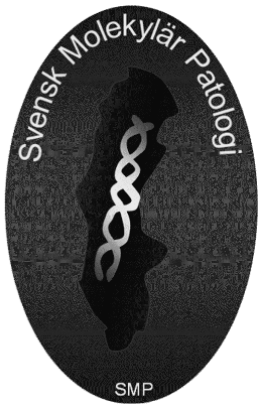
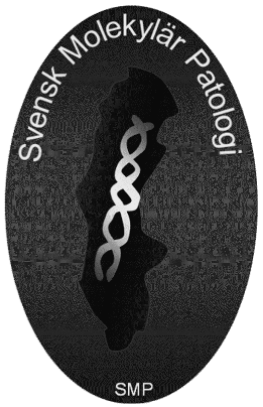
QA in Predictive Molecular Pathology



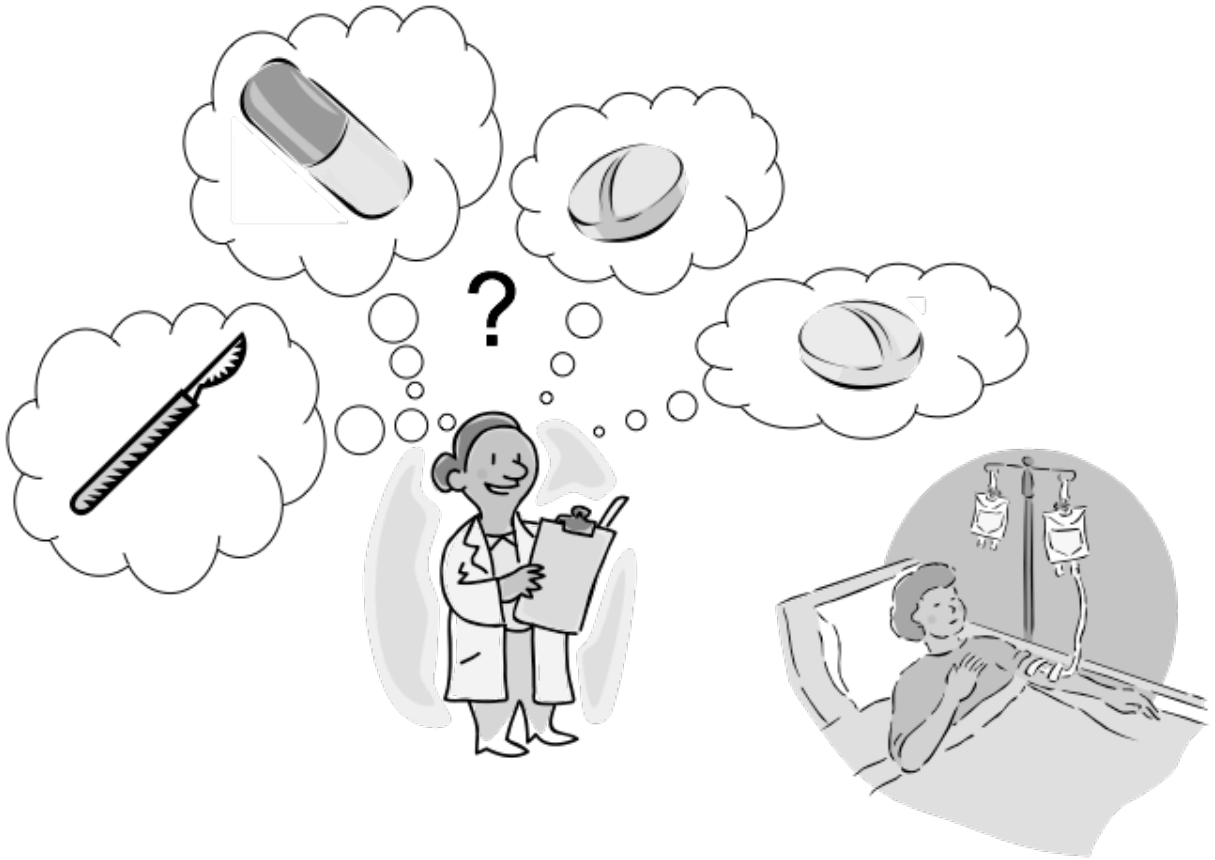
Anders Edsjö
Uppsala 2012-09-25

QA in Predictive Molecular Pathology

Why?
What?
How?
Results
Future?



Predictive Molecular Pathology



Predictive Molecular Pathology - Need for QA

- Rational use of targeted drugs a necessity
- Tight link between genotype and treatment response
- No backup from histology or traditional ancillary methods
- Medicolegal issues

QA in Molecular Pathology
- Freedom of Choice for ESP labs

- CE-labelling vs IVD
- Generic testing vs companion Dx
- In-house assays vs commercial kits

Generic Testing vs Companion Diagnostics

Generic Testing

e.g. in house/kit-based *BRAF* pyrosequencing +/- Sanger

Pros: Flexible, open

Cons: More extensive internal validation needed

Companion Diagnostics

e.g. 4800 BRAF V600 Mutation Test for vemurafenib treatment

Pros: Closeness to clinical studies

Thorough validation

Cons: Closed system

Harder to trouble shoot

Might potentially lead to a multitude of platforms

Dependence on individual suppliers

In House vs Dx Kits
- *EGFR* in NSCLC

In House

Pros: Possibility to create flexible, open and sensitive systems

Cons: Technically difficult to cover all relevant mutations with high sensitivity

Extremely costly to validate assays for rare mutations

Dx Kits

e.g. Allele-specific PCR & pyrosequencing kits

Pros: Well validated – (CE-, IVD-approval)

Usually high sensitivity

Cons:

Closed systems

Harder to trouble shoot – black box

Dependence on individual suppliers

QC/QA/EQA?

(External) Quality Assessment

Quality Assessment

Proficiency testing
Rechecking testing
On site evaluation

Follow-up of results

Quality Assurance

Quality assessment
Information
Education / Training

Molecular Pathology - Workflow

Histology / cytology

Synthesis
Report

Preparation
Analysis

Molecular Pathology – Proficieny testing

Swedish EQA Activities

DNA Samples *KRAS* 2008
FFPE Samples *KRAS* CRC 2008
Part of European *KRAS* EQA for CRC within ESP 2009-

FFPE samples *BRAF* Malignant Melanoma 2011

Annual follow-up of genotyping *KRAS*, *BRAF* &
EGFR genotyping statistics 2011-

National recommendations ”Mutation analysis
on tumor tissue” 2012

Long term: Database

Swedish 2011 *BRAF* EQA Scheme

BRAF-genotyping of malignant melanoma

FDA approval Aug 2011
FFPE MM samples distributed in Nov 2011
Deadline Dec 24 2011
Results presented and discussed Jan 2012
Vemurafenib EU-approved Feb 2012

