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EGFR mutation prevalence in Asia-Pacific and Russian patients with advanced NSCLC of adenocarcinoma and non-adenocarcinoma histology: The IGNITE study

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ABSTRACT

Objectives: Limited understanding exists of epidermal growth factor receptor (*EGFR*) mutation frequency in less common subgroups of advanced non-small-cell lung cancer (aNSCLC) (e.g. squamous cell carcinoma [SCC]), and to what extent local practices exclude patients from *EGFR* testing based on their clinical characteristics.
Materials and methods: IGNITE (non-comparative/-interventional; NCT01788163) was conducted in 90 centres (Asia-Pacific/Russia). Eligible patients: local/metastatic aNSCLC; chemotherapy-naïve, newly-diagnosed/recurrent disease after resection; ineligible for curative treatment. Patients provided a tissue/cytology (all) and a blood plasma (China/Russia/South Korea/Taiwan) sample. Primary endpoint: *EGFR* mutation frequency in aNSCLC patients (adenocarcinoma [ADC]/non-ADC), as per local practices.
Results: 3382 patients were enrolled. *EGFR* mutation frequencies for evaluable tissue/cytology samples in Asia-Pacific and Russian patients: 49.3% (862/1749) and 18.0% (90/500) for ADC tumours; 14.1% (74/525) and 3.7% (15/402) for non-ADC; 9.9% (40/403) and 3.7% (13/349) for SCC. Of Russian patients with SCC tumours harbouring common, activating *EGFR* mutations, 6/9 were never-/former-smokers. Mutation status concordance between 2581 matched tissue/cytology and plasma samples: 80.5% (sensitivity 46.9%, specificity 95.6%).
Conclusion: *EGFR* mutation testing should be considered in all Asian aNSCLC patients. Also, as activating *EGFR*

Abbreviations: ADC, adenocarcinoma; aNSCLC, advanced non-small-cell lung cancer; ASR, age-standardised rate; ctDNA, circulating free tumour-derived DNA; EGFR, epidermal growth factor receptor; LNA, locked nucleic acid; NE, neuroendocrine; NSCC, non-small-cell carcinoma; NSCLC, non-small-cell lung cancer; NPV, negative predictive value; PCR, polymerase chain reaction; PNA, peptide nucleic acid; PPV, positive predictive value; SCC, squamous cell carcinoma; SCCA, small-cell carcinoma; TKI, tyrosine kinase inhibitor; TTF-1, thyroid transcription factor 1; WHO, World Health Organization

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mutations were observed in a small number of Caucasian squamous NSCLC patients, testing here may be appropriate, particularly in those with no/remote smoking history. Circulating free tumour-derived DNA is feasible for mutation analysis employing well-validated and sensitive methods, when tumour samples are unavailable.

1. Introduction

Statistics indicate that, in Asia, lung cancer is the most common cancer in men (age-standardised rate [ASR; per 100,000] 35.2) and the third most common cancer in women (ASR 12.7) [1]. Similarly, in Russia, lung cancer is the most common cancer in men (ASR 51.4) and the eighth most common cancer in women (ASR 6.8) [1].

Adenocarcinoma (ADC) is among the most common histological subtypes of non-small-cell lung cancer (NSCLC) [2]. NSCLC of ADC histology is reported to be associated with mutations in the epidermal growth factor receptor (*EGFR*) gene in approximately 14–19% of Western patients and 40–48% of Asian patients (corresponding data for non-ADC: 3% and 8%, respectively) [3,4]. Data for Russia specifically have indicated that *EGFR* mutations may occur in 13–20% of Russian patients with NSCLC of ADC histology [5,6].

EGFR tyrosine kinase inhibitors (TKIs) specifically target the protein encoded by the *EGFR* oncogene [7,8], and it is now accepted that response to EGFR TKIs is mainly limited to patients with tumours harbouring activating, targetable, *EGFR* mutations (most common: exon 19 deletion or L858R mutation) compared with wild-type *EGFR* [9]. Furthermore, EGFR TKIs have demonstrated superior efficacy to doublet-chemotherapy in patients with *EGFR* mutation-positive advanced NSCLC (aNSCLC) [10–15].

