optimal patient outcomes. This report summarises the resulting consensus statements, while proposing an algorithm for T790M testing and subsequent NSCLC treatment, which is intended to serve as a guidance for clinicians regarding best practices in *EGFR* T790M testing.

Methods

A panel of seven oncologists and a pathologist was convened to participate in the development of a consensus document regarding best practices in T790M testing in Hong Kong. During the initial face-to-face meeting, the panel members reviewed current *EGFR* T790M testing practices in Hong Kong Hospital Authority hospitals, then discussed relevant evidence and practical considerations. After the identification of knowledge gaps and differences in T790M testing practices within Hong Kong, the panel proposed key questions regarding the timing and procedures of testing, along with relevant clinical scenarios.

A second meeting was convened to discuss the best practices for T790M testing, in response to the key questions previously drafted. After each member's queries and comments had been considered by the panel, the members indicated their agreement with, or selection among, the responses presented (online supplementary Appendix). If the majority of the panel agreed with a response, it was regarded as a current best practice and adopted as a consensus statement. Individual members' comments based on practical experience in the field were integrated with the chosen responses to formulate the final consensus statements.

香港的晚期非小細胞肺癌表皮生長因子受體T790M測試的最佳做法

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表皮生長因子受體基因的T790M突變在晚期非小細胞肺癌的一線或二線表皮生長因子受體的酪氨酸激酶抑制劑造成大多數的獲得性抗藥性。T790M的測試結果有助引導其後的治療。雖然多個國際組織已訂立指引，但香港的T790M測試做法必須簡化，並須因應醫院管理局的情況而調整。為回應這議題，一群腫瘤及病理學專家聚首討論關於本港的T790M測試做法的重要題目，包括適合進行測試及重測的時間及最佳的測試方法。所有專家小組成員就討論結果投票以達成共識，獲得大比數票數的項目被採納為本港T790M測試的現行最佳做法的共識聲明。在眾多討論題目中，專家小組同意T790M測試應該按放射學進展而開展，包括有症狀的疾病進展或僅與中樞神經系統有關的進展。各專家亦偏好使用大行其道的數碼聚合酶鏈反應平台以活體液態方式進行初次測試。本文提供最終的共識聲明以及測試及治療流程，供本港醫生用作T790M測試的指引。

Recommendations

When to test for the *EGFR* T790M mutation

The panel members unanimously agreed that patients with *EGFR*-mutated NSCLC treated with first- or second-generation TKIs should undergo T790M testing upon radiological disease progression (eg, asymptomatic progression, symptomatic disease progression, or central nervous system [CNS]–only progression) [Table 1 and Fig]. Testing is warranted because these events indicate progression that may require modified treatment. Although the underlying premise of T790M testing involves assessing eligibility for third-line TKI inhibition, clinicians should concurrently investigate the feasibility of local therapy for oligoprogressive disease.

Biochemical progression (eg, an increasing carcinoembryonic antigen [CEA] level) may prompt clinicians to perform additional investigation of tumour status; however, biochemical progression alone is insufficient to indicate a need for T790M testing. Radiological progression is usually defined by

the Response Evaluation Criteria in Solid Tumours, which are typically used for objective assessment of tumour burden in clinical trials.[3] Testing is indicated upon radiological progression, but panel members acknowledged that the definition of radiological progression may differ among clinicians.

Patients with symptomatic disease progression can experience rapid deterioration; thus, immediate assessment of T790M mutation status is needed to plan subsequent treatments that are likely to confer benefit, such as osimertinib.[14,15] Similarly, regardless of the patient's clinical state (symptomatic or asymptomatic), immediate testing is indicated for CNS-only progression because the condition carries a poor prognosis. Patients with T790M-positive CNS progression may also benefit from osimertinib, which has compelling efficacy data with respect to CNS metastases, including asymptomatic cases.[16-18] In the AURA3 trial, osimertinib showed superior CNS efficacy compared with platinum- or pemetrexed-

TABLE 1. When to test for the T790M mutation

T790M testing should be conducted when patients experience radiological progression, symptomatic disease progression, or central nervous system–only progression.
Supplementary statements
1. Oligoprogression alone is insufficient to initiate T790M testing.
2. An elevated carcinoembryonic antigen level alone is not a reliable marker of progression and should not motivate T790M testing.

