EUROPEAN LUNG CANCER CONFERENCE 2016

Plasma ctDNA analysis for detection of EGFR T790M mutation in patients with EGFR mutation-positive advanced non-small cell lung cancer

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Disclosures

Suzanne Jenkins, Susie Weston, Mireille Cantarini, Sabina Patel – Employees and shareholders: AstraZeneca

Rachael Lawrance – Shareholder and former employee: AstraZeneca

James C-H Yang – Advisory board: Boehringer Ingelheim, Eli Lilly, Bayer, Roche/Genentech, AstraZeneca, Astellas, Bayer, MSD, Merck Serono, Pfizer, Novartis, Clovis Oncology, Celgene

Suresh S Ramalingam – Consultancy: AstraZeneca, Boehringer Ingelheim, Celgene, Genentech, Novartis, Eli Lilly, Merck, Bristol-Myers Squibb

Karen Yu – Employee: Roche Molecular Systems

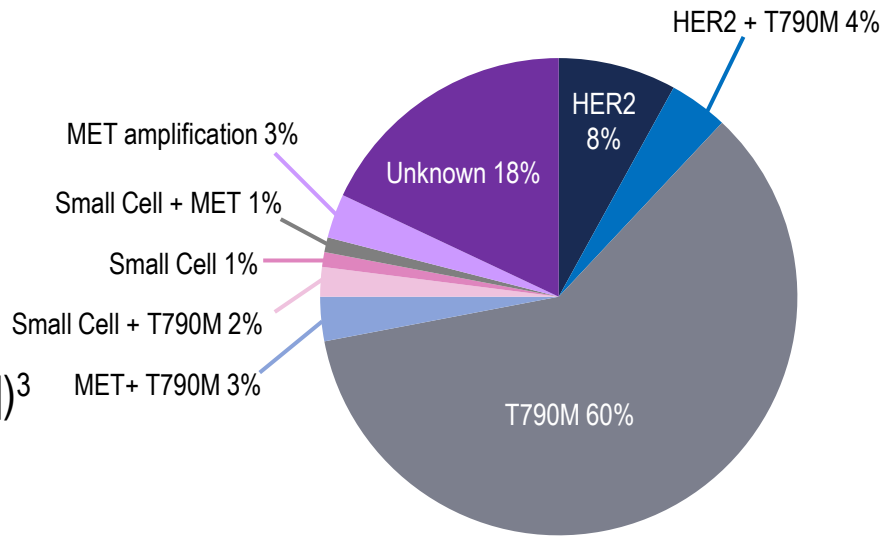
Pasi A Jänne – Consultancy: AstraZeneca, Pfizer, Roche; research support: AstraZeneca, Astellas; stock ownership: Gatekeeper Pharmaceuticals; other: Post-marketing royalties on Dana-Farber Cancer Institute-owned patent on EGFR mutations licensed to Lab Corp

Tetsuya Mitsudomi – Advisory board: AstraZeneca, Boehringer Ingelheim, Chugai, Pfizer; honoraria: AstraZeneca, Chugai, Boehringer Ingelheim, Pfizer; research funding: Boehringer Ingelheim, Chugai, Pfizer

Background

- The EGFR T790M resistance mutation is observed in ~60% of patients with EGFR mutation positive (EGFRm) advanced NSCLC who progress on first-line EGFR-TKIs[1]
- Osimertinib (AZD9291) is an irreversible EGFR-TKI that is selective for sensitising EGFRm and T790M resistance mutations[2]
- Osimertinib treatment has demonstrated efficacy in patients with T790M positive advanced NSCLC (ORR 66% [95% CI 61,71])[3]

Relative frequency of mechanisms of acquired resistance to approved EGFR-TKIs[1,*]



*Data shown are from an analysis of tumour specimens from 155 patients at the time of acquired resistance to gefitinib or erlotinib therapy[1]

1. Yu HA et al. Clin Cancer Res 2013;19:2240–2247; 2. Cross DAE et al. Cancer Discov 2014;4:1046–1061; 3. Yang et al. LBA_2 PR, European Lung Cancer Conference 2016

CI, confidence interval; EGFR, epidermal growth factor receptor; EGFRm, EGFR mutation-positive; NSCLC, non-small cell lung cancer; TKI, tyrosine kinase inhibitor; ORR, objective response rate

