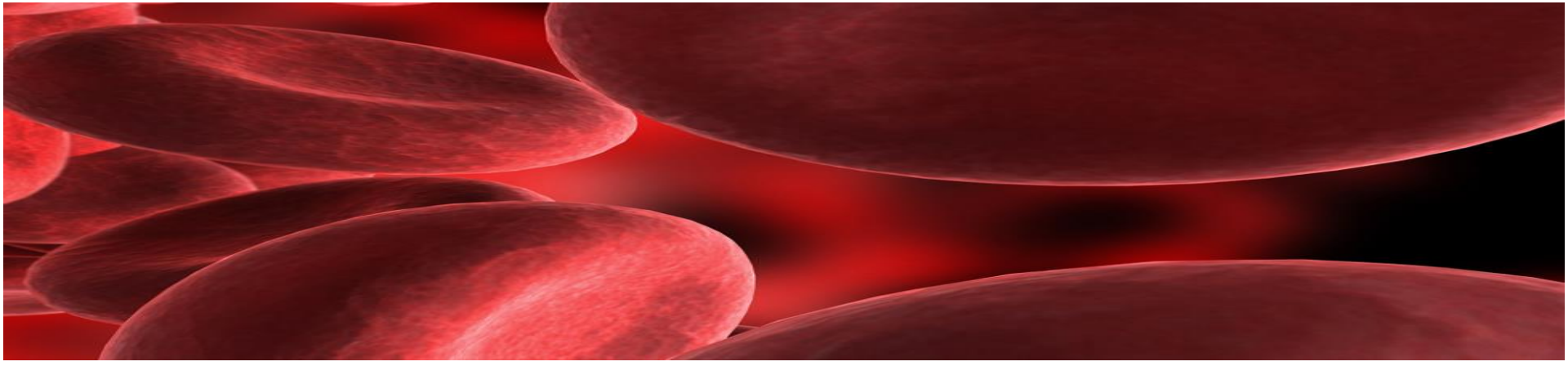
cobas® EGFR Mutation Test for Use with Plasma
13 October 2016

Roche Molecular Systems

Lesley Farrington



Overview of Lung Cancer

Leading cause of cancer death worldwide

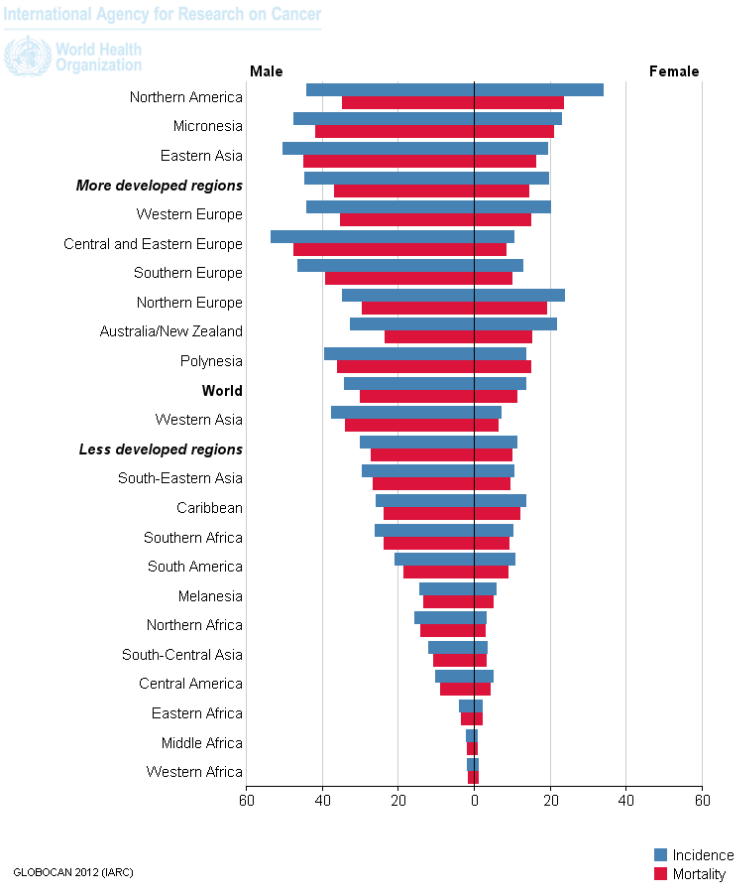
–Incidence Worldwide **1.83 M**

–Deaths Worldwide: **1.59 M**

–Most common cancer in men (1.2M cases) and 3rd most common cancer in women worldwide

–Leading cause of cancer death in the US for men and women

–Non-small cell lung cancer accounts for 85% of all lung cancer in the U.S.