Swedish 2011 EQA Scheme
- *BRAF* Genotypes

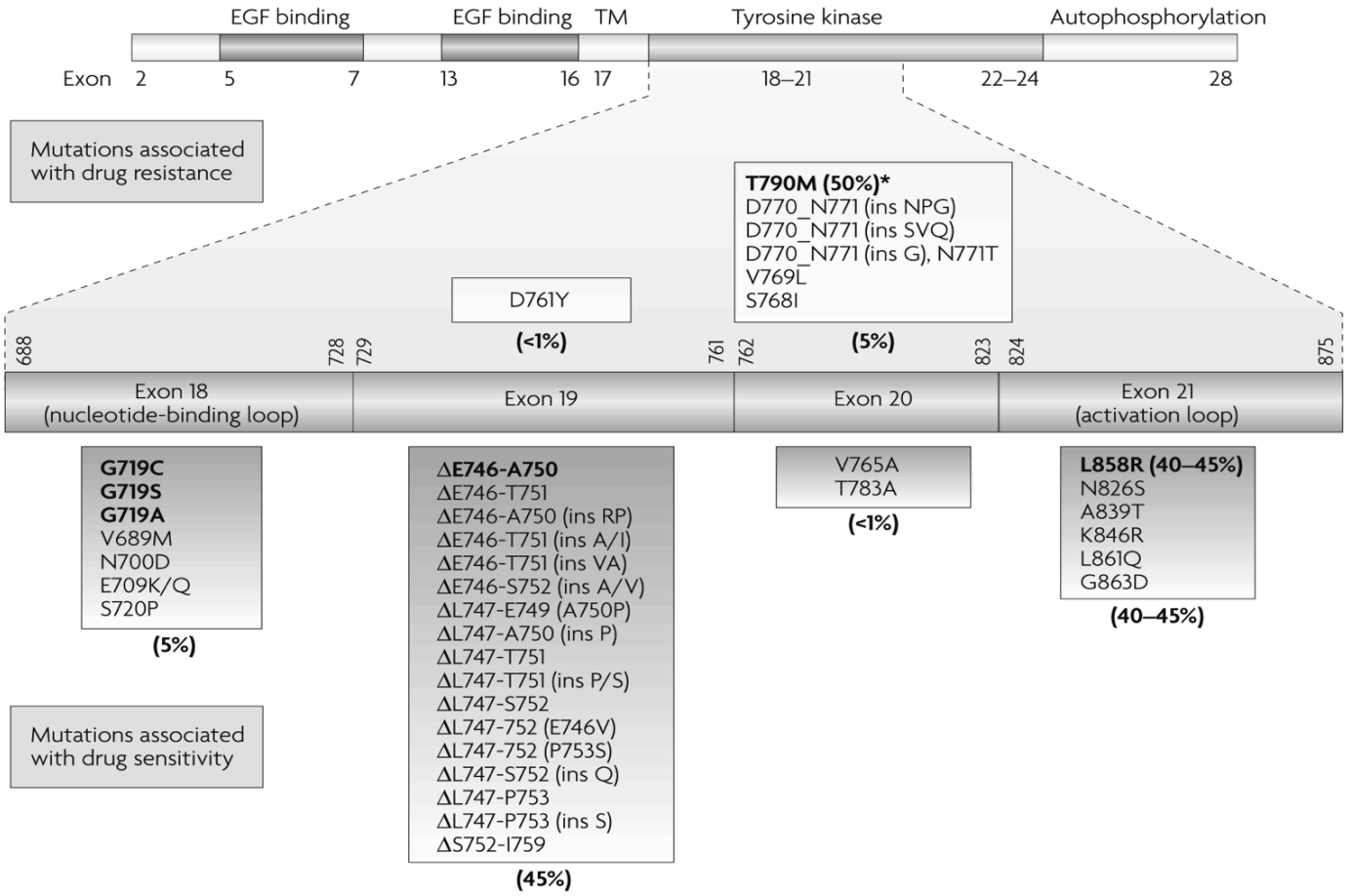
Sample	Genotype	AA Change
BRAF1	wt	wt
BRAF2	c.1799T>A	p.V600E
BRAF3	c.1799T>A	p.V600E
BRAF4	wt	wt
BRAF5	c.1798_1799delinsAA	p.V600K
BRAF6	wt	wt
BRAF7	wt	wt
BRAF8	wt	wt
BRAF9	c.1799T>A	p.V600E
BRAF10	c.1799T>A	p.V600E

Swedish 2011 *BRAF* EQA Scheme - Genotyping Platforms

Primary Method	Secondary	# of labs
cobas® 4800 BRAF V600 Mutation Test	-	2
Pyrosequencing	-	2
Pyrosequencing	Sanger Sequencing	1
Pyrosequencing	EntroGen B-Raf V600E Mutation Analysis Kit	1
ViennaLab Strip assay	Sanger Sequencing	1
Sanger Sequencing	-	1