Current clinical guidelines (National Comprehensive Cancer Network, National Institute for Health and Care Excellence) [16–18] and several working groups [19,20] now advocate mutation testing of tumour samples from patients with non-squamous aNSCLC (and in specific patients with squamous NSCLC [e.g. never-smokers]; European Society for Medical Oncology guidelines) [21] to confirm their suitability for EGFR TKI treatment. Prior to the association with *EGFR* mutation-positive status and response to EGFR TKIs, certain clinical characteristics associated with a high frequency of activating, sensitising *EGFR* mutations (female gender, Asian ethnicity, never-smokers, and ADC histology [3,22]) drove patient selection for mutation testing [23]. However, it is now acknowledged that *EGFR* mutations may occur in any patient [24,25]. Indeed, the number of facilities that conduct mutation testing has risen, reflecting increased clinician demand [26,27].

As the availability of testing becomes more widespread, understanding of the frequency of *EGFR* mutations (particularly in groups that have not previously been widely tested) needs to be updated. Moreover, it is important to assess real-world diagnostic practices to identify areas for improvement, as the methodologies used are highly diverse [16–18,21,26,28], with differences in tumour sampling and *EGFR* mutation testing methodologies not well-documented. Optimum testing methodologies for alternative sample types are, therefore, under investigation, such as circulating free tumour-derived DNA (ctDNA) obtained from blood serum or plasma [4,10,29,30]. Overall, this knowledge will help to ensure that as many patients as possible have access to mutation testing and are treated appropriately based on the molecular characteristics of their disease.

1.1. Objectives

The large, multinational, diagnostic, non-comparative, non-interventional IGNITE study (NCT01788163) assessed *EGFR* mutation frequency in patients with aNSCLC of ADC or non-ADC histologies in a real-world diagnostic setting.

2. Methods

2.1. Study design and patients

Eligible patients (aged ≥18 years) had newly diagnosed, locally advanced (not eligible for curative treatment)/metastatic treatment-naïve NSCLC, or had recurrent disease and surgical resection with/without adjuvant chemotherapy. Provision of a diagnostic tissue/cytology sample was mandatory upon inclusion for all patients, and provision of a routine blood (plasma) sample was mandatory for patients from China, Russia, South Korea, and Taiwan only (other countries included were Australia, Indonesia, Malaysia, Singapore, and Thailand).

The primary endpoint of IGNITE was *EGFR* mutation frequency in patients with aNSCLC of ADC and non-ADC histologies. Secondary endpoints included: *EGFR* mutation testing practices; level of concordance in *EGFR* mutation status between matched tissue/cytology and blood (plasma) samples; correlations between *EGFR* mutation status and demographic data/disease status; and treatment decisions following *EGFR* mutation testing (not reported).

All patients provided written, informed consent. Study approval was obtained from independent ethics committees at each institution. The study was conducted in accordance with the Declaration of Helsinki, the International Conference on Harmonisation/Good Clinical Practice, applicable regulatory requirements for non-interventional studies, and AstraZeneca's policy on bioethics and human biological samples.

2.2. Procedures

EGFR mutation testing and results data for tumour samples obtained prior to enrolment in IGNITE were used where available. For tests conducted in IGNITE, diagnostic tissue/cytology samples underwent *EGFR* mutation testing as per local practices, following histopathologic review (World Health Organization [WHO] classification) to ensure that samples were adequate for use. Plasma samples were obtained from patients from China, Russia, South Korea, and Taiwan only, as countries deemed most likely to provide sufficient plasma samples to support the concordance analysis: these patients provided 10-mL blood samples, which were processed to plasma, frozen and transported to designated laboratories for testing. In all countries, academic, hospital, or commercial laboratories were utilised for tissue/cytology-based testing; central/regional expert laboratories were utilised for blood (plasma)-based testing.

2.3. Outcomes

Testing methodologies, sample types and availability, and testing turnaround time/success rate/mutation detection rate were captured to assess *EGFR* mutation testing practices. *EGFR* mutation frequency (primary endpoint) was assessed overall, by ADC and non-ADC histologies, and by country/region. *EGFR* mutation concordance between matched tissue/cytology and plasma samples was assessed via: concordance rate; sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV); and exact two-sided 95% confidence interval.

2.4. Statistical analyses

EGFR mutation testing practices (enrolled population) and *EGFR* mutation frequency (evaluable tumour [tissue/cytology]/plasma

populations) were summarised using appropriate descriptive statistics. It was estimated that 2500 patients from Asia-Pacific and 1000 patients from Russia would need to be tested to give similar precision of the mutation frequency estimate in patients with aNSCLC of non-ADC histology.

EGFR mutation status concordance between matched tissue/cytology and plasma samples was calculated for the evaluable population (all eligible patients with known tumour [tissue/cytology] and plasma sample *EGFR* mutation status).