Rate per 100,000 population

Source: WHO IARC Globocan in 2012 Cancer Facts & Figures, 2014

cobas®
Life needs answers

Most Lung Cancer is Diagnosed at Advanced Stages

Extent of Disease at Diagnosis

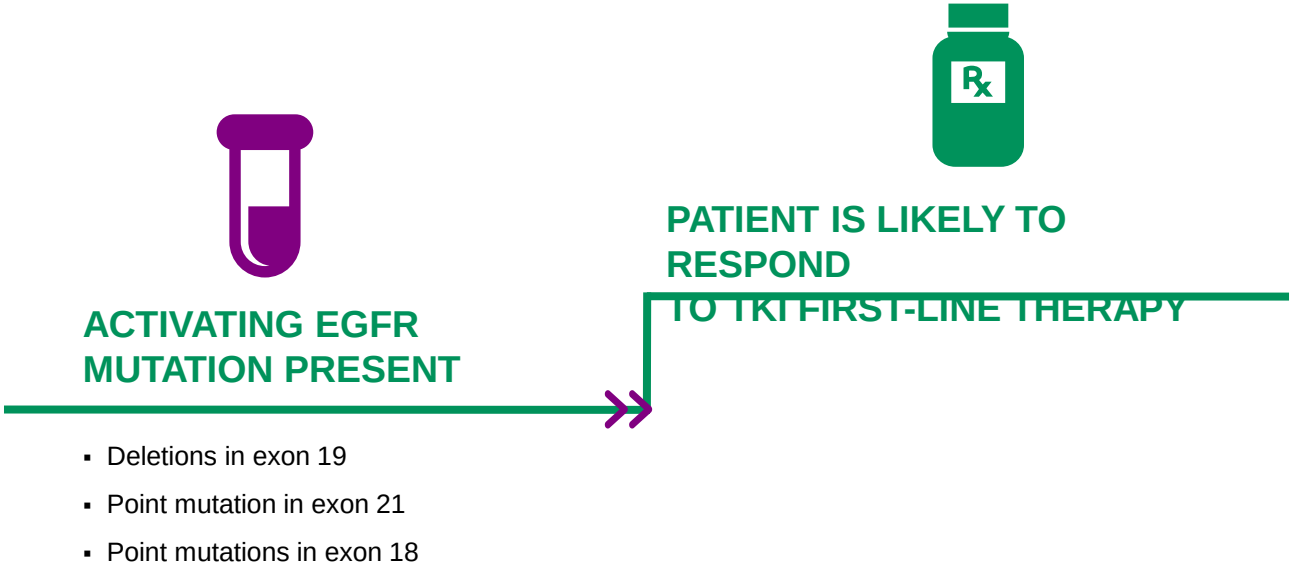
Most patients present with stage IIIB or stage IV disease[2]

5–6 month median survival for untreated stage IIIB/IV NSCLC[3,4]

1. Jemal et al. *CA Cancer J Clin*. 2006;56:106–130.
2. Schrump et al. Non-small cell lung cancer. In: *Cancer: Principles and Practice of Oncology*. 7th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2005.
3. Spiro et al. *Thorax*. 2004;59:828-836.
4. Albain et al. *J Clin Oncol*. 1991;9:1618-1626.