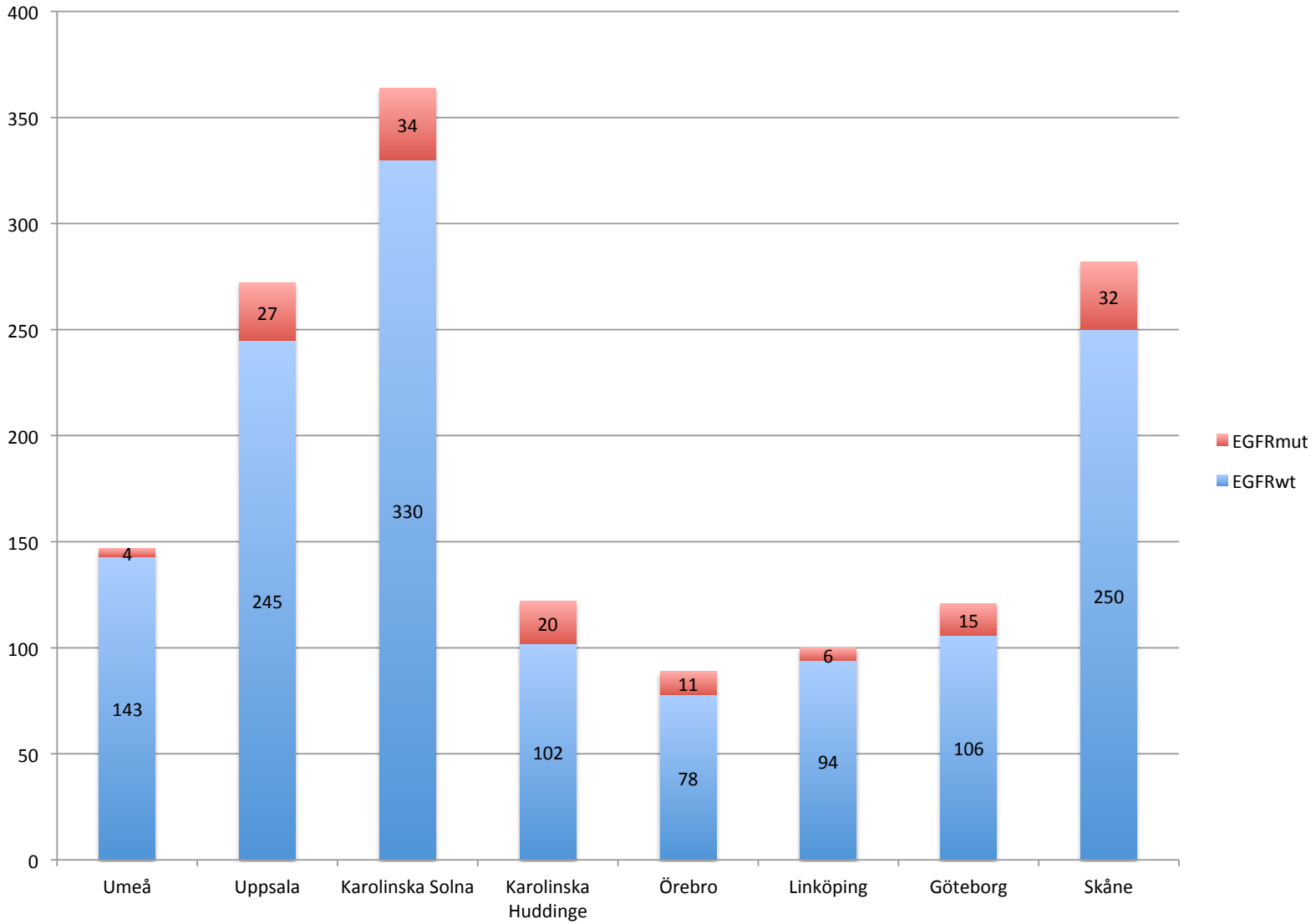
Total agreement on genotypes

Swedish Genotyping Results
- *EGFR* in NSCLC

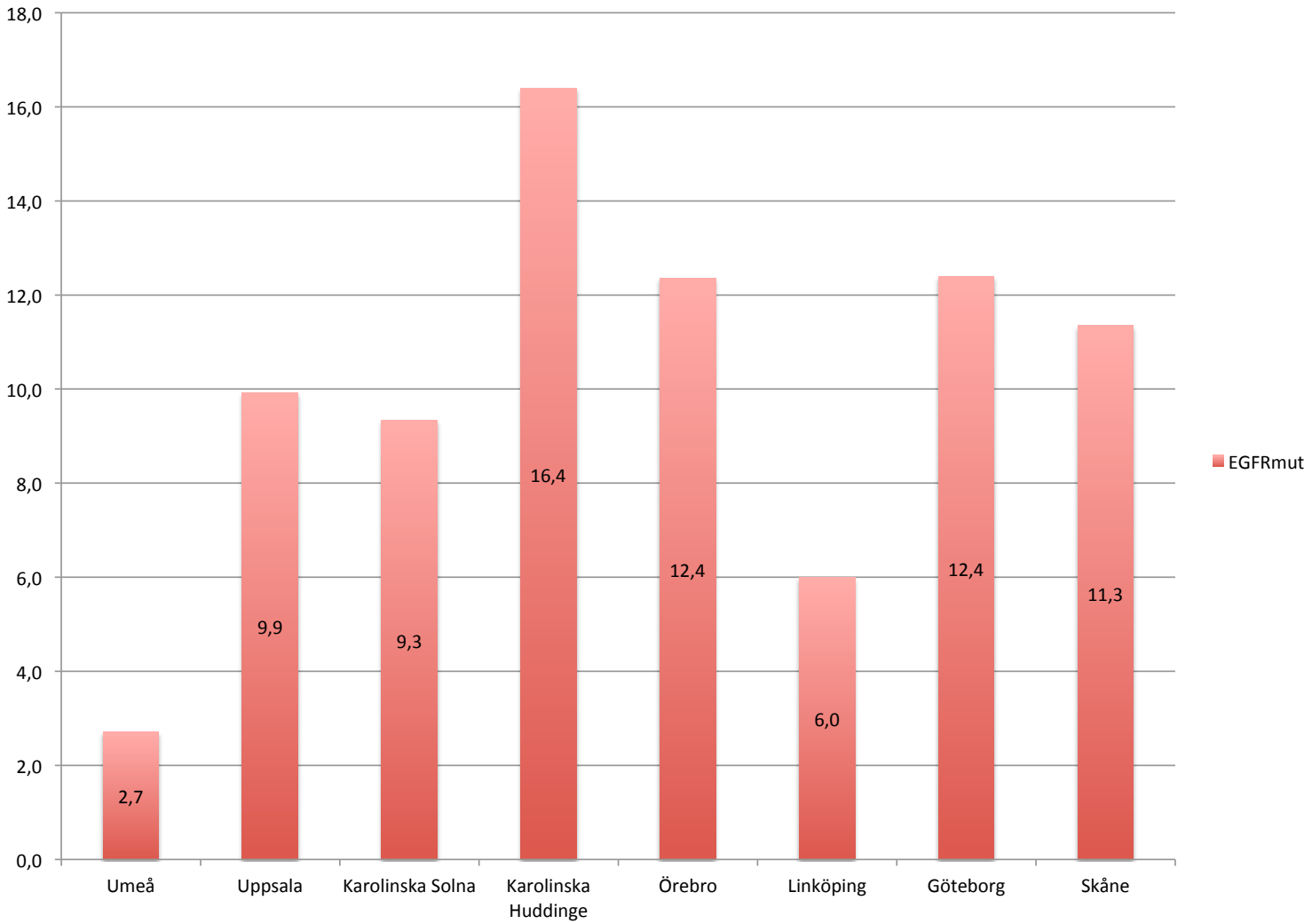


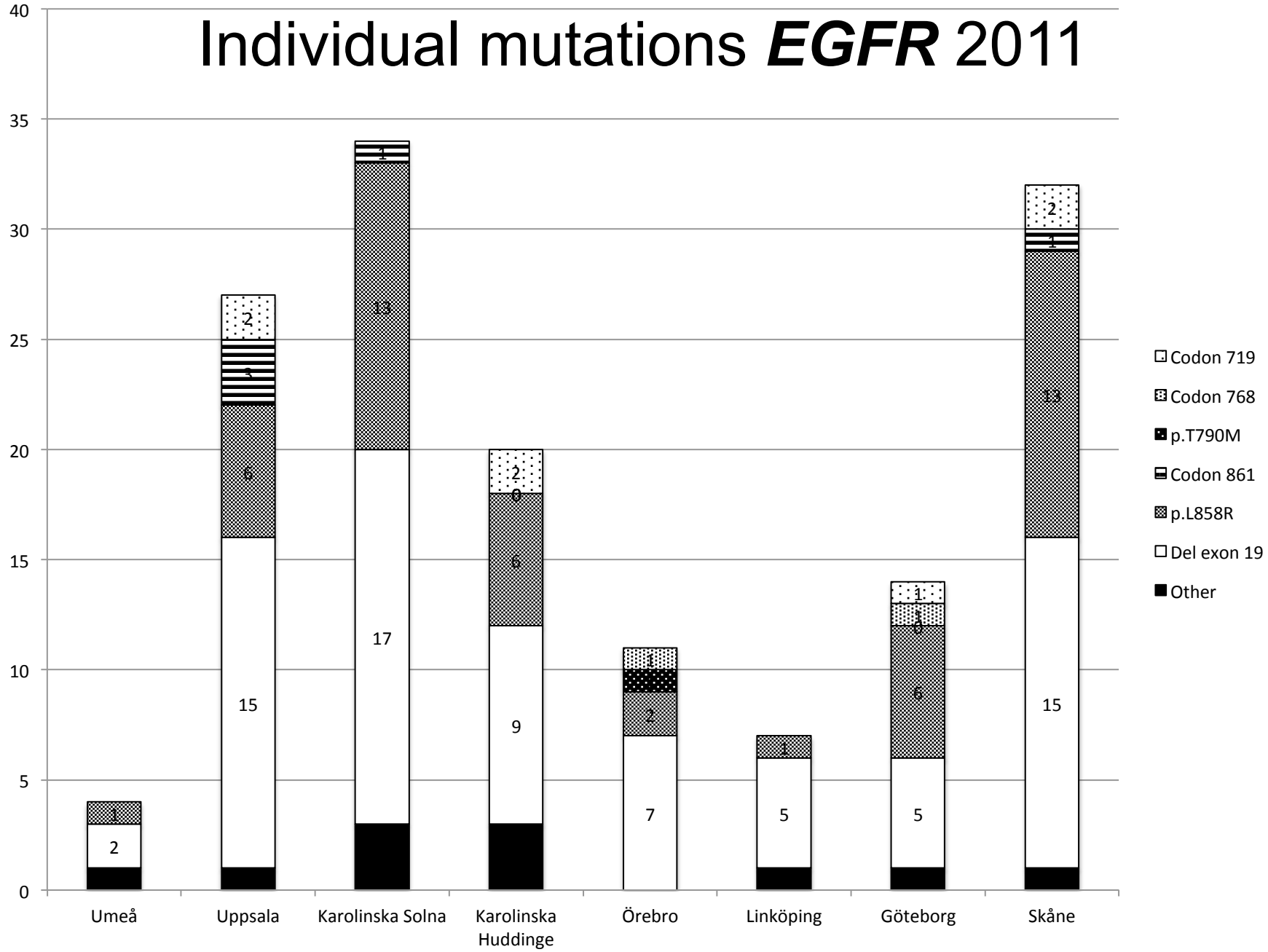
Nat Rev Cancer 2007; 7: 169-81

Number of *EGFR* analyses 2011