The correlation between *EGFR* mutation status and demographic characteristics and disease status was analysed using a multivariate logistic regression model of *EGFR* mutation status at baseline in the evaluable populations with the following covariates: region (Asia-Pacific, Russia; as indicative of ethnicity), histology (ADC, non-ADC), smoking status (never-, ever-smoker), gender (female, male), age (≤ 65, > 65 years), WHO performance score (0–1, 2), and key disease status characteristics (evaluable tumour [tissue/cytology]/plasma populations).

3. Results

3.1. Patients

From 27 February, 2013 to 25 August, 2014, 3382 patients were enrolled (Fig. 1). Demographics and baseline characteristics were generally well-balanced between patients with data available for tissue/cytology and plasma samples (Supplementary Table 2).

3.2. Sampling methodologies and EGFR mutation testing practices

Tissue/cytology samples were mostly collected during current diagnosis (Asia-Pacific 93.7%, Russia 74.1%; Supplementary Fig. 1A), and sample tissue was most often derived from the primary tumour (Asia-Pacific 67.1%, Russia 80.3%; Supplementary Fig. 1B). The most common sampling sites were the lungs/lymph nodes (Asia-Pacific 68.3%/14.1%, Russia 79.8%/10.2%; Supplementary Fig. 1C). The majority of samples were collected by bronchoscopy (Asia-Pacific 22.4%, Russia 44.9%; Supplementary Fig. 1D).

In terms of mutation testing, a wide range of methods for tissue/cytology samples were observed across Asia-Pacific (most common: 24.2% with AmoyDX™ EGFR 29 Mutation Detection Kit [Amoy Diagnostics Co., Ltd., Xiamen, China]), and less so in Russia (most common: 37.5%, with QIAGEN therascreen® EGFR RGQ PCR Kit [QIAGEN, Manchester, UK]; Fig. 2A). With regards to plasma sample testing, methodologies were relatively limited in number and generally similar in type across both regions (Fig. 2B).

The median *EGFR* test turnaround time for tissue/cytology samples was 6 days for Asia-Pacific (range: 1–197 days) and 9 days for Russia (range: 1–401 days). Across Asia-Pacific countries, the median test turnaround was generally within 2 weeks, aside from Thailand where it was 70.0 days (range: 4–197 days). Tumour mutation testing success rates for Asia-Pacific and Russia were 99.5% (2291/2302) and 98.7% (924/936), respectively. Tumour mutation tests were not performed on samples of 144 patients. The most common reason for not testing, where provided, was insufficient material provided for the test (Asia-Pacific 92.6% [100/108 responses], Russia 66.7% [24/36 responses]).

3.3. EGFR mutation frequency

For tissue/cytology samples, the overall *EGFR* mutation frequencies in Asia-Pacific and Russian patients with tumours of ADC histology were 49.3% and 18.0%, respectively; and for non-ADC, 14.1% and 3.7%, respectively (Table 1). Corresponding data for plasma samples generally reflected a similar pattern, albeit with lower overall mutation frequencies (Table 1).

EGFR mutation status by non-ADC subtype in tissue/cytology samples is presented in Table 2. *EGFR* mutation frequency in squamous cell carcinoma (SCC) was 9.9% (40/403) in Asia-Pacific and 3.7% (13/349) in Russia; and in non-small-cell carcinoma (NSCC; not otherwise specified) was 27.5% (19/69) and 7.4% (2/27) respectively. Also in the non-ADC group, 21 patients had tumours classified as NSCC with squamous cell and ADC patterns; in Asia-Pacific, 53.3% (8/15) of these tumour samples carried *EGFR* mutations.

Across IGNITE, 67 patients (54 Asian) with *EGFR* mutation-positive tumours were histologically classified as having SCC, or NSCLC with a squamous component. Of these, 74.6% (50/67) were male, 34.3% (23/67) were never-smokers and 31.3% (21/67) were current smokers. Of 9 Russian patients with activating exon 19 deletions or L858R mutations in tumours histologically classified as having SCC/NSCLC with a squamous component, 66.7% (6/9) were never- or former-smokers (3/9 current smokers).

Of interest, where relevant testing was locally conducted, 43.9% (351/799) of thyroid transcription factor 1 (TTF-1)-positive and 9.8% (25/256) of TTF-1-negative tissue/cytology samples were *EGFR* mutation-positive.

