

Circulating Tumor DNA T790M Testing as a Predictor of Osimertinib Efficacy in Epidermal Growth Factor Receptor Mutant Non-small Cell Lung Cancer: A Single Center Experience

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ABSTRACT: **Background:** The main acquired resistance mechanism to first- and second-generation epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) in EGFR mutant non-small cell lung cancer (NSCLC) is the propagation of T790M clones, which can be detected in circulating tumor DNA (ctDNA).

Objectives: To analyze osimertinib outcomes according to T790M testing method.

Methods: The study comprised 33 consecutive patients with advanced EGFR mutant NSCLC who were diagnosed with a T790M mutation after progression on first- or second-generation EGFR TKIs and treated with osimertinib. The patients were divided into groups A (diagnosed by tumor testing) and B (by ctDNA testing). Osimertinib outcomes were compared between the groups.

Results: Objective response rate with osimertinib comprised 54% and 62% in groups A and B, respectively ($P = 0.58$). Median progression-free survival (PFS) with osimertinib was 8.9 months (95% confidence interval [95%CI] 1.8–17.5) and 9.1 months (95%CI 5.3–12.6) in groups A and B, respectively (log-rank test 0.12, $P = 0.73$). Median overall survival (OS) was 13.8 months (95%CI 4.9–25.5) and 13.8 months (95%CI 7.7–27.7) in groups A and B, respectively (log-rank test 0.09, $P = 0.75$). T790M testing technique did not affect PFS (hazard ratio [HR] 1.16, 95%CI 0.50–2.69, $P = 0.73$) or OS (HR = 1.16, 95%CI 0.45–3.01, $P = 0.76$). The proportion of patients diagnosed by ctDNA grew from 56% in 2015 to 67% in 2016–2017.

Conclusions: Our study provides a ctDNA validation for the purpose of T790M testing in EGFR mutant NSCLC.

IMAJ 2019; 21: 394–398

KEY WORDS: circulating tumor DNA (ctDNA), epidermal growth factor receptor (EGFR), non-small cell lung cancer (NSCLC), osimertinib, T790M

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Epidermal growth factor receptor (EGFR) is a transmembrane receptor with tyrosine kinase activity involved in the regulation of cell proliferation, survival, and differentiation [1]. Somatic mutations in the gene encoding EGFR are detected in approximately 15% of non-small cell lung carcinomas (NSCLC) in Caucasians [2,3]. Among patients with advanced EGFR-mutant NSCLC, treatment with the first- and second-generation epidermal growth factor receptor tyrosine kinase inhibitors (EGFR TKIs including gefitinib, erlotinib, and afatinib) is associated with an objective response rate (ORR) of 56–74% and a median progression-free survival (PFS) of 10–14 months [4–7]; however, resistance inevitably develops.

In approximately 60% of patients, acquired resistance results from the propagation of a tumor clone driven by a different *EGFR* mutation: T790M [8]. A phase 1/2 AURA study conducted in patients who progressed after first- and second-generation *EGFR* TKIs, osimertinib (a third generation *EGFR* TKI and a potent T790M inhibitor) was associated with an ORR and median PFS of 61% and 9.6 months in T790M-positive tumors, in contrast to 21% and 2.8 months in T790M-negative tumors [9]. Results from a phase 3 AURA3 trial established osimertinib as a standard of care for this subset of patients [10].

Genotyping for T790M in cases of progression on first- and second-generation *EGFR* TKIs has become a crucial step for guiding treatment decisions. In a retrospective analysis of the AURA study, patients with the T790M mutation detected in plasma using BEAMing digital polymerase chain reaction (PCR) had ORR (63%) and median PFS (9.7 months) that was comparable with Osimertinib and with positive tissue-based assay results (ORR 62%; median PFS 9.7 months) [11].

The predictive value of plasma T790M genotyping using the Cobas® EGFR Mutation Test (Roche Molecular Systems, USA) was prospectively confirmed in the AURA3 trial, which

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demonstrated similar outcomes with osimertinib in the tumor and plasma T790M-positive subgroups [10]. Different ctDNA techniques used for T790M detection are associated with different sensitivity. For example, sensitivity of plasma T790M genotyping with BEAMing digital PCR is 70–80%, whereas sensitivity of the Cobas® EGFR Mutation Test is only 41–73% [10,12]. Droplet Digital™ PCR (ddPCR) (Bio-Rad Laboratories, Inc., USA), a highly sensitive plasma method for T790M detection (sensitivity 71% [11]), which is largely adopted in Israel, has not been tested in a large validation study correlating the results with the outcomes.