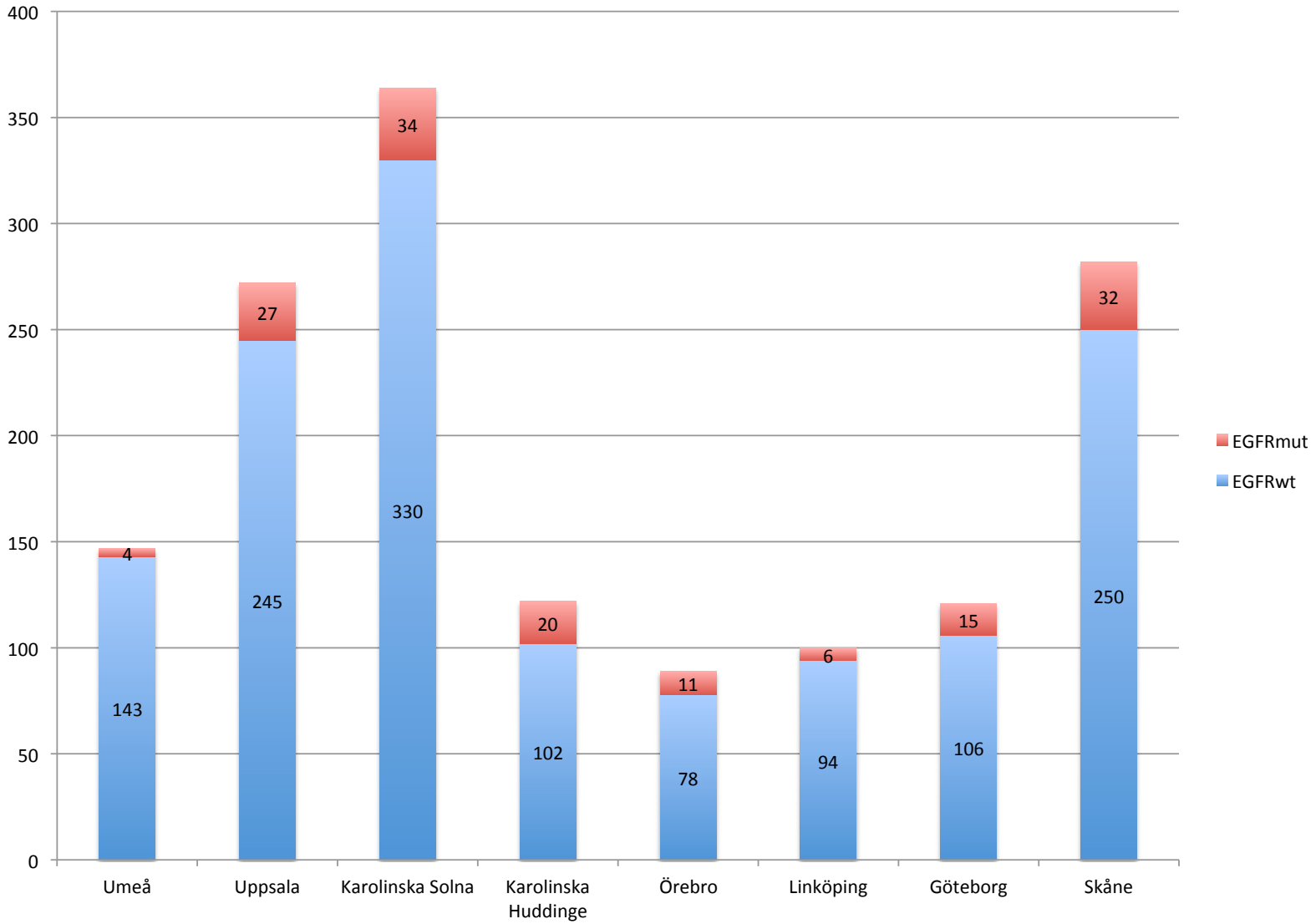


% *EGFR* mutants 2011




Individual mutations EGFR 2011
40
35
30
25
20
15
10
5
0
Umeå
Uppsala
Karolinska Solna
Karolinska Huddinge
Örebro
Linköping
Göteborg
Skåne
Codon 719
Codon 768
p.T790M
Codon 861
p.L858R
Del exon 19
Other