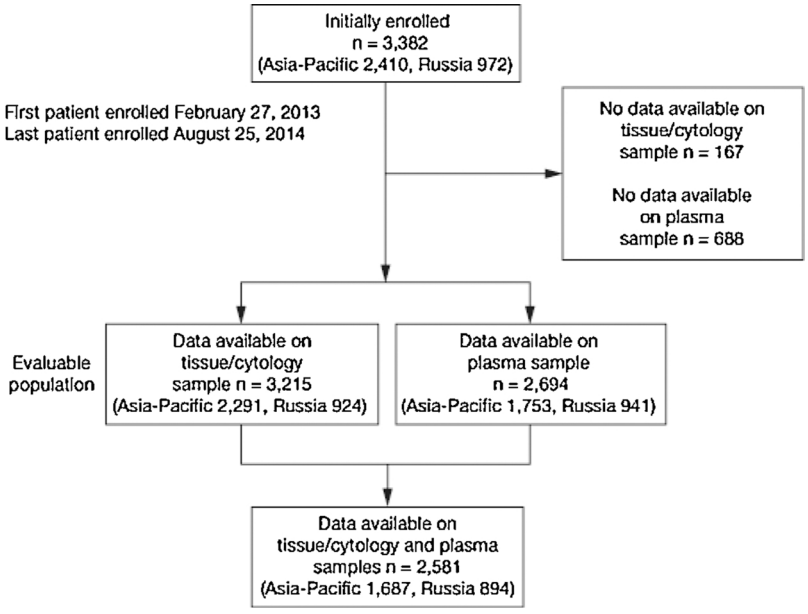

Fig. 1. Patient flow diagram.

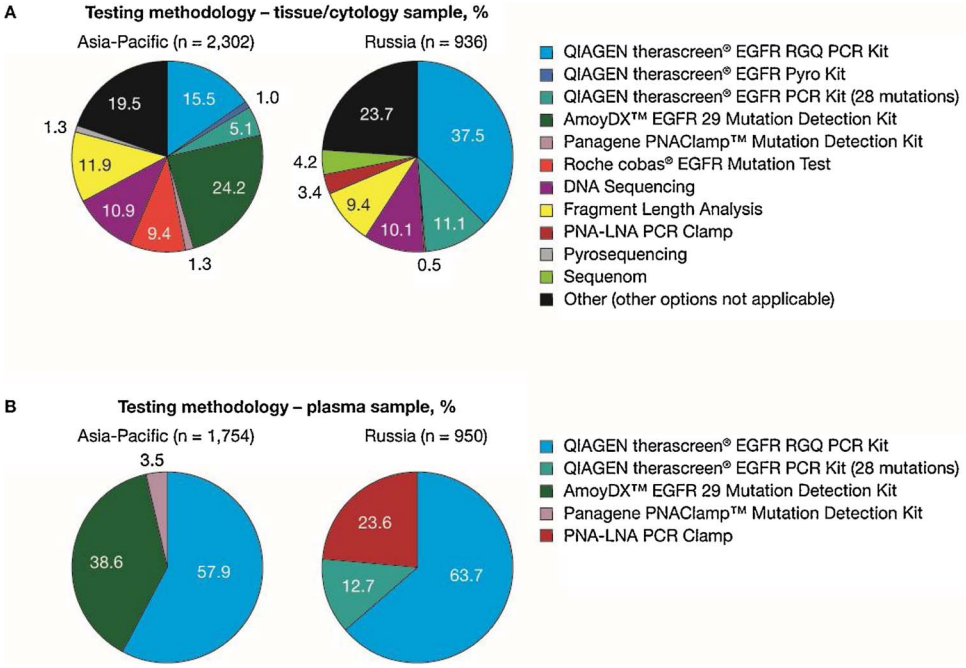

Fig. 2. Mutation testing methods employed for (A) tissue/cytology, and (B) plasma samples in Asia-Pacific and Russia (enrolled population).
Abbreviations: EGFR, epidermal growth factor receptor; LNA, locked nucleic acid; PCR, polymerase chain reaction; PNA, peptide nucleic acid.

Table 1
E*GFR* mutation frequency by sample type, region, and histology (tissue/cytology and/or plasma evaluable population).