EGFR mutations influence therapy choice

- *Common mutations confer sensitivity to TKIs*[1-6]



- Deletions in exon 19
- Point mutation in exon 21
- Point mutations in exon 18

[1] Mok TM et al. N Engl J Med 2009;361:947–957;

[2] Maemondo M et al. N Engl J Med 2010;362:2382–2388;

[3] Rosell R et al. N Engl J Med 2009;361:958–967;

[4] Lynch TJ et al. N Engl J Med 2004;350:2129–2139;

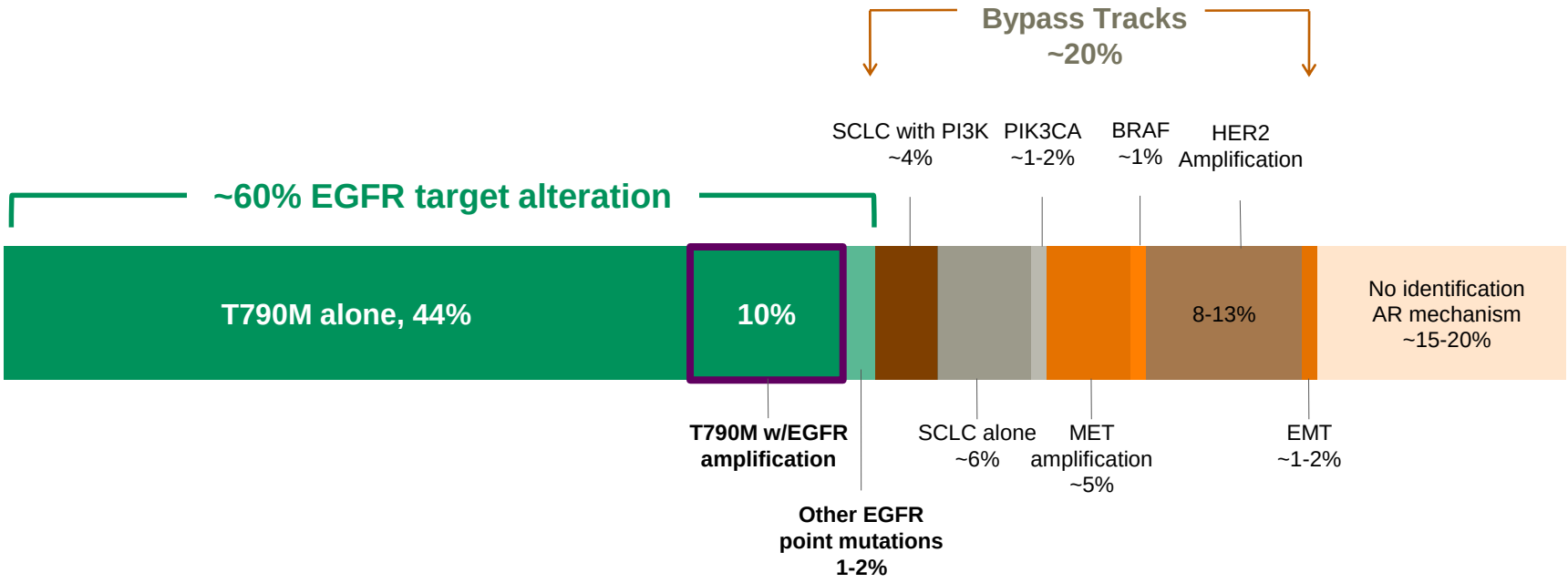
[5] Jackman DM et al. Clin Cancer Res 2009;15:5267–5273;

[6] Maheswaran S et al. N Engl J Med 2008;359:366–377.

cobas® Life needs answers

Resistance to EGFR TKIs

Most prevalent mutation is T790M

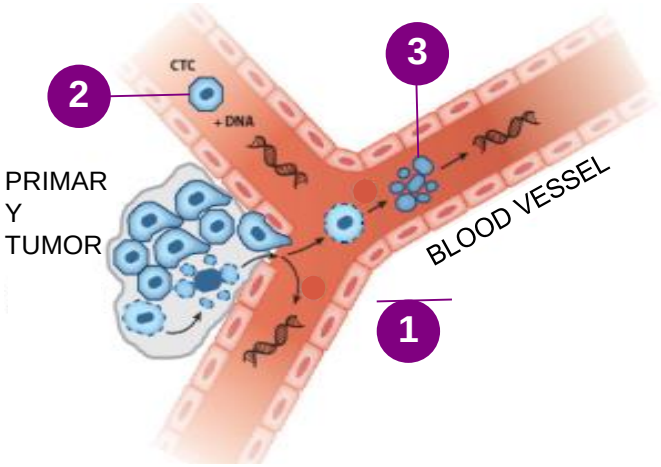


Camidge, D. R. et al. (2014) Acquired resistance to TKIs in solid tumours: learning from lung cancer
Nat. Rev. Clin. Oncol.
doi:10.1038/nrclinonc.2014.104