The main purpose of this study was to determine the predictive value of ctDNA T790M testing with the techniques currently used in Israel (mainly ddPCR) in a real-life cohort of patients. In addition, we were eager to determine whether the quantity of T790M-positive DNA found during ctDNA testing predicted the efficacy of osimertinib treatment. Finally, trends in T790M testing in Israel were assessed.

PATIENTS AND METHODS

We collected data from consecutive patients with advanced EGFR-mutant NSCLC who were treated at the Davidoff Cancer Center, Rabin Medical Center, Petah Tikva, Israel, between June 2015 and August 2017. The patients had been diagnosed with a T790M mutation after progression on first- and second-generation *EGFR* TKIs (either erlotinib, gefitinib, or afatinib). Patients receiving osimertinib were selected. These patients were divided into two groups: patients who were diagnosed with a T790M mutation in the tumor tissue specimen (group A) and patients with a T790M mutation that was detected by ctDNA testing (group B). Patient charts and electronic medical records were retrospectively reviewed. Baseline demographics as well as clinical and pathological characteristics were retrieved.

Tissue T790M testing was conducted using AmoyDx® EGFR 29 Mutations Detection Kit (Amoy Diagnostics Co., LTD, China). Plasma T790M testing was conducted either by ddPCR, Guardant360 (Guardant Health, USA), or Biocept (Biocept, Inc., USA).

The ddPCR method is a technology that is based on amplification of the selective DNA fragments using complementary primers. The ddPCR method has been available in several molecular pathology laboratories in Israel since 2017 (Rambam Health Care Campus, Haifa; Hadassah Medical Center, Jerusalem; and Assaf Harofeh Medical Center, Zerifin).

Guardant360 is a next-generation sequencing (NGS) panel of 70 clinically actionable genes (including T790M) to conduct digital sequencing. This method uses a preparation of a digital library of individually tagged ctDNA molecules combined with post-sequencing bioinformatic reconstruction.

Biocept technology is another method of ctDNA analysis using NGS and hybridization capture technique. This technol-

ogy is designed to suppress amplification of wild-type targets, while not suppressing amplification of mutant sequences. Both Guardant360 and Biocept became commercially available before ddPCR. Those tests, however, are not reimbursed by the Israeli national health insurance funds. Before the reimbursement of the ctDNA testing, tissue testing was the only method that was reimbursed, and therefore, largely available for the purpose of T790M diagnosis. At that time, however, there were some individuals who were willing to pay privately for the ctDNA testing.

In patients who had adequate computed tomography (CT)/positron emission tomography/computed tomography (PET/CT) scans for radiological assessment, the images were reviewed and the response was assessed using response evaluation criteria in solid tumors (RECIST v.1.1) [13]. ORR was defined as the proportion of patients reaching partial response or complete response. PFS was calculated from the day of osimertinib initiation until disease progression, death, or start of another systemic treatment, whichever occurred first. The outcome was censored if a patient was alive without known progression of disease at the time of the last follow-up. Overall survival was calculated from the day of osimertinib initiation until death. The outcome was censored if a patient was alive at the time of the last follow-up. ORR, PFS, and overall survival with osimertinib were analyzed in correlation with T790M type of testing (tissue vs. ctDNA). In group B, ORR and PFS were analyzed in relation to ctDNA T790M testing method (BIOCEPT vs. ddPCR vs. Guardant360). Quantitative analysis of T790M on ctDNA testing was performed and a correlation between the T790M fraction and osimertinib efficacy was analyzed. Univariate analysis of PFS and overall survival was performed using the Cox proportional hazard regression model. In addition, the trends in using tissue vs. ctDNA methods for T790M testing were analyzed.

Statistical analyses were performed using SAS 9.4 software (SAS Institute Inc., Cary, NC, USA). The sample size was determined by the number of available patients who met the inclusion criteria. Categorical variables were presented by numbers and percentiles. Medians and ranges were reported for continuous variables. All reported *P* values were based on two-sided hypothesis tests; two-sided *P* values < 0.05 were considered statistically significant. *T*-tests and chi-square tests were used to compare the baseline characteristics between the groups. ORR was compared using Fisher's exact test. Median PFS and overall survival were estimated using the Kaplan–Meier method, and curves were compared by the log-rank test. Association between the T790M-fraction and response was analyzed by logistic regression model. Association between the T790M-fraction and PFS was analyzed using Cox proportional-hazards regression model.

The study was conducted in accordance with the principles of good clinical practice, and institutional review board approval was obtained before reviewing the patient charts and electronic medical records.