		Tissue/cytology samples		Plasma samples	
		ADC n/N (%)	Non-ADC n/N (%)	ADC n/N (%)	Non-ADC n/N (%)
Overall		952/2249 (42.3)	89/927 (9.6)	397/1814 (21.9)	60/854 (7.0)
Country					
Asia-Pacific		862/1749 (49.3)	74/525 (14.1)	342/1301 (26.3)	31/445 (7.0)
Russia		90/500 (18.0)	15/402 (3.7)	55/513 (10.7)	29/409 (7.1)
Mutation subtype					
Exon 19 deletion only	Asia-Pacific	420/862 (48.7)	29/74 (39.2)	173/342 (50.6)	14/31 (45.2)
	Russia	53/90 (58.9)	6/15 (40.0)	38/55 (69.1)	21/29 (72.4)
L858R only	Asia-Pacific	366/862 (42.5)	41/74 (55.4)	143/342 (41.8)	14/31 (45.2)
	Russia	23/90 (25.6)	3/15 (20.0)	17/55 (30.9)	7/29 (24.1)
Exon 20 insertions only	Asia-Pacific	20/862 (2.3)	0/74 (0.0)	6/342 (1.8)	0/31 (0.0)
	Russia	0/90 (0.0)	0/15 (0.0)	0/55 (0.0)	1/29 (3.4)
G719X only	Asia-Pacific	10/862 (1.2)	1/74 (1.4)	5/342 (1.5)	0/31 (0.0)
	Russia	0/90 (0.0)	0/15 (0.0)	0/55 (0.0)	0/29 (0.0)
L861Q only	Asia-Pacific	11/862 (1.3)	1/74 (1.4)	4/342 (1.2)	1/31 (3.2)
	Russia	0/90 (0.0)	2/15 (13.3)	0/55 (0.0)	0/29 (0.0)
Other rare mutations/double mutations[a]	Asia-Pacific	35/862 (4.1)	2/74 (2.7)	11/342 (3.2)	2/31 (6.5)
	Russia	14/90 (15.6)	4/15 (26.7)	0/55 (0.0)	0/29 (0.0)

Abbreviations: ADC, adenocarcinoma; EGFR, epidermal growth factor receptor.
[a] Including L858R + any other or exon 19 deletion + any other mutation.

3.4. *EGFR* mutation subtypes

In the majority of tissue/cytology samples from Asia-Pacific patients with tumours of ADC histology, activating exon 19 deletions (48.7%) and L858R mutations (42.5%) were observed (Table 1). In corresponding Russian samples, as has been seen previously for Caucasian populations, the proportion of exon 19 deletions was substantially higher (58.9%) versus L858R mutations (25.6%). In non-ADC tissue/cytology samples, these common mutations were observed in 94.6% of Asia-Pacific *EGFR* mutation-positive patients (exon 19 deletion: 39.2%; L858R mutation: 55.4%); and in only 60.0% of Russian *EGFR* mutation-positive patients (exon 19 deletion: 40.0%; L858R mutation: 20.0%). Corresponding data for plasma samples are reported in Table 1.

Considering overall tissue/cytology sample *EGFR* mutation subtype frequencies, rare mutations were more frequently seen in Russia versus Asia-Pacific (Supplementary Table 3). In Russia, a total of 19.1% of mutation-positive tissue/cytology samples (21/110 mutation subtypes detected) were neither exon 19 deletions nor L858R-only mutations; in Asia-Pacific samples, the corresponding percentage was 8.6% (81/941). Among the Russian rare mutations, eight were double mutations including exon 19 deletions, and five were designated as R836R.

3.5. Concordance of *EGFR* mutation status between matched tissue/cytology and plasma samples

Overall mutation status concordance was 80.5% (sensitivity 46.9%, specificity 95.6%, PPV 82.6%, NPV 80.0%) (Table 3). The sensitivity (30.3%) and specificity (93.5%) were noticeably lower in Russia compared with Asia-Pacific (49.6% and 97.2%, respectively).

Table 2
EGFR mutation status by non-ADC histological subtype (tissue/cytology evaluable population).

	Asia-Pacific samples		Russian samples	
	EGFR mutation-positive N = 939 n (%)	*EGFR* mutation-negative N = 1348 n (%)	*EGFR* mutation-positive N = 109 n (%)	*EGFR* mutation-negative N = 810 n (%)
Non-ADC group	74 (7.9)	451 (33.5)	15 (13.8)	387 (47.8)
SCC	40 (4.3)	363 (26.9)	13 (11.9)	336 (41.5)
NSCC, favour SCC	6 (0.6)	12 (0.9)	0 (0.0)	12 (1.5)
SCCA	0 (0.0)	3 (0.2)	0 (0.0)	0 (0.0)
NSCC, NOS	19 (2.0)	50 (3.7)	2 (1.8)	25 (3.1)
NSCC with NE morphology (positive NE markers)	0 (0.0)	10 (0.7)	0 (0.0)	1 (0.1)
NSCC with NE morphology (negative NE markers)	0 (0.0)	1 (< 0.1)	0 (0.0)	1 (0.1)
NSCC with squamous cell and ADC patterns	8 (0.9)	7 (0.5)	0 (0.0)	6 (0.7)
Poorly differentiated NSCLC with spinal and/or giant cell carcinoma	1 (0.1)	5 (0.4)	0 (0.0)	6 (0.7)

Abbreviations: ADC, adenocarcinoma; EGFR, epidermal growth factor receptor; NE, neuroendocrine; NOS, not otherwise specified; NSCC, non-small-cell carcinoma; NSCLC, non-small-cell lung cancer; SCC, squamous cell carcinoma; SCCA, small-cell carcinoma.