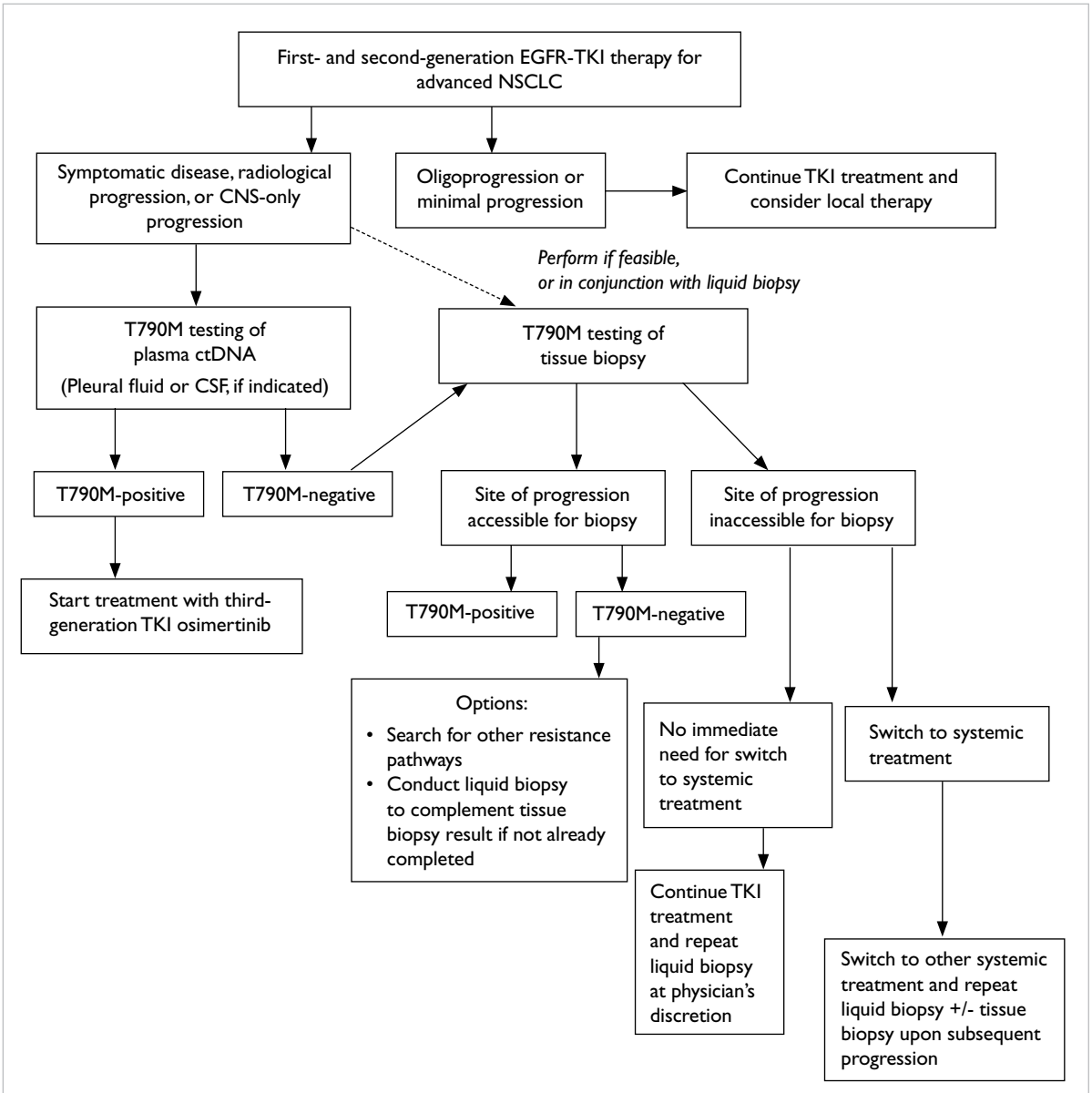

FIG. Proposed algorithm for T790M testing in Hong Kong

Abbreviations: CNS = central nervous system; CSF = cerebrospinal fluid; ctDNA = circulating tumour DNA; EGFR = epidermal growth factor receptor; NSCLC = non–small-cell lung cancer; TKI = tyrosine kinase inhibitor

based chemotherapy; it also demonstrated activity against leptomeningeal metastasis.[16,18] In the phase 3 FLAURA study, osimertinib had superior CNS efficacy compared with gefitinib or erlotinib.[17]

Oligoprogression (new lesions or regrowth in a few areas)[19] alone does not warrant T790M testing and can be managed by local ablative therapy. Local therapy may prolong disease control. For example, two studies of patients with oligometastatic NSCLC while on standard TKI therapy revealed a median time to progression of 6.2 to 10.0 months from the initiation of local therapy and continuation of previous TKI.[20,21]

The panel members agreed that CEA level is not a reliable marker of disease progression[1,2]; CEA analysis alone should not be used to determine the need for T790M testing. However, an elevated CEA level suggests that disease progression should be closely monitored by other investigation methods. The level may be elevated in conjunction with radiological progression; consideration of CEA level and any evidence of radiological progression can help clinicians to determine subsequent management.

