Österreichische Gesellschaft für Pathologie
Österreichische Division der IAP

Ring Trial
Molecular Diagnostics

EGFR mutations in NSCLC
Series II – March 2012
results

design

- national external quality control instrument
- 4 distributions per year
- 3 unstained slides from 3 resection-specimen with or without EGFR-mutation
- one slide should be stained H&E, which is referred to in an explaining letter
- form with questions to be filled in
  - turnaround time
  - technical indicators
  - simulation of definitive reports
  - histological diagnosis of tumor
- confirmation of participation edited by AG Pulmopathology of the Austrian Society of Pathology
- offer to discuss problems

participants

- 10 Austrian institutes of pathology
- 3 Hungarian institutes of pathology
- 1 Croatian institute of pathology

report form

Laboratory	
address	
ID	
Responsible MD	
Responsible technician	
Tel	
e-mail	
Date received	
Date of report	

	Case 1	Case2	Case 3	Comment for QA/questions
Percentage of tumor cells within section (as in regular report)				
how was percentage calculated?				
Tumor cell enrichment – yes/no : method of enrichment (e.g. macrodissection by needle, scratching)				
Method of DNA extraction				
Method of measurement of DNA				
Amount of DNA extracted (as in regular report)				
Quality of DNA				
Evaluation of DNA-quality				
Method of sequencing (as in regular report)				
What types of mutations can be detected by your method? Sensitivity of your method, if known?				
Result (as in regular report)				
Interpretation of result as in regular report				
Additional comments to oncology department or recommendations				
Histologic diagnosis of case incl. patterns and percentage, as done in regular reports				

definition of cases

- Case 1
  - Lung adenocarcinoma,
    - Predominant acinar with
    - papillary and
    - minor micropapillary component,
    - G2
  - mutations
    - activating mutation in exon 21 – p.L858R (c.2573T>G) - present in 71% alleles tested and
    - mutation in exon 20 – p.T790M (c.2369C>T) – present in 59% alleles tested – associated with resistance to TKI
  - tested by
    - conventional pyro-sequencing
    - allele specific RT-PCR

definition of cases/comments

- Case 2
  - Lung adenocarcinoma,
    - adenocarcinoma
      - predominant acinar with
      - papillary and
      - minro micropapillary component,
      - G2
    - mutation
      - activating mutation in exon 21 - p.L858R (c.2573T>G)- 36% alleles
  - tested by
    - next generation sequencing (Roche 454)