3.6. Correlations between EGFR mutation status and demographic data and disease status

Significant correlations were observed between the following patient characteristics and an *EGFR* mutation-positive tissue/cytology and plasma sample: ADC histology, never-smoking status, and Asia-Pacific ethnicity (Table 4).

4. Discussion

To our knowledge, IGNITE is the largest study of real-world *EGFR* mutation analysis, including observations of both tumour- and blood-based testing practices, in Asia-Pacific and Russia.

EGFR mutation frequency, as determined by local testing of tissue/cytology samples, was consistent with reported data in Asian and Caucasian populations [3–6,31–33]. Of interest, a higher than expected proportion of Russian patients had reports indicating unusual *EGFR* mutations (15.6% [14/90] ADC tumour samples and 26.7% [4/15] non-ADC samples), of which five were R836R – an unusual mutation which would be anticipated to be silent – and eight were exon 19 deletions combined with another mutation.

Of the patients with *EGFR* mutation-positive NSCLC with a squamous component (including SCC), the majority were of Asian ethnicity, male, and current or ex-smokers. This is a notable finding in a group frequently not tested, as they are considered unlikely to have *EGFR* mutations; although, in Russia, it has been shown that SCC is more frequent than ADC histology [34], likely due to high tobacco consumption [35]. Furthermore, although *EGFR* mutation-positive status of both tissue/cytology and plasma samples was aligned with previously characterised associations (ADC histology, never-smoking status, Asia-Pacific ethnicity, increasing number of metastases) [3,36], interestingly, a significant correlation was also observed between being aged ≤ 65 years and having an *EGFR* mutation-positive plasma sample (p = 0.0009), independent of other covariates. In addition, *EGFR* mutations were observed in some TTF-1-negative samples.

Together, these results support mutation testing in all Asian patients with NSCLC. For Caucasian patients of non-ADC histology, testing may warrant consideration on a case-by-case basis, particularly in never- or former-smokers. Moreover, when reporting *EGFR* mutations to clinicians, the nature of the mutation and whether it is activating and/or targetable by TKIs should be made clear, to assist with appropriate treatment decisions. Whilst the functional consequences of unusual or rare *EGFR* mutations are currently less well understood when compared with exon 19 deletions and L858R mutations, consistent reporting of these may assist in closing this gap in knowledge.

The mutation status concordance between plasma and tumour observed in Asia-Pacific (78%, with a sensitivity of 50% and specificity of 97%) suggests that ctDNA is a feasible sample for *EGFR* mutation analysis in real-world practice, if robust and sensitive DNA extraction and mutation analysis methodologies that are able to detect even low levels of mutations are employed. Clinical studies using centralised, validated ctDNA testing have shown that a plasma sensitivity of over 80% can be achieved with the latest technologies, with high specificity [37–40]. Although tumour samples should remain the preferred choice, due to the potential occurrence of false negative results by plasma testing, plasma-based mutation analysis represents a promising alternative for patients with unavailable tumour samples.

There was a difference in the sensitivity and specificity of plasma testing in Russia compared with Asia-Pacific, to the extent that, in Russia, the majority of plasma positive results (51/84) were not confirmed by tumour results. Further investigation showed that some of these 51 cases were due to incomplete coverage of key exons in tumour

Table 3
EGFR mutation status concordance between matched tissue/cytology and plasma samples (tissue/cytology and plasma evaluable population).