How to test for the *EGFR* T790M mutation

Initial testing

All panel members supported the use of liquid biopsy for initial T790M testing (Table 2). Liquid biopsy was the preferred method because it allows non-invasive assessment of tumour biology, is readily available, and has a short turnaround time.[9,10,22] Conditions that may support the use of liquid biopsy as the first choice for T790M testing include limited tumour tissue availability, low tissue sample quality, poor patient health that precludes tissue biopsy, and patient refusal of tissue biopsy.[1,2,9,10] Published guidelines from Australia, the United States National Comprehensive Cancer Network, and the European Society for Medical Oncology also recommend liquid biopsy for initial T790M testing.[1,9,13]

A liquid biopsy is generally conducted by collecting plasma ctDNA. The detection of *EGFR* mutations in plasma ctDNA has high concordance with tissue-based detection (up to 74%).[11] Analyses of plasma ctDNA have high specificity but moderate sensitivity; thus, negative plasma results should be confirmed by tissue biopsy.[9,10,13,23]

Other biological fluids (eg, pleural fluids and cerebrospinal fluid [CSF]) can be used to provide ctDNA for liquid biopsy. The majority (88%) of panel members would send pleural fluid (when available) for liquid biopsy. The *EGFR* mutations can be detected via ctDNA from pleural effusion fluid; however, if a sufficient number of cells is collected, cell block analysis may be an alternative diagnostic method.[24,25]

The majority (80%) of panel members would also request CSF-based liquid biopsy in the event of CNS metastasis. Cerebrospinal fluid is suitable for ctDNA analysis of tumour mutations in patients with CNS metastasis or leptomeningeal metastasis.[26-28] Although CSF sampling for T790M testing requires the invasive lumbar puncture procedure, CSF is considered an accessible representation of *EGFR* mutation status in the brain and leptomeningeal metastases, which are typically inaccessible; therefore, CSF analysis is regarded as a useful adjunct to plasma analysis.[26,27]

If a tissue sample is available, tissue sample–based T790M testing can be performed in parallel with liquid biopsy–based testing. This approach is supported by the Canadian guideline panel, the International Association for the Study of Lung Cancer, and a Pan-Asian group that adopted the European Society for Medical Oncology guidelines.[2,10,12]

TABLE 2. How to test for the epidermal growth factor receptor T790M mutation

Initial testing Liquid biopsy should be performed first to identify T790M mutations in non–small-cell lung cancer with progression or acquired resistance to EGFR-TKIs.
Supplementary statements 1. T790M testing using a tissue specimen from a site of progression (if available) may be performed in parallel with liquid biopsy. 2. In some clinical situations, pleural fluid and cerebrospinal fluid may be appropriate samples for liquid biopsy.
Re-testing for the *EGFR* T790M mutation Perform tissue biopsy if the result of the initial liquid biopsy is T790M-negative.
Supplementary statements 1. Liquid biopsy with a different platform may also be regarded as a re-test. 2. Tissue biopsy remains necessary as a re-test if only sensitising mutations were detected during the initial liquid biopsy. 3. If re-testing cannot be conducted via tissue biopsy, assess the patient's clinical condition, then either continue therapy with the EGFR-TKI or switch to chemotherapy. 4. If treatment was continued without re-testing via tissue biopsy, perform repeated liquid biopsy upon progression or after around 8 weeks. 5. Perform liquid biopsy if the result of the initial tissue biopsy is T790M-negative.

Abbreviation: EGFR-TKI = epidermal growth factor receptor–tyrosine kinase inhibitor

The panel members agreed that all tissue samples for T790M testing should be collected from accessible and untreated sites of progression. Any type of tissue is acceptable, except necrotic tissue. Furthermore, if a bone lesion sample is used for biopsy, it should have minimal decalcification to ensure that DNA quality is sufficient for molecular analysis.

Tissue biopsy–based analyses have some limitations. For example, lung biopsy is an invasive procedure with potential complications, such as intrapulmonary haemorrhage and pneumothorax.[29,30] Additionally, intratumour and intermetastatic heterogeneity in biopsied tissue may lead to false-negative results.[31]

Re-testing

The panel members agreed that re-testing should be performed if the initial liquid biopsy is T790M-negative. Considering that plasma liquid biopsy has a false-negative rate of 30%, tissue biopsy is warranted to confirm T790M mutation status if the result of the initial plasma liquid biopsy is T790M-negative (Table 2).[23] Failure to detect the original sensitising mutation via liquid biopsy may be related to various factors, including suboptimal sample preservation or a non-secretory tumour, and further testing is highly recommended. If the initial liquid biopsy was performed with a less sensitive assay (eg, real-time PCR), a more sensitive assay such as dPCR or NGS should be considered. If tissue biopsy is indicated, it should be collected from a site of progressive disease.