Liquid Biopsy

- *Tumors release proteins, nucleic acids and cells into blood*

Tumor releases a multitude of biomarkers into blood



Tumor biomarkers in blood

1. Cell-free DNA (cfDNA)
2. Circulating tumor cells (CTCs)
3. Exosomes[1] & micro vesicles

Liquid biopsies can provide real-time treatment prediction and resistance detection

[1] Exosomes = lipid vesicles containing protein and nucleic acid; found in both blood and urine

Source: Speicher et al., Tumor signatures in blood, Nature 2014.

cobas® Life needs answers

cobas® EGFR Mutation Test v2 Intended Use

Clinical Claims

The **cobas®** EGFR Mutation Test v2 is a real-time PCR test for the qualitative detection of defined mutations of the epidermal growth factor receptor (EGFR) gene in non-small cell lung cancer (NSCLC) patients. **Defined EGFR mutations are detected using DNA isolated from formalin-fixed paraffin-embedded tumor tissue (FFPET) or circulating-free tumor DNA (cfDNA) from plasma derived from EDTA anti-coagulated peripheral whole blood.**

The test is indicated as a **companion diagnostic** to aid in selecting NSCLC patients for treatment with the targeted therapies listed in Table 1 below in accordance with the approved therapeutic product labeling:

Drug	FFPET	Plasma
TARCEVA® (erlotinib)	Exon 19 deletions and L858R	Exon 19 deletions and L858R
TAGRISSO™ (osimertinib)	T790M	T790M*

Patients with positive **cobas®** EGFR Mutation Test v2 test results using plasma specimens for the presence of EGFR exon 19 deletions or L858R mutations are eligible for treatment with TARCEVA® (erlotinib). ***Patients who are negative for these mutations by this test should be reflexed to routine biopsy and testing for EGFR mutations with the FFPET sample type.***

*The efficacy of TAGRISSO™ (osimertinib) has not been established in the EGFR T790M plasma-positive, tissue-negative or unknown population and clinical data for T790M plasma-positive patients are limited; therefore testing using plasma specimens is most appropriate for consideration in patients from whom a tumor biopsy cannot be obtained.

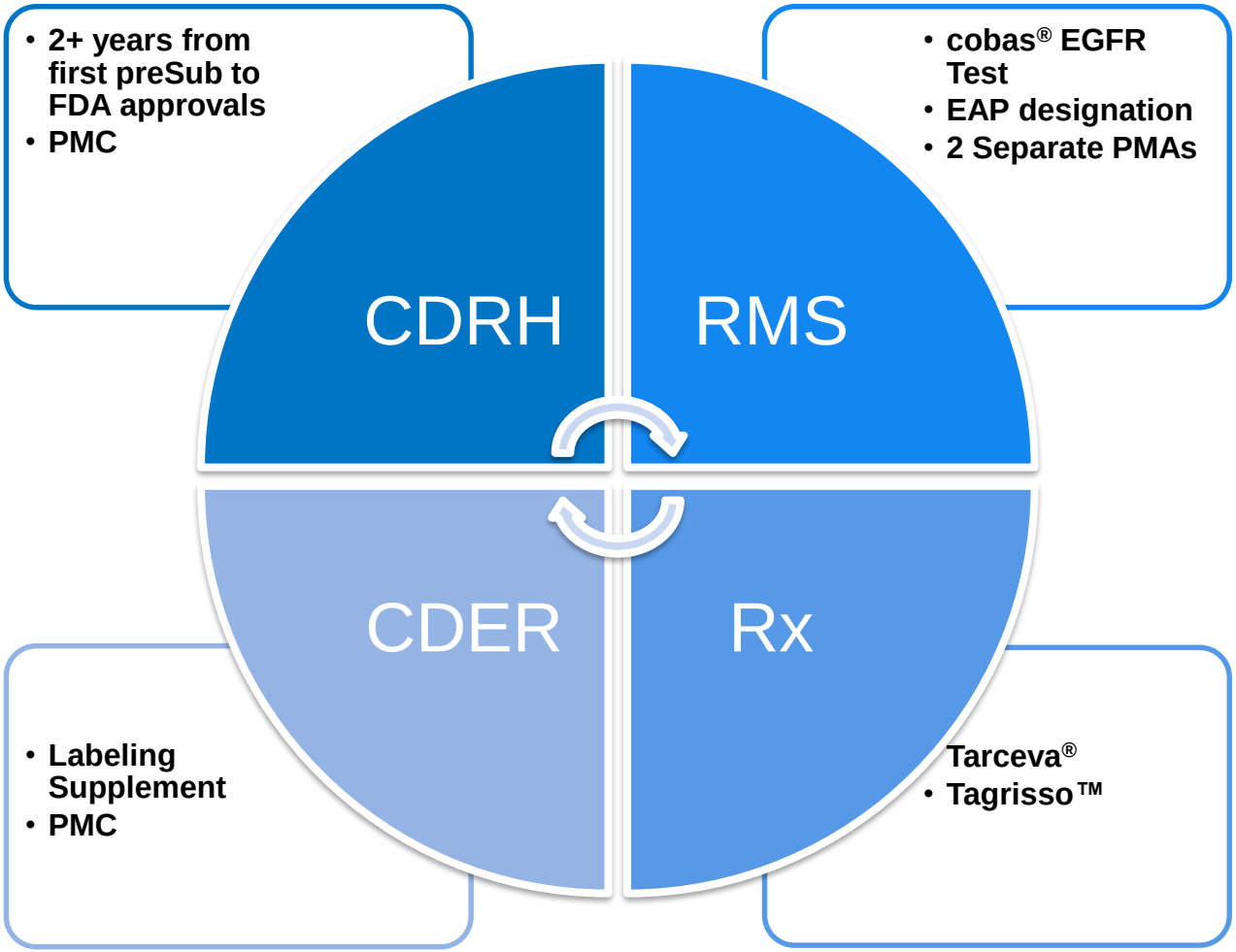
cobas® EGFR Mutation Test v2 Intended Use