	Concordance rate		Sensitivity		Specificity		PPV		NPV	
	n (%)	95% CI	n/N (%)	95% CI	n/N (%)	95% CI	n/N (%)	95% CI	n/N (%)	95% CI
Overall (N = 2581)	2077/2581 (80.5)	78.9, 82.0	376/801 (46.9)	43.4, 50.5	1701/1780 (95.6)	94.5, 96.5	376/455 (82.6)	78.8, 86.0	1701/2126 (80.0)	78.2, 81.7
Russia (N = 894)	767/894 (85.8)	83.3, 88.0	33/109 (30.3)	21.8, 39.8	734/785 (93.5)	91.5, 95.1	33/84 (39.3)	28.8, 50.5	734/810 (90.6)	88.4, 92.5
Asia-Pacific (N = 1687)	1310/1687 (77.7)	75.6, 79.6	343/692 (49.6)	45.8, 53.4	967/995 (97.2)	96.0, 98.1	343/371 (92.5)	89.3, 94.9	967/1316 (73.5)	71.0, 75.8
China (n = 1355)	1051/1355 (77.6)	75.2, 79.8	267/548 (48.7)	44.5, 53.0	784/807 (97.1)	95.8, 98.2	267/290 (92.1)	88.3, 94.9	784/1065 (73.6)	70.9, 76.2
South Korea (n = 61)	51/61 (83.6)	71.9, 91.8	6/14 (42.9)	17.7, 71.1	45/47 (95.7)	85.5, 99.5	6/8 (75.0)	34.9, 96.8	45/53 (84.9)	72.4, 93.3
Taiwan (n = 271)	208/271 (76.8)	71.3, 81.6	70/130 (53.8)	44.9, 62.6	138/141 (97.9)	93.9, 99.6	70/73 (95.9)	88.5, 99.1	138/198 (69.7)	62.8, 76.0

Abbreviations: CI, confidence interval; EGFR, epidermal growth factor receptor; NPV, negative predictive value; PPV, positive predictive value.

Table 4
Correlations between *EGFR* mutation status derived from tissue/cytology and plasma-based testing (tissue/cytology and/or plasma evaluable population).

Demographic/disease status	Tissue/cytology				Plasma (China/South Korea/Russia/Taiwan only)			
	%	*p*-value	OR	95% CI	%	*p*-value	OR	95% CI
ADC vs. non-ADC	952/2249 (42.3) vs. 89/927 (9.6)	< 0.0001	3.973	2.943, 5.364	397/1814 (21.9) vs. 60/854 (7.0)	0.0002	1.955	1.377, 2.774
Asia-Pacific vs. Russia	941/2291 (41.1) vs. 110/924 (11.9)	< 0.0001	3.929	2.997, 5.151	375/1754 (21.4) vs. 87/941 (9.2)	< 0.0001	2.084	1.525, 2.848
Never- vs. ever-smoker	705/1352 (52.1) vs. 346/1863 (18.6)	0.0001	2.515	1.957, 3.233	298/1133 (26.3) vs. 164/1561 (10.5)	< 0.0001	2.077	1.624, 2.656
Female vs. male	571/1088 (52.5) vs. 480/2127 (22.6)	0.0075	1.409	1.096, 1.811	234/891 (26.3) vs. 228/1803 (12.6)	N/A	N/A	N/A
Greater number of organs with metastases, % of patients with *EGFR* mutation-positive NSCLC with 1, 2, 3, ≥4 metastatic organs	One metastatic organ: 458/1359 (33.7); two metastatic organs: 266/674 (39.5); three metastatic organs: 127/276 (46.0); ≥ four metastatic organs: 74/153 (48.4)	0.0909	1.086	0.987, 1.195	One metastatic organ: 167/1123 (14.9); two metastatic organs: 120/540 (22.2); three metastatic organs: 67/222 (30.2); four metastatic organs: 54/130 (41.5)	< 0.0001	1.386	1.242, 1.546
≤65 vs. > 65 years old	N/A	N/A	N/A	N/A	337/1791 (18.8) vs. 125/903 (13.8)	0.0009	1.561	1.201, 2.028

Abbreviations: ADC, adenocarcinoma; CI, confidence interval; EGFR, epidermal growth factor receptor; N/A, not available; NSCLC, non-small-cell lung cancer; OR, odds ratio.
Ethnicity correlation (Asia-Pacific vs. Russia) as per region.

testing. Notably, for three patients with exon 19 deletions in plasma, exon 19 had not been screened in the tumour sample. This is of concern, as exon 19 deletions can predict response to TKIs and should be tested for routinely in first-line aNSCLC.