Number of *EGFR* analyses 2011



ESP working groups for GI & Mol Path
ESP Guidelines on *KRAS* testing (2008)
Proficieny testing (annually 2009-, 33 countries)
Information website http://kras.eqascheme.org

Virchows Arch (2008) **453**: 417-431
Oncologist (2011) **16**(4): 467-78

KRAS EQA Scheme 2012

FFPE samples
8 samples on slides
2 artificial samples

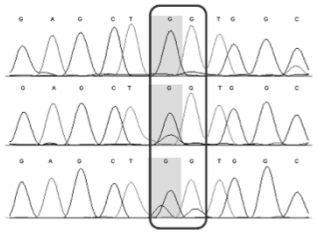
KRAS network

Assessment of results

General report
Individual feedback
Listing on website

% neoplastic cells

Analysis



Genotype

Written reports (Genotype, interpretation)
Raw data

Participating lab

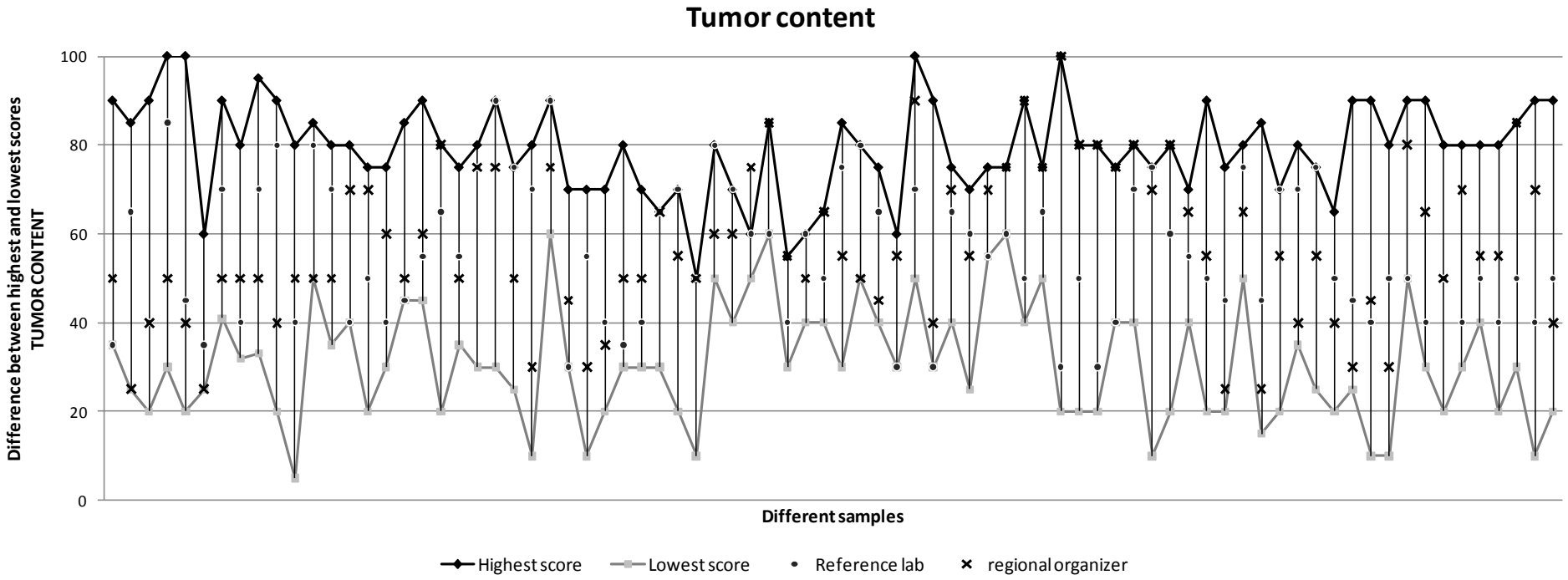
KRAS EQA Scheme Results

High quality genotyping in most labs
Estimations neoplastic cell content vary
Initially poor adherence to HVGS nomenclature

Column-based DNA prep kits dominate
Several genotyping platforms with varying sensitivities

Labs get better!

2009 KRAS EQA scheme - % neoplastic cells



Lack of appreciation of tumor complexity
Disregard of what genotyping assays actually measure
Communication errors

KRAS
European EQA program

EGFR in NSLC 2011

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Email : nicnorm@yahoo.com

VALIDATED RESULTS

Scheme: EGFR (2011)

NOTE:

- Mutation nomenclature is according to GenBank accession number NM_005228.3 with numbering starting at the A of the ATG initiation codon according to Human Genome Variation Society guidelines (www.hgvs.org).
- All dates of birth are given in the format dd/mm/yyyy
- The EMQN is co-ordinating the scheme and all correspondence about it should be directed via them at the address above.

VALIDATED RESULTS

CASE	BLOCK No.	PATIENT NAME	SEX	DATE OF BIRTH (dob)	CONFIRMED GENOTYPE
1	101	David JONES	Male	23/07/1971	Mutation in exon 19 of the *EGFR* gene (NM_005228.3): c.2235_2249del15 (p.Glu746_Ala750del)
2	102	Camilla DAVIES	Female	10/08/1945	Two mutations in exons 20 and 21 of the *EGFR* gene (NM_005228.3): c.[2369C>T (p.Thr790Met) (;) 2573T>G (p.Leu858Arg)]
3	103	Jon THOMPSON	Male	28/06/1960	Mutation in exon 19 of the *EGFR* gene (NM_005228.3): c.2235_2249del15 (p.Glu746_Ala750del)
4	104	Michael GOODE	Male	11/11/1951	No mutation(s) in exons 18-21 of the *EGFR* gene (NM_005228.3)
5	105	Becky WINSTANLEY	Female	08/10/1945	No mutation(s) in exons 18-21 of the *EGFR* gene (NM_005228.3)
6	106	Russel BERTRAND	Male	15/12/1964	No mutation(s) in exons 18-21 of the *EGFR* gene (NM_005228.3)
7	107	Josephine BAKER	Female	08/03/1952	No mutation(s) in exons 18-21 of the *EGFR* gene (NM_005228.3)
8	108	Petra FELLOWES	Female	17/07/1967	Mutation in exon 18 of the *EGFR* gene (NM_005228.3): c.2155G>A (p.Gly719Ser)
9	109	Douglas ADAMS	Male	01/06/1950	Two mutations in exons 20 and 21 of the *EGFR* gene (NM_005228.3): c.[2369C>T (p.Thr790Met) (;) c.2573T>G (p.Leu858Arg)]
10	110	Micha BAYLISS	Male	12/06/1954	Mutation in exon 18 of the *EGFR* gene (NM_005228.3): c.2155G>A (p.Gly719Ser)

EGFR in NSLC 2011

Testing for *EGFR* gene mutation status to determine suitability for EGFR-TKI therapy.