Testing for T790M at disease progression to direct treatment decisions

- In the US and Japan, osimertinib is indicated for treatment of patients with metastatic EGFR T790M mutation-positive NSCLC, as detected by an approved test, who have progressed on or after EGFR-TKI therapy[1,2]
- In the EU, osimertinib is indicated for the treatment of adult patients with locally advanced or metastatic EGFR T790M mutation-positive NSCLC[3]
- At the time of disease progression, not all patients are able to provide tumour biopsies for EGFR T790M testing
- Plasma circulating tumour DNA (ctDNA) testing from blood samples provides a less invasive alternative
- Here we present analysis of T790M by plasma from patients enrolled in the AURA Phase II studies (AURA extension [NCT01802632] and AURA2 [NCT02094261])

1. TAGRISSO™ (osimertinib) prescribing information, available at http://www.accessdata.fda.gov/drugsatfda_docs/label/2015/208065s000lbl.pdf;
2. TAGRISSO (osimertinib) Japan prescribing information, March 2016 Version 1;
3. TAGRISSO™ Summary of Product Characteristics; available at http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/004124/WC500202022.pdf
ctDNA, circulating tumour DNA; FDA, Food and Drug Administration

A plasma test can complement a tissue test to optimise diagnostic practices

Potential barriers to testing	Tumour tissue	ctDNA
Patient willingness to undergo tissue biopsy[1]	x	✔
Patient fitness to undergo tissue biopsy[2]	x	✔
Contraindication due to concomitant therapy (e.g. anticoagulants)[1]	x	✔
Ease of obtaining a sample for testing	x	✔
Tumour tissue heterogeneity[2–4]	x	✔
Tumour tissue sample size/quality[2,4]	x	✔
Tumour burden[4]	✔	x
ctDNA shedding[4]	✔	x
Risk of complications[1,2,4]	x	✔
Cost[2]	x	✔
Turnaround time[4]	x	✔
Provider / laboratory familiarity with methodology	✔	x

1. Chouaid C et al. Lung Cancer 2014;86:170–173; 2. Korpanty G et al. Oncol Ex 2012;11:8–10;
3. Huang WL et al. Biomed Res Int 2015; 2015:1–11; 4. Diaz L Jr and Bardelli AJ. Clin Oncol 2014;32:579586

Plasma analyses in AURA trials

- Across the AURA trials (NCT01802632, NCT02094261), plasma was collected for analysis

	AURA Phase I	Phase II studies: AURA extension and AURA2
Treatment / dosing	Osimertinib dose escalation and dose expansion cohorts (20–240mg QD)	Osimertinib 80 mg QD
T790M status	T790M positive and negative	Only T790M positive
Analysis	Exploratory post-hoc analysis	Intention to treat for regulatory submission
Plasma assay	BEAMing	cobas
Method of comparison	ddPCR or cobas	NGS
ELCC presentation	Oxnard G. et al; 135O	Jenkins S. et al; 134O [Yang J. presenting]

The **cobas**® EGFR Mutation Test is not available for use with plasma samples in U.S
BEAMing, Beads, Emulsification, Amplification and Magnetics; ddPCR, droplet digital PCR; NGS, next generation sequencing; QD, once daily

Osimertinib AURA studies

AURA – NCT01802632

- A Phase I and II extension study in patients with advanced NSCLC who have progressed on prior therapy with an EGFR-TKI

AURA2 – NCT02094261

- A Phase II single-arm study of osimertinib 80 mg qd in patients with locally advanced/metastatic NSCLC who have progressed following prior EGFR-TKI therapy