Confirmatory re-testing is intended to guide clinicians in the selection of appropriate therapy; although tissue biopsy is the preferred re-test approach, factors such as site accessibility, patient symptoms, and performance status should be considered when determining re-test timing. The following treatment options may be suitable alternatives to early tissue repeated biopsy: continue EGFR-TKI therapy and perform repeated liquid biopsy later, or switch to chemotherapy and perform repeated liquid biopsy upon progression.

The optimal timing for repeated liquid biopsy is unknown. Most panel members (86%) would perform repeated liquid biopsy if there was evidence of further progression, including worsening symptoms. In contrast, for asymptomatic patients or patients with slowly progressing disease who continued to receive EGFR-TKI therapy, panel members suggested a minimum of 8 weeks between repeated liquid biopsies. In real-world setting, 8 weeks is the typical interval for further progression from the time that a patient continues TKI therapy after the first progression event; further progression at that time would suggest a need for systemic treatment, rather than TKIs. Additionally, in phase 2 studies, tumour assessments are typically performed at around 8-week intervals to coincide with the end of a treatment cycle.[32] For example, in the phase 2 ASPIRATION study that included a cohort of patients with advanced NSCLC who continued TKI therapy after progression, plasma analysis was generally conducted every 8 weeks. The study showed that the median time between the first and the second progression events was approximately 3 months.[33]

Most panel members (88%) agreed that, when tissue biopsy is used as the initial test, a T790M-negative result should be confirmed by liquid biopsy. Although the standard of care constitutes tissue biopsy using an adequate sample from a site of progression, tumour heterogeneity may lead to a false-negative result. Subsequent liquid biopsy using ctDNA may complement the T790M-negative findings of initial tissue biopsy.

The most effective method for *EGFR* T790M testing

In Hong Kong, plasma samples are generally tested by validated targeted assays, such as real-time PCR, ddPCR, or NGS. The assay used for liquid biopsy depends on the hospital's laboratory infrastructure, but all assays should be able to detect T790M in ≤5% of viable cells.[9,10]

All panel members expressed a preference for dPCR to detect T790M via liquid biopsy (Table 3). An important consideration is that dPCR platforms have higher sensitivity than real-time PCR—ddPCR has a sensitivity of approximately 80% or higher for T790M.[11,34] In patients who showed progression while receiving EGFR-TKIs, ddPCR had a positivity rate of 66% for T790M, whereas Cobas real-time PCR had a positivity rate of 24%.[35] Next-generation sequencing has also shown high sensitivity for T790M.[11,22,34] Furthermore, NGS can be used to analyse other genes implicated in the EGFR-TKI resistance (eg, MET, BRAF, ERBB2 [HER2], and KRAS), in conjunction with T790M testing.

Tissue samples can also be tested by real-time PCR or NGS. In Hong Kong, real-time PCR is commonly used for T790M testing. As mentioned above, NGS has a high sensitivity for T790M[34] and can provide additional genetic information

TABLE 3. The most effective method for T790M testing

Digital polymerase chain reaction is the preferred platform for T790M testing because of its high accuracy, availability, and rapid turnaround times.
Supplementary statements
1. Real-time polymerase chain reaction is widely available and may also be used for T790M testing, but it has lower sensitivity for T790M compared with digital platforms.
2. Next-generation sequencing may be useful in situations that require analysis of multiple mutations.

regarding the mechanism of EGFR-TKI resistance. For repeated liquid biopsy, ddPCR is the preferred assay, but liquid-based NGS can also be considered.

Conclusion

Molecular profiling of T790M mutation status in NSCLC with acquired resistance provides important guidance for clinicians with respect to next-line treatment. It can identify patients who are candidates for second- or third-line treatment with osimertinib, which has demonstrated superior efficacy, compared with chemotherapy, in the management of advanced NSCLC refractory to first- or second-generation TKIs.[8]

In Hong Kong, liquid biopsy assessment by a sensitive ctDNA platform is recommended as the first-line option for T790M testing to facilitate clinical decision making. Because of its accuracy and availability, dPCR is the preferred platform for this assessment. This expert panel developed consensus statements (Tables 1 to 3) and a corresponding workflow for T790M testing (Fig). Clinicians in Hong Kong can use the proposed workflow to guide the T790M testing process from the initial step of liquid biopsy to the determination of clinically appropriate situations for re-testing, followed by selection of treatment approaches.

In the future, T790M testing guidelines can be refined by adding the experience of multidisciplinary experts and new knowledge gained from research in Hong Kong and other countries.

Author contributions

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Critical revision of the manuscript for important intellectual content: All authors.

All authors had full access to the data, contributed to the study, approved the final version for publication, and take responsibility for its accuracy and integrity.

Conflicts of interest

All authors have disclosed no conflicts of interest.

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