Referring clinician: Consultant Oncologist.

Patient: David Jones, 23/07/1971.

Analyzed material: Primary tumor, lung adenocarcinoma. Formalin-fixed paraffin-embedded biopsy material. Material identity EMQN 101.103.

Tumor cell percentage: >25% (according to information in test request).

Dissection: None.

Analysis method used: Therascreen® EGFR Pyro® Kit, Qiagen, Germany.

Detectable *EGFR* mutations:
Point mutations in exon 18 (codon 719).
Exon 19 deletions.
Point mutations in exon 20 (codons 768 and 790).
Point mutations in exon 21 (codons 858 and 861).

Sensitivity: The limit of detection for the various mutations range between a mutation frequency of 0.6 to 10.7 %.

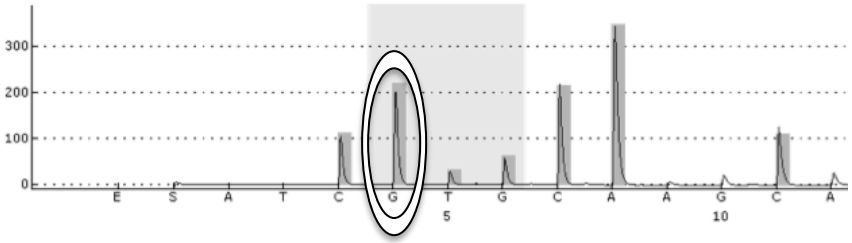
***EGFR* genotype:** p.Glu746_Ala750del, please see comment.

Comment: The deletion is activating, fulfilling the molecular criteria for EGFR-TKI therapy.

Dr. A E, 2012-01-27. √

+

Well: A4
Assay: EGFR codon858-861
Sample ID: 109-80



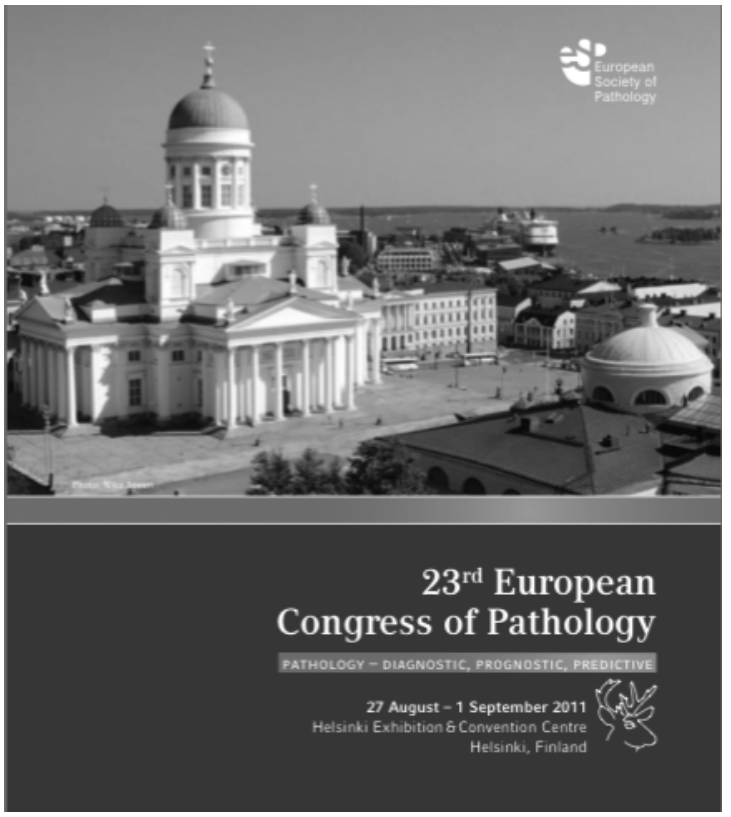
Result	**Mutation (Codon 858)**
Frequency (mut)	70,8% (LOD: 2,6%)
Codon Change (mut)	CTG>CGG
Amino Acid Substitution (mut)	L858R

European EQA Guidelines

Clinical oncologists
Pathologists
Molecular biologists
EQA providers
Pharma industry representatives

ECP, Helsinki, Sep 2011
Naples, Italy, Mar 2012
Ms in preparation

Focus on tests based on extracted DNA



European EQA Guidelines

Issues covered:

Organization

Reference labs

Test samples – quality, quantity

Scoring

Consequences of poor performance

Communication of results

EQA databases

ISO 17043:2010

Eurepean EQA Guidelines - Samples

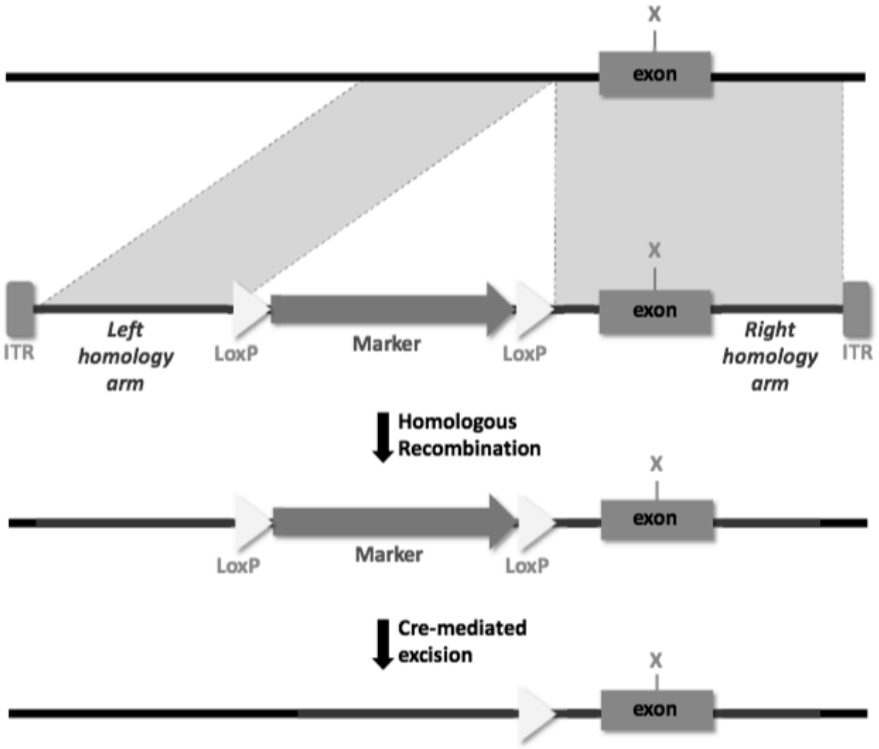
Surgical pathology samples vs artificial?
Routine or borderline?
Legal implications
Numbers needed to verify adequate performance