AURA3 – NCT02151981

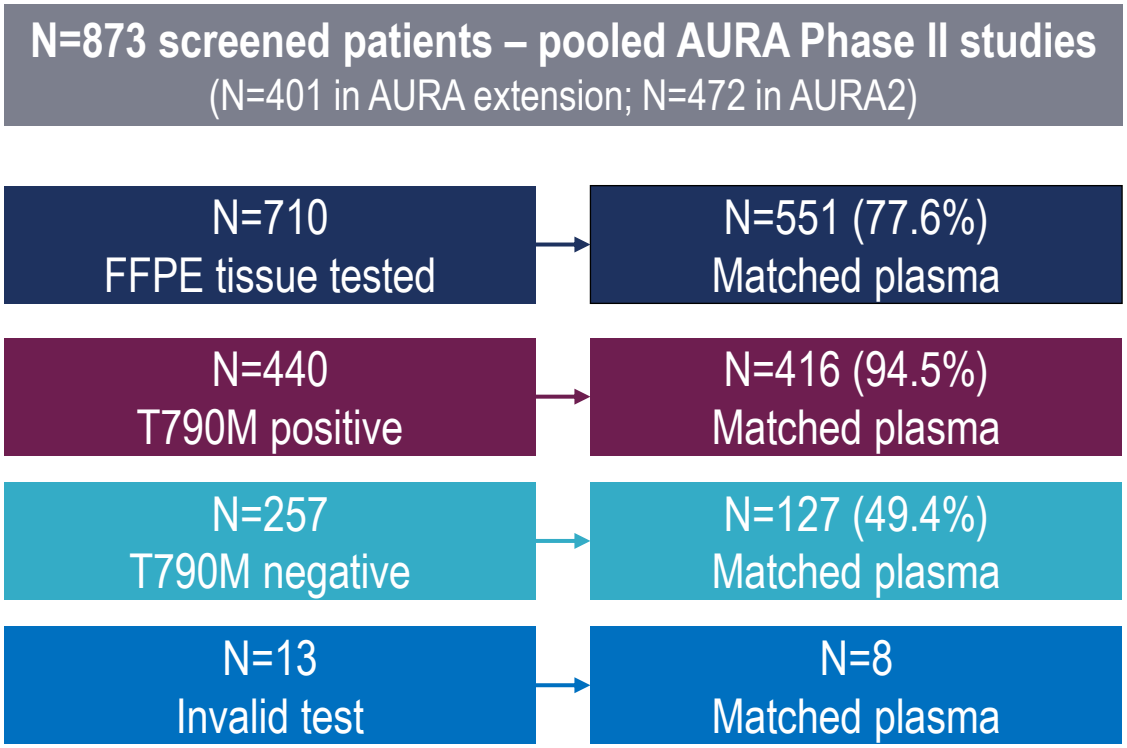
- A Phase III randomised study to compare osimertinib with platinum-based doublet chemotherapy in patients with locally advanced / metastatic T790M positive NSCLC who have progressed following prior EGFR-TKI therapy

- Patients were selected for the AURA Phase II studies using the Roche **cobas**® EGFR Mutation Test (IUO version) using tumour tissue. Plasma samples were analysed using the cobas test
- Testing flow:
  - Manual sample preparation to obtain DNA from FFPET or plasma
  - PCR amplification and detection of target DNA using complementary primers and oligonucleotide probes labeled with fluorescent dyes
  - Mutation detection through PCR analysis with the **cobas**® z 480 analyzer. A mutant and negative control are included in each run to confirm validity
- The test identifies 42 mutations: exon 19 deletions, L858R, T790M, G719X, exon 20 insertions, S768I, L861Q

The **cobas**® EGFR Mutation Test is not available for use with plasma samples in U.S.
FFPET, formalin-fixed, paraffin-embedded tumour tissue

Plasma sample collection

- Matched plasma samples were collected from screened patients in the Phase II AURA studies (AURA extension and AURA2) for retrospective analysis



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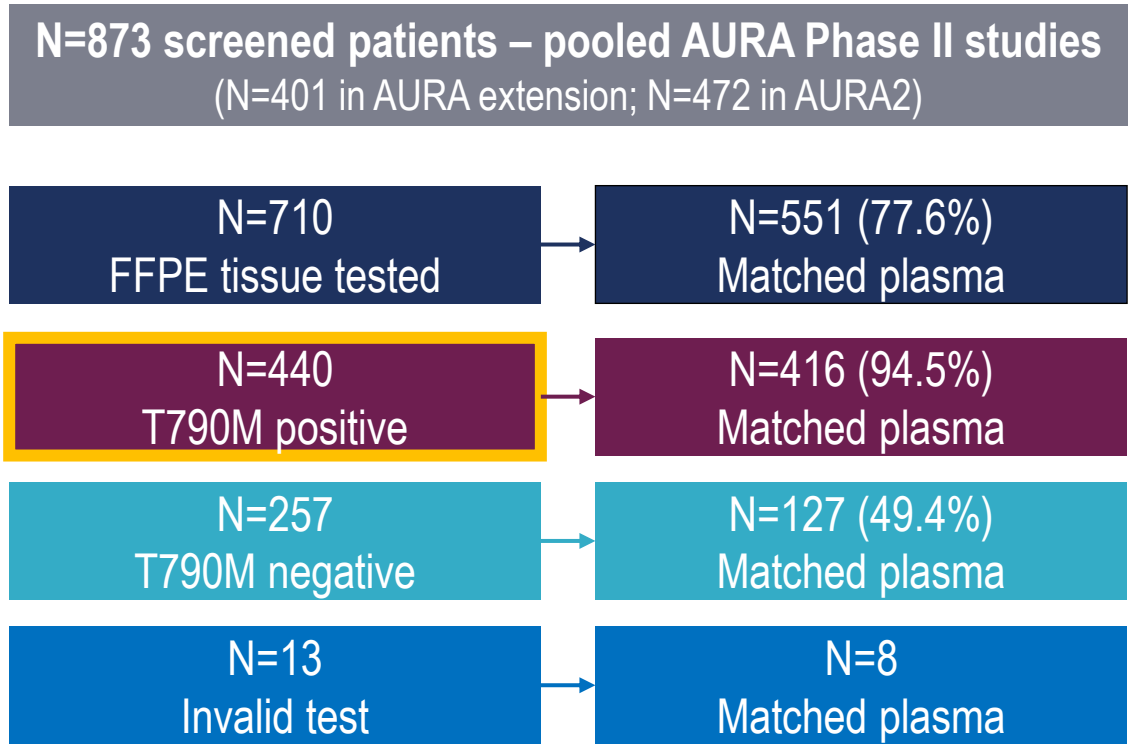