RESULTS

DEMOGRAPHICS AND BASELINE CLINICAL CHARACTERISTICS

Thirty-three patients with advanced *EGFR*-mutant NSCLC who met the inclusion criteria were identified. The patients were divided into two groups: patients who were diagnosed with *T790M* mutation in the tumor tissue specimen (group A, n=12) and patients with a *T790M* mutation that was detected by ctDNA testing (group B, n=21). None of the patients had both tests performed (after *T790M* was diagnosed, treatment proceeded with osimertinib). There were no differences between the groups in terms of baseline demographic and clinical characteristics [Table 1].

EFFICACY

In 32 patients who had adequate CT–PET/CT scans for radiological assessment, the images were reviewed and the response was assessed. ORR comprised 54% (6/11) and 62% (13/21) in groups A and B, respectively ($P = 0.58$) [Table 1].

With median follow-up of 8.6 months since the start of osimertinib (interquartile range [IQR] 4.1–10.5), 24 (73%) patients progressed or died (75% and 72% in groups A and B, respectively). Median PFS with osimertinib was 8.9 months (95%

confidence interval [95%CI] 1.8–17.5) and 9.1 months (95%CI 5.3–12.6) in groups A and B, respectively (log-rank test 0.12, $P = 0.73$) [Figure 1A]. With a median follow-up of 10.2 months after the start of osimertinib (IQR 7.0–13.8) 19 patients (57%) died (58% and 57% in groups A and B, respectively). Median overall survival with osimertinib was 13.8 months (95%CI 4.9–25.5) and 13.8 months (95%CI 7.7–27.7) in groups A and B, respectively (log-rank test 0.09, $P = 0.75$) [Figure 2]. *T790M* testing type (tissue biopsy or ctDNA) did not affect PFS (hazard ratio [HR] 1.16, 95%CI 0.50–2.69, $P = 0.73$) or overall survival with osimertinib (HR = 1.16, 95%CI 0.45–3.01, $P = 0.76$).

In group B, 14, 4, and 3 patients were diagnosed using ddPCR, Guardant360, and Biocept technology, respectively. ORR comprised 57% (8/14), 75% (3/4), and 67% (2/3) in

Figure 1. Kaplan–Meier curves for progression-free survival. Progression-free survival with osimertinib according to type **[A]** tissue biopsy vs. ctDNA (n=33) **[B]** ddPCR vs. Guardant 360 vs. Biocept (n=21), *T790M* testing