Analytical Claims

Safety and efficacy have not been established for the following EGFR mutations also detected by the **cobas**® EGFR Mutation Test v2:

Drug	FFPET	Plasma
TARCEVA® (erlotinib)	G719X, exon 20 insertions, T790M, S768I and L861Q	G719X, exon 20 insertions, T790M, S768I and L861Q
TAGRISSO™ (osimertinib)	G719X, exon 19 deletions, L858R, exon 20 insertions, S768I, and L861Q	G719X, exon 19 deletions, L858R, exon 20 insertions, S768I, and L861Q

cobas® EGFR Test v2 for Use with Plasma



Life needs answers

cobas® EGFR Test v2 for Use with Plasma

EAP Designation

Expedited Access for Premarket Approval and De Novo Medical Devices Intended for Unmet Medical Need for Life Threatening or Irreversibly Debilitating Diseases or Conditions

Guidance for Industry and Food and Drug Administration Staff

Document issued on April 13, 2015.

The draft of this document was issued on April 23, 2014.

cobas® EGFR Test v2 for Use with Plasma
EAP Designation

The following 3 criteria should be met:

- The device is intended to treat or diagnose a life-threatening or irreversibly debilitating disease or condition.
- The device meets at least one of the following criteria for addressing an unmet need:
  - No appropriate alternative treatment or means of diagnosis exists.
  - The device represents a breakthrough technology that provides a clinically meaningful advantage over existing legally marketed technology.
  - The availability of the device is in the best interest of patients (e.g., addresses an unmet medical need).
- The sponsor submits an acceptable draft Data Development Plan.

cobas® EGFR Test v2 for Use with Plasma

EAP Designation

- Request for designation as an EAP Device
- Agreement on a Data Development Plan
- Review of a PMA or de novo request for an EAP Device
- Postmarket data collection an evaluation

A few points to consider when developing a plasma-based test for tumor mutations

Customer and Product Requirements

- Physician and patient needs – Fast TAT, high accuracy
- Analytical and Clinical Sensitivity and Specificity required to be clinically useful
- Robust, consistent, reliable, cost-effective

Regulatory requirements

- Collaboration with FDA for novel diagnostic indication and claims, as well as Global Registration and Use
- Specimen acquisition for Development and Technical Performance Verification – Adequate real world sample availability? Alternatives?
- Reference Methods – How is truth established with regard to tissue, and plasma results?