T790M concordance between cobas and NGS

- The cobas test showed good concordance with NGS reference methods using both tissue and plasma samples

cobas tissue test - tissue vs tissue	AURA2 (N=383)
Using MiSeq NGS of tissue as reference	
PPA / sensitivity	88.3% (83.8–91.7)
NPA / specificity	97.3% (92.4–99.1)
OPA / concordance	91.0% (87.7–93.5)

cobas ctDNA test - plasma vs plasma	AURA2 plasma samples (N=344)
Using MiSeq NGS of plasma as reference	
PPA / sensitivity	91.5% (85.7–95.1)
NPA / specificity	91.1% (86.0–94.4)
OPA / concordance	91.3% (87.6–94.1)

Using NGS of plasma ctDNA as a reference method, the cobas plasma test is highly sensitive and specific for T790M detection

The **cobas**® EGFR Mutation Test is not available for use with plasma samples in U.S.

NGS, next generation sequencing; NPA, negative percentage agreement; OPA, overall percentage agreement; PPA; positive percentage agreement

cobas plasma test versus cobas tissue test as a reference method

cobas plasma test performance	Pooled AURA Phase II studies (AURA extension and AURA2)		
	L858R	Exon 19 deletion	T790M
Using cobas tissue test as reference			
PPA / sensitivity	75.6%	85.1%	61.4%
NPA / specificity	98.1%	98.0%	78.6%
OPA / concordance	90.9%	90.0%	65.4%

Differences in detection of T790M using tissue and plasma are thought to reflect tumour biology and molecular heterogeneity in the resistance setting

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Objective response rate based on tissue and plasma mutation results

ORR (95% CI)	AURA extension	AURA2	Pooled AURA Phase II studies (AURA extension and AURA2)[1]
ctDNA T790M positive subset	59.1% (50.0, 67.7)	69.7% (60.2, 78.2)	64.0% (57.5, 70.1)
Evaluable for response set (tissue T790M positive)	61.3% (54.2, 68.1)	70.9% (64.0, 77.1)	66.1% (61.2, 70.7)

In the AURA Phase II pooled analysis, the ORR for the plasma T790M-positive subset was similar to that of the evaluable for response set (selected using tissue testing)

Data cut-off: 1 May 2015

1. Yang et al. LBA_2 PR, European Lung Cancer Conference 2016

Conclusions

- A plasma-based companion diagnostic has been developed for osimertinib*
- Plasma and tissue-based tests are similarly sensitive and specific compared with an NGS reference method
- In the EU, when considering osimertinib treatment, T790M mutation status can be determined using either a tissue-based or plasma-based test[1]
- Following a negative T790M result from a plasma-based test, it is advisable to follow-up with a tissue-based test

*A CE-IVD test for use with tissue and plasma is already launched in the EU, pre-market approval application for the plasma-based test is under review by the FDA

[1]TAGRISSO™ EU Summary of Product Characteristics. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/004124/WC500202022.pdf accessed 2 March 2016

CE-IVD, Conformité Européenne In vitro Diagnostic

Acknowledgements

- With thanks to
  - The investigators and patients involved in the AURA Phase II studies
  - The team at AstraZeneca
  - The team at Roche Molecular Systems
- This work was funded by AstraZeneca

These studies were sponsored by AstraZeneca, NCT01802632 and NCT02094261
We thank Donna Tillotson, PhD, from iMed Comms, an Ashfield Company, who provided technical editing support funded by AstraZeneca