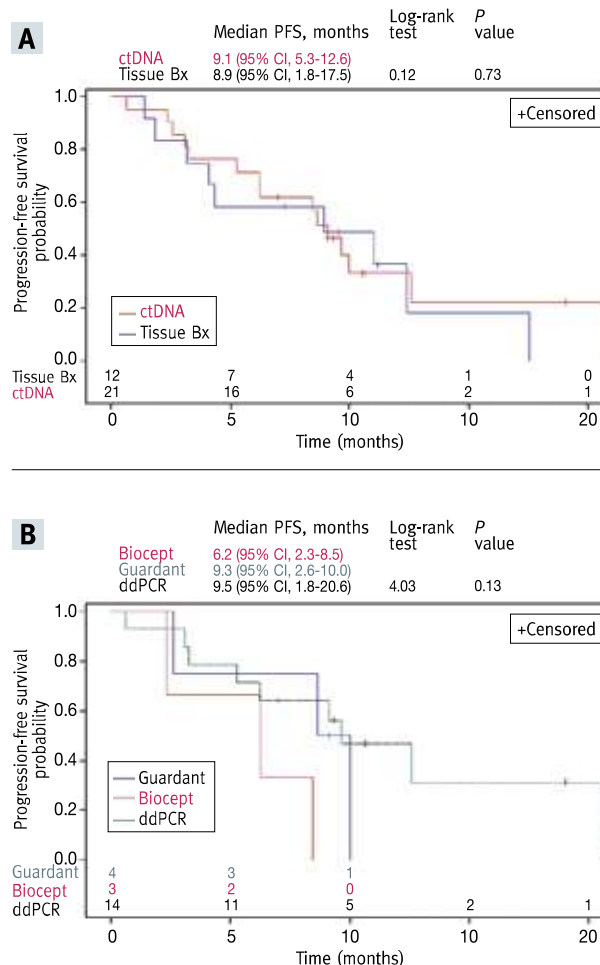


Table 1. Baseline patient characteristics and response to osimertinib

Variable	Group A	Group B	P value
Age in years, median (range)	68 (57–90)	73 (41–94)	0.88
Gender, n (%)			
Male	6 (50)	7 (33)	0.46
Female	6 (50)	14 (67)	
Smoking history, n (%)			
Current/past smokers	5 (42)	3 (14)	0.11
Never smokers	7 (58)	18 (86)	
ECOG PS, n (%)			
0–2	11 (92)	20 (95)	1
3/4	1 (8)	1 (5)	
EGFR mutation type, n (%)			
Exon 19 delition	5 (42)	13 (62)	0.38
L858R	2 (16)	1 (5)	
Others (uncommon and complex)	5 (42)	7 (33)	
Prior chemotherapy, n (%)			
Yes	4 (33)	11 (52)	0.47
No	8 (67)	10 (48)	
Prior lines of EGFR TKIs, median (range)	1 (0–2)	1 (1–3)	0.34
ORR*, n (%)			
Complete response*	6 (54)**	13 (62)	0.58
Partial response*	0 (0)	0 (0)	
	6 (55)	13 (62)	
Stable disease*, n (%)	1 (9)	4 (19)	
Progressive disease*, n (%)	4 (36)	4 (19)	
Total, n	12	21	

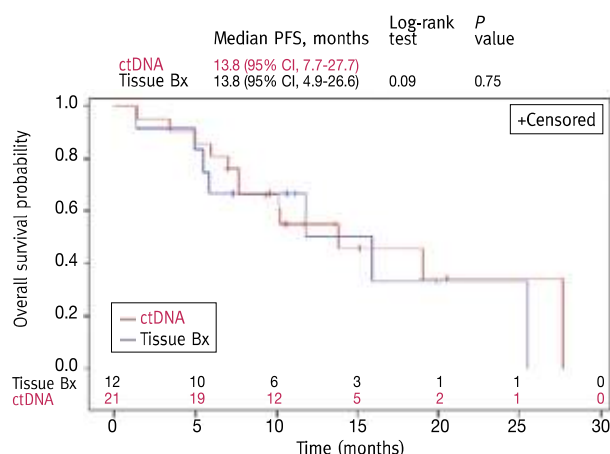
*Using response evaluation criteria in solid tumors (RECIST v.1.1) [13]

**In group A, 11 patients were evaluable for response assessment

ECOG PS = Eastern Cooperative Oncology Group Performance Status score, EGFR TKIs = epidermal growth factor receptor tyrosine kinase inhibitors, ORR = objective response rate

95%CI = 95% confidence interval, Bx = biopsy, ctDNA = circulating tumor DNA, ddPCR = digital droplet polymerase chain reaction, PFS = progression-free survival

Figure 2. Kaplan–Meier curves for overall survival. Overall survival according to type of T790M testing (tissue biopsy vs. ctDNA, n=33)



95%CI = 95% confidence interval, Bx = biopsy, ctDNA = circulating tumor DNA, OS = overall survival

patients diagnosed by ddPCR, Guardant360, and BIOCEPT, respectively, with no differences between the groups ($P = 1.0$). Median PFS with osimertinib was 9.6 months (95%CI 3.2–20.6), 9.3 months (95%CI 2.6–10.0), and 6.2 months (95%CI 2.3–8.5) in patients diagnosed by ddPCR, Guardant360, and BIOCEPT, respectively, with no differences between the groups (log-rank test 4.03, $P = 0.13$) [Figure 1B].

Quantitative assessment of T790M found in plasma was performed in 13 cases (median T790M-fraction comprised 1%, range 0.01–60%). No association between the T790M-fraction and PFS was seen (HR 1.03, 95%CI 0.99–1.07, $P = 0.15$). In addition, there was no significant relationship between the T790M fraction and clinical benefit with osimertinib (response or disease stabilization vs. progression) (odds ratio 3.04, 95%CI 0.32–29.11, $P = 0.33$).

TRENDS IN T790M TESTING

During 2015, 44% (4/9) and 56% (5/9) of patients were diagnosed by tissue biopsy and ctDNA testing, respectively. In 2016–2017, the proportion of patients diagnosed by ctDNA grew to 67% (16/24), whereas only 33% (8/24) were diagnosed by tissue biopsy.

DISCUSSION

In this study, we summarized a single-center experience with T790M testing. Our major observation was that in an Israeli cohort of patients, osimertinib had similar efficacy regardless of the T790M testing type (whether T790M was detected in tissue or in ctDNA), which is in line with the previously reported data [11]. The fact that, in our cohort, ctDNA testing in several cases was paid privately by the patient, while the tissue testing was

reimbursed by the national health insurance, in our opinion, is unlikely to bias the results.