Plasma and Tissue Specimen Acquisition/Use

Impractical to Use Clinical Specimens for all Development and TPV Studies

Practical considerations

- Prospective collection and time to screen samples = very expensive!
  - ~$2 million spent on cell line DNA and specimens
- Large volumes needed for non-clinical performance study panels
- Low mutation prevalence and low cfDNA concentrations when present
  - Purchased ~1000 specimens (commercial vendors) to find ~80 specimens with detectable mutations
  - Most had EGFR mutation cfDNA concentrations < 200 copies/mL

Ethical considerations

- HIPAA, IRB, ICF
- Patient population is very ill
- IRB typically permits 30 – 40mL whole blood to be drawn (15 – 20mL plasma)

Drives need for constructing appropriate contrived samples to evaluate analytical test performance

Plasma cfDNA Reference Methods

The Challenge of defining "Truth"

How to define biological truth?

- No validated reference methods readily available
- No international standards
- Partially dependent on ***concurrent*** tissue and plasma collection
- Dependent on tumor heterogeneity, disease stage and metastatic progression
- Clinical drug response the ultimate indicator

Quantitative reference methods as options

- Must validate sensitivity, specificity, reportable range of reference methods
- Most methods to detect rare mutations are affected by the amount of background, *normal* cfDNA present (eg from lymphocyte lysis)
- ddPCR sensitivity is not easily multiplexed, FDA recommended using a non-PCR method
  - **cobas**® EGFR Mutation Test v2 detects at least **42** mutations
- NGS sensitivity depends on ***absolute*** and ***relative*** mutant cfDNA concentration
  - Must increase read depth to detect low mutant cfDNA concentrations

Non-Clinical Performance Studies

Clinical and Contrived Plasma Samples

Contrived sample performance directly traceable to clinical specimen results

- Determined ***Limit of Detection (LoD)*** with contrived samples consisting of sheared cell line DNA containing EGFR mutations diluted in healthy donor (HD) K2-EDTA plasma

- Demonstrated ***commutability:*** sheared cell line DNA diluted in HD plasma or NSCLC K2-EDTA plasma yield equivalent results at concentrations near LoD

- Confirmed LoD in clinical setting using NSCLC plasma panels

Non-Clinical Performance Studies

LoD Study Results (according to CLSI EP17-A2)

EGFR Exon	EGFR Mutation Group	EGFR Nucleic Acid Sequence	LoD (copies/mL)	COSMIC ID
18	G719X	2156 G>C	**100**	6239
19	Exon 19 Deletion	2235_2249del15	**75**	6223
20	T790M	2369 C>T	**100**	6240
	S768I	2303 G>T	**25**	6241
	Exon 20 Insertion	2307_2308insGCCAGCGTG	**25**	12376
21	L858R	2573 T>G	**100**	6224
	L861Q	2582T>A	**30**	6213

Notes:

- **LoD study tested 72 replicates across 3 lots per level in healthy donor plasma**
- Limit of Blank (LoB) determined to be zero for all mutations reported by the test (N=198 across 3 lots for each of 33 Healthy Donor samples)
- Samples used in this study had a *wild-type* DNA background of approximately 100,000 copies/mL **(~0.025-0.1% mutation)**

Non-Clinical Performance Studies

LoD Confirmation in Clinical Setting

Study Objective: Confirm the LoD of EGFR mutations in NSCLC patient plasma

- **Panel Design:**
  - NSCLC clinical specimens with known EGFR mutations diluted into NSCLC EGFR *wild-type* plasma
  - **11 member panel (1X LoD & 2X LoD):**
    - Three most prevalent exon 19 deletion mutations
    - One L858R mutation sample
    - One T790M mutation sample
    - One *non-mutant EGFR normal* sample
- **Test plan**
  - Three testing sites (two external and one internal, two operators per site)
  - Three reagent lots (two non-identical lots per site)
  - Two non-consecutive testing days
  - Two replicates per panel member per run

Clinical Reproducibility Study

Plasma

- **Study Objective:** Evaluate the reproducibility for the detection of mutations in exons 18, 20, and 21 of the EGFR gene across the following factors:
  - 3 manufactured lots of reagents, 2 non-identical lots per site
  - 3 **cobas z** 480 instruments, 1 per site
  - 3 sites, 2 operators per site
  - 3 non-consecutive days
- **Specimen panel design:** 648 total replicates
  - **9 panel members**, 7 different mutations: exon 18 G719X; exon 20 T790M; exon 20 S768I; exon 20 insertion; exon 21 L861Q; 1 WT
  - For each mutation: **100 and 300 copies/ml**
  - Each panel member tested in **duplicate**