Starting mtrl

Surgical pathology samples
Pros:
Tumor cell heterogeneity
Real-life DNA damage
(*in vivo* and lab-induced)
Cons:
Difficult to standardize
Limited supply

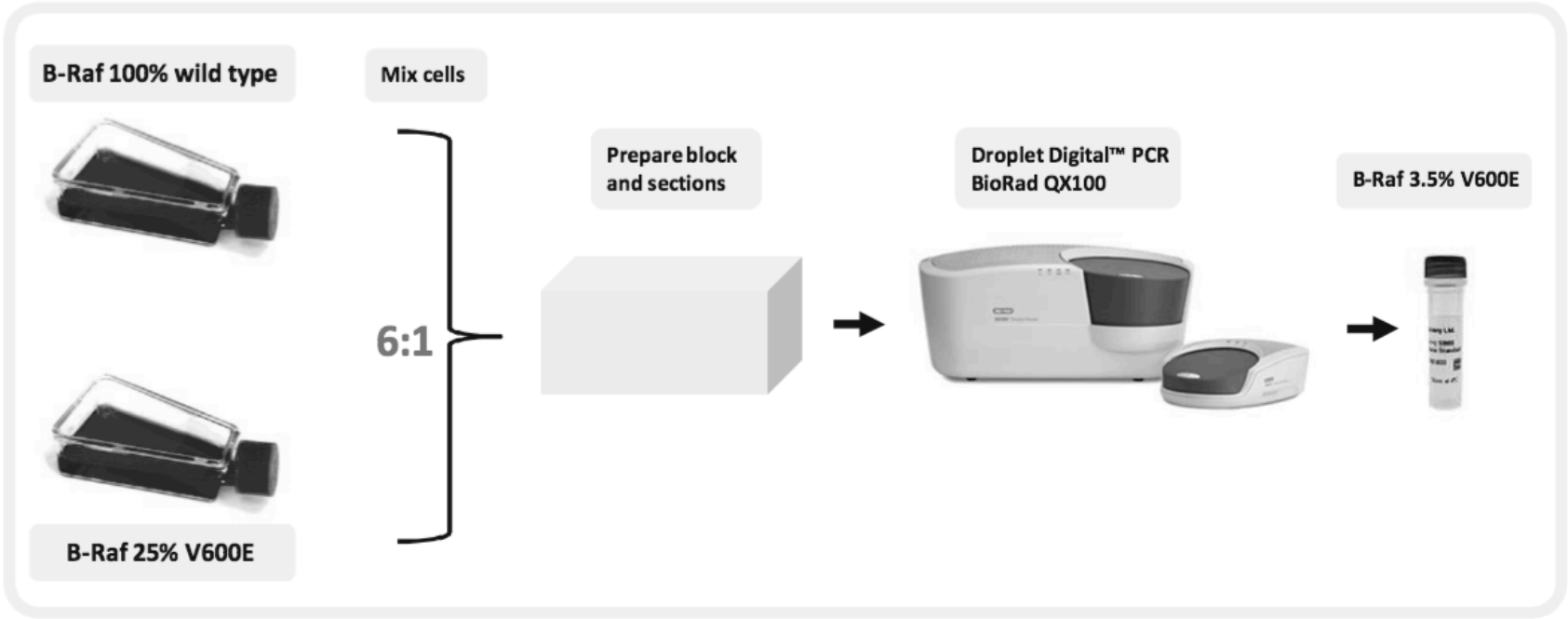
Artifical samples
Pros:
Well defined genotypes
Standardized allelic frequencies
Unlimited supply
Cons:
Difficult to introduce real-life DNA damages

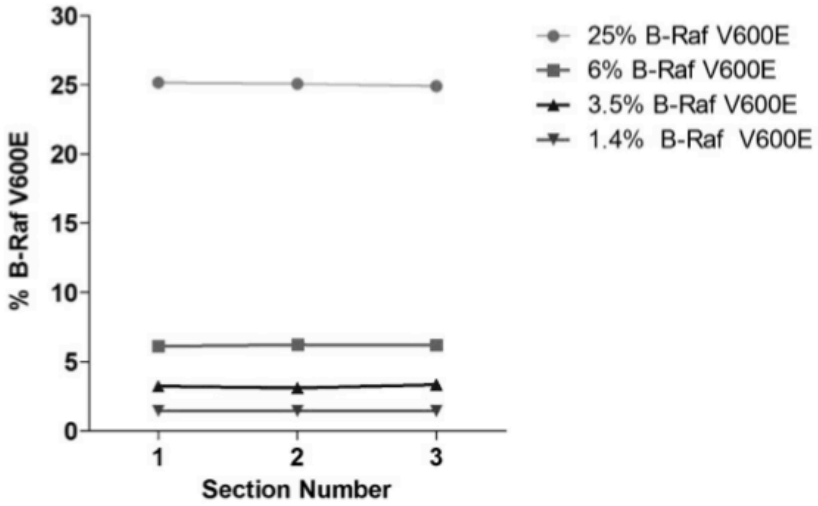
Artificial Samples



Gene	Mutation				
B-Raf	V600E	25%	5%	3.5%	1%
B-Raf	V600K	50%	5%	1%	---
K-Ras	G12A	50%	5%	---	---
K-Ras	G12C	50%	5%	---	---
K-Ras	G12D	50%	5%	---	---
K-Ras	G12R	50%	5%	---	---
K-Ras	G12S	50%	5%	---	---
K-Ras	G12V	50%	5%	---	---
K-Ras	G13D	50%	5%	---	---
EGFR	G719S	33%	8%	---	---
EGFR	ΔE746-A750	50%	5%	---	---
EGFR	T790M	50%	5%	---	---
EGFR	L858R	50%	20%	5%	---
EGFR	L861Q	50%	5%	---	---
PI3Kα	E542K	50%	---	---	---
PI3Kα	E545K	50%	---	---	---
PI3Kα	H1047R	50%	---	---	---

Horizon Diagnostics





Artificial Samples - Consistency

horizon diagnostics

Routine vs Borderline

EQA schemes might help labs push their limits

Enrichment for difficult cases skews performance statistics

e.g. *KRAS* c.34G>C, p.Gly12Arg in the 2009 *KRAS* EQA Scheme

Roughly 1/100 routine CRC samples
Included as 1/10 samples in the EQA scheme
Missed by one of the Swedish labs