Moreover, osimertinib efficacy in a real-life cohort, comprised of 60% of patients diagnosed by ctDNA, was comparable to the efficacy reported in the randomized controlled trial. This result shows the accuracy of T790M testing techniques, which are currently used in Israel. Furthermore, we observed that, as ctDNA testing availability for T790M detection improved, physician preferences changed in favor of liquid biopsy as a less invasive technique.

According to our results, all the three technologies used for T790M testing (ddPCR, Guardant360, and Biocept) have the same predictive ability in terms of ORR and PFS with osimertinib treatment. Therefore, each of the techniques listed may be used for the purpose of T790M testing in EGFR mutant NSCLC patients after failure of first- and second-generation EGFR TKIs. The value of the observation, however, is limited by the small number of patients tested by each technique.

We assumed that amount of T790M detected in ctDNA (T790M-fraction) might correlate with osimertinib efficacy, but no association was observed. A small number of patients in whom T790M quantitative testing was performed; however, limits the value of the observation.

Our study has several limitations that should be acknowledged. First, it is a retrospective analysis with a small number of patients included. In addition to sample selection bias (patients from only one medical center were selected), there was a group allocation bias (different physicians may have chosen different testing types and techniques). The absence of a central radiological review along with the absence of uniform intervals for tumor response assessment introduces further uncertainties into the osimertinib efficacy assessment. These limitations have reduced the external validity of our study.

CONCLUSIONS

Our study provides results of local validation of ctDNA for the purpose of T790M testing in EGFR-mutant NSCLC patients in Israel.

Conflict of Interest

Dr. E. Dudnik reported grants from Roche, Boehringer Ingelheim and personal fees for consulting or advisory services from Boehringer Ingelheim, Roche, Astra Zeneca, Pfizer, MSD, BMS, Novartis. Dr. N. Peled reported grants from Astra Zeneca and BMS; and personal fees for consulting or advisory services from Boehringer Ingelheim, Roche, Astra Zeneca, Pfizer, MSD, BMS, Novartis, Eli Lilly. Dr. A. Zer reported grants from BMS and personal fees for consulting or advisory services from Roche, Astra Zeneca, MSD.

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Capsule

Risk of incident chronic obstructive pulmonary disease in rheumatoid arthritis

Using administrative health data, **Mecguire** and colleagues studied a population-based incident rheumatoid arthritis (RA) cohort with matched general population controls. All incident RA cases in British Columbia who first met RA definition between January 1996 and December 2006 were selected using previously published criteria. General population controls were randomly selected, matched 1:1 to RA cases on birth year, sex, and index year. COPD outcome was defined as hospitalization with a primary COPD code. Incidence rates, 95% confidence intervals (95% CIs), and incidence rate ratios (IRRs)

were calculated for RA and controls. The cohorts included 24,625 RA individuals and 25,396 controls. The incidence of COPD hospitalization was greater in RA than controls (IRR 1.58, 95%CI 1.34–1.87). After adjusting for potential confounders, RA cases had a 47% greater risk of COPD hospitalization than controls. The increased risk remained significant after modeling for smoking and with varying COPD definitions.

Arthritis Care Res 2019; 71: 602
Eitan Israeli

Capsule

Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases

Inflammatory bowel diseases, which include Crohn's disease and ulcerative colitis, affect several million individuals worldwide. Crohn's disease and ulcerative colitis are complex diseases that are heterogeneous at the clinical, immunological, molecular, genetic, and microbial levels. Individual contributing factors have been the focus of extensive research. As part of the Integrative Human Microbiome Project (HMP2 or iHMP), **Lloyd-Price** et al. followed 132 subjects for 1 year each to generate integrated longitudinal molecular profiles of host and microbial activity during disease (up to 24 time points each; in total 2965 stool, biopsy, and blood specimens). The results provided a comprehensive view of functional dysbiosis in the gut microbiome during inflammatory bowel disease activity. The authors demonstrate a characteristic increase in facultative anaerobes at the expense of obligate anaerobes, as well as

molecular disruptions in microbial transcription (for example, among clostridia), metabolite pools (acylcarnitines, bile acids, and short-chain fatty acids), and levels of antibodies in host serum. Periods of disease activity were also marked by increases in temporal variability, with characteristic taxonomic, functional, and biochemical shifts. Finally, integrative analysis identified microbial, biochemical, and host factors central to this dysregulation. The study's infrastructure resources, results, and data, which are available through the Inflammatory Bowel Disease Multi-omics Database (<http://ibdmdb.org>), provide the most comprehensive description to date of host and microbial activities in inflammatory bowel diseases.

Nature 2019; 569: 655
Eitan Israeli

“Language exerts hidden power, like the moon on the tides”

Rita Mae Brown (born 1944), American author and screenwriter