A root-cause analysis of the plasma sample mutation testing methodologies of the Russian laboratories was conducted in order to understand possible reasons for the low sensitivity and specificity yielded. It was confirmed that plasma processing and handling in Russia had been performed in accordance with the laboratory manual, ruling out pre-analytical factors as contributors to the low sensitivity and specificity, several issues were found with subsequent DNA extraction and analysis. Regarding sensitivity, none of the Russian laboratories used a DNA extraction kit specifically optimised for ctDNA (i.e. suitable to detect low-concentration fragmented DNA found in the blood). Data from the ASSESS study [4] showed that use of a non-optimised DNA extraction method can significantly lower the sensitivity of plasma testing. Furthermore, a high proportion of rare mutations was detected in the Russian tumour samples (e.g. R836R) that are not targeted by the polymerase chain reaction (PCR)-based methods used for ctDNA testing, thus also reducing the apparent sensitivity of plasma testing. Regarding specificity, the proportion of false positives differed between laboratories. The laboratory with the highest proportion of false positives used a peptide nucleic acid-locked, nucleic acid PCR-based method, with no lowest cut-off for percentage of mutant ctDNA. In the absence of prospective clinical studies assessing whether these extremely low levels of mutant ctDNA predict response to TKIs, it is recommended that a plasma cut-off be defined that maximises specificity relative to tumour mutations, which have been shown to predict response.

The IGNITE study revealed substantial differences in sampling within and between Asia-Pacific and Russia. In particular, biopsy sample origin varied considerably within Asia-Pacific: a higher proportion of samples in Thailand were from metastases (64.9% [61/94]) compared with 38.0% (19/50) in Malaysia and < 30% in other countries. Associated with this, a higher proportion of samples in Thailand were from lymph nodes (27.7% [26/94]) compared with other countries (< 17%). Needle biopsy was common in Indonesia (51.0% [154/302]) and Thailand (40.4% [38/94]), but rare in other countries (< 20%). A wider range of *EGFR* mutation testing methodologies were also observed across Asia-Pacific, particularly for tissue/cytology samples. The results of the IGNITE study demonstrate that standardisation of the practical aspects of real-world mutation testing, particularly with regards to plasma-based ctDNA testing (highlighted by the anomalous IGNITE Russian plasma data), does still warrant further guidance and improvement.

With the increase in studies evaluating more sensitive mutation testing methodologies, there is the opportunity for global and local guidelines to be developed to facilitate a consensus on optimal mutation analysis of both tissue/cytology and plasma samples. Notably, ctDNA mutation testing offers the potential of real-time monitoring of tumour mutation status during TKI treatment via regular and minimally invasive blood sampling [41]. This may facilitate detection of TKI resistance-inducing mutations, such as T790 M [9], for which third-generation TKIs that target such mutations are available in the United States and European Union [42,43].

5. Conclusions

These real-world data indicate that *EGFR* mutation testing should be considered in all Asian patients with aNSCLC of ADC or non-ADC histology. Also, as activating *EGFR* mutations were observed in a small number of Caucasian patients with squamous NSCLC, testing here may be appropriate, particularly in those who have no history or a remote history of smoking. Continued education is required to ensure accurate testing and clarity in reporting of relevant *EGFR* mutations in some regions. ctDNA is a feasible, suitable sample for mutation analysis when

tumour samples are unavailable, if robust and sensitive mutation testing methods are employed; local in-house assays must be thoroughly validated before use in a clinical setting. Consensus of optimal tumour and plasma-based testing methods will ensure that patients receive the most appropriate treatments to address the molecular characteristics of their disease.

Conflict of interest statement

BH has participated in speakers' bureau for, and received consulting fees from, AstraZeneca, has participated in speakers' bureau for Roche, and has received consulting fees from Pfizer. ST has participated in speakers' bureau for AstraZeneca, Pfizer, and Sanofi-Aventis. KH has participated in speakers' bureau for AstraZeneca, Pfizer, and Chugai Pharmaceuticals, and has a patent with LSI Medience. NN has received grants, research, and consulting fees from AstraZeneca. LW has participated in speakers' bureau for AstraZeneca and has received consulting fees from Boehringer Ingelheim. KL has participated in speakers' bureau for Eli Lilly, AstraZeneca, Pfizer, Boehringer Ingelheim, and BMS. AH, YH, Y-PZ, M-ZW, and CYL have no relationships to disclose. MR and RM are employed by, and have stock or other ownership of, AstraZeneca. MR has participated in speakers' bureau for, and received consulting fees from, Hoffmann-La Roche, Lilly, BMS, AstraZeneca, Pfizer, Boehringer Ingelheim, and MSD, and has received consulting fees from Daiichi-Sankyo.

Role of the funding source

This study was sponsored by AstraZeneca and co-ordinated by Worldwide Clinical Trials, who also managed the database and performed the primary analyses. In collaboration with AstraZeneca, the study results were interpreted by the study steering committee. The corresponding author had full access to the study data and final responsibility for the decision to submit for publication. A full list of the IGNITE study principal investigators and study centres is included in Supplementary Table 1.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.lungcan.2017.08.021.

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