Number of Samples – Statistics

Bayesian statistics used to decide scheme size

For a 90% CI ≥95%, 58 samples are needed

N samples	10	10	14	20	30	30
# correct answers	# correct answers	90% CI	90% CI	90% CI	# correct answers	90% CI
n/n	10/10	76.2 - 99.5	81.9 - 99.7	86.7 - 99.8	30/30	90.8 - 99.8
n-1/n	9/10	63.6 - 96.7	72.1 - 97.6	79.3 - 98.3	29/30	85.6 - 98.8
n-2/n	8/10	53.0 - 92.1	63.7 - 94.3	72.9 - 96.0	28/30	81.1 - 97.3
n-3/n	7/10	43.6 - 86.5	56.0 - 90.3	67.1 - 93.2	27/30	76.8 - 95.5
n-4/n	6/10	35.0 - 80.0	48.9 - 85.8	61.6 - 90.1	26/30	72.9 - 93.4
n-5/n	5/10	27.1 - 72.9	42.3 - 80.9	56.3 - 86.8	25/30	69.0 - 91.2
n-6/n	4/10	20.0 - 65.0	36.0 - 75.6	51.3 - 83.2	24/30	65.3 - 88.9
n-7/n	3/10	13.5 - 56.4	30.0 - 70.0	46.4 - 79.4	23/30	61.7 - 86.5
n-8/n	2/10	7.9 - 47.0	24.4 - 64.0	41.7 - 75.5	22/30	58.2 - 83.9
n-9/n	1/10	3.3 - 36.4	19.1 - 57.7	37.2 - 71.4	21/30	54.8 - 81.3
n-10/n	0/10	0.5 - 23.8	14.2 - 51.1	32.8 - 67.2	20/30	51.5 - 78.7

Number of Samples – Statistics

10 cases is a typical setup

90% CI for 8/10 correct answers ranges from 53 – 92%

>95% success rate in clinical genotyping thus unlikely
-> 9/10 recommended as cut-off

Number of Samples
- Practial Solutions

If less than 10 samples is preferred, a set up with e.g. 3 cases in 3 annual rounds (still 9/10 needed) can be used

A database with added results from multiple schemes is planned

Uniform scoring to aid in forming such a database is presented

Larger and larger schemes?

KRAS European EQA program

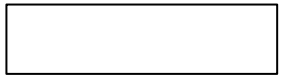
UKNEQAS

EMQN The European Molecular Genetics Quality Network

Current schemes include:

KRAS, *BRAF*, *EGFR*, *ALK-EML4*

DNA sequencing



Organization

European schemes

Pros:

Costs shared

Results comparable

Networks built

Cons:

Limited availability of samples

Written reports translated berore assessment

Schemes not adapted to regional needs and lab practices

National/regional schemes

Pros:

Flexibility (what & how to assess)

Meaningful assessment of written reports

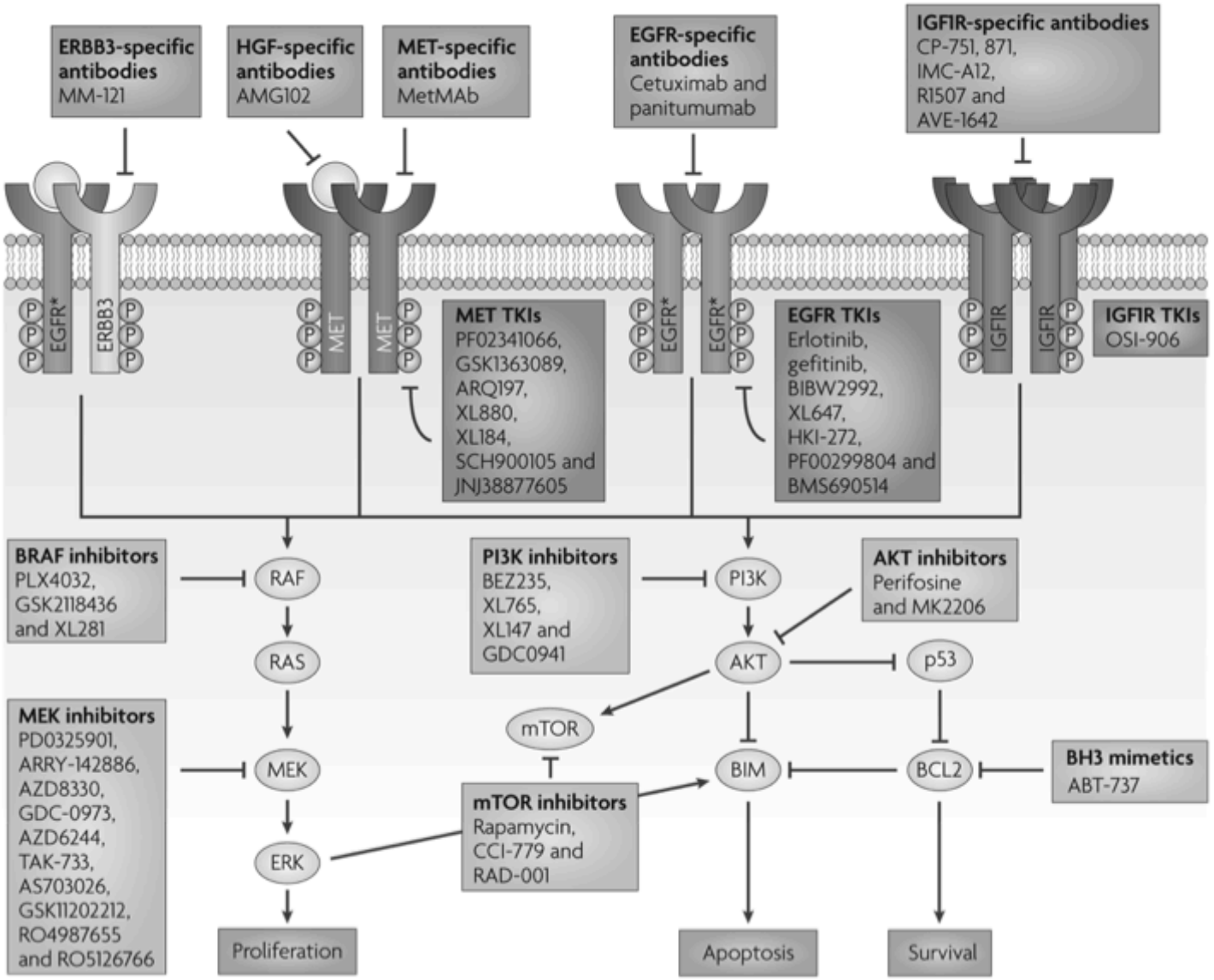
Can be combined with other diagnostic QA schemes

Cons:

Huge task for a small lab

Transfer of information from continental labs lost

Future targets - NSCLC



Nat Rev Cancer
2010, 10 760-74

Future Development

Proficiency testing:
More targets
Multiple targets
Platform tests (genotyping, sequencing, bioinformatics)

Quality assurance:
Network of clinical labs
Close collaboration with basic research
Education
Recruitement

Thank you!

Svensk Molekylär Patologi
SMP

Cancerfonden

VÄSTRA GÖTALANDSREGIONEN