Clinical Studies

Correlation to NGS, FFPET; Clinical Outcome

Analytical Accuracy:
Correlation to NGS

Clinical Outcome

Correlation between
Plasma and FFPET

cobas® EGFR Test v2 for Use with Plasma

FDA Approvals

Tarceva: 02 June 2016

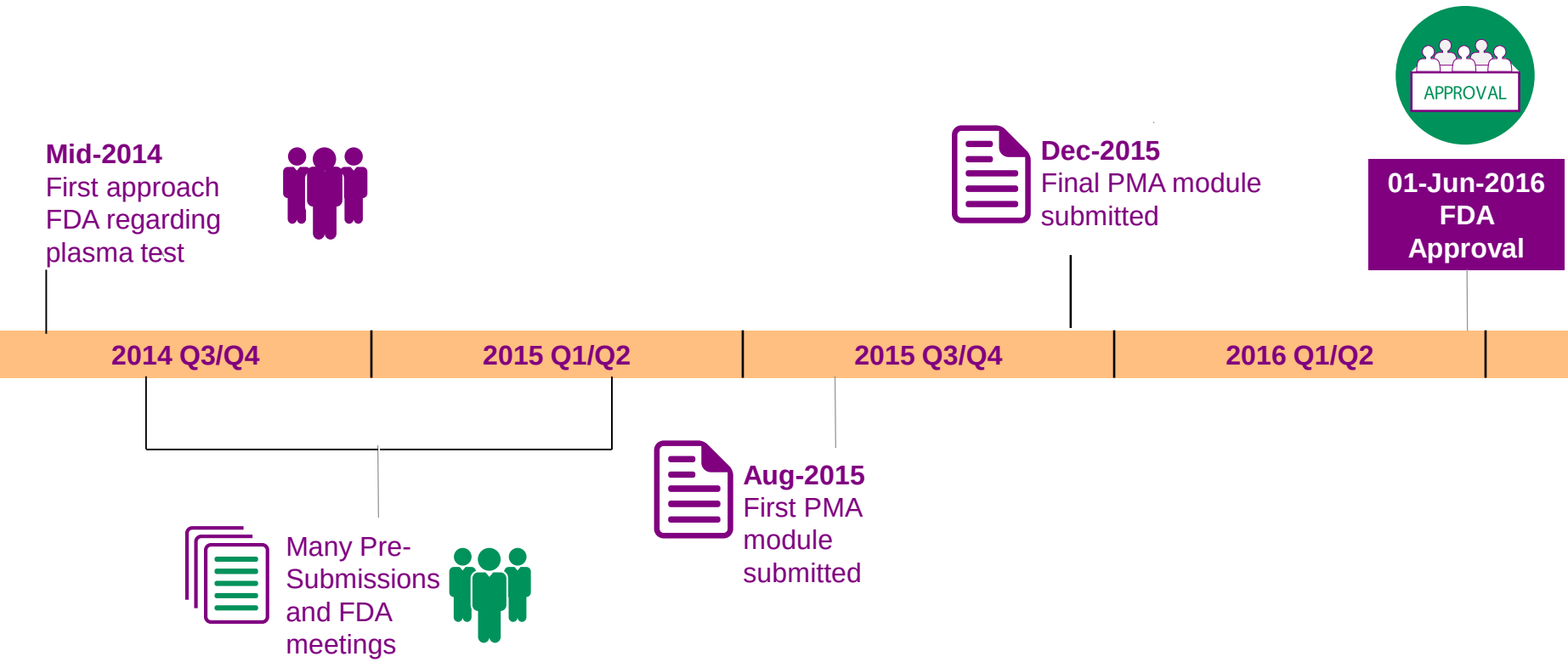
Tagrisso: 28 September2016

Doing now what patients need next

Life needs answers

PMA Submission Timeline

- *~2 Years from initiating discussions with FDA to approval!*



Roche

cobas®
Life needs answers

Doing now what patients need next

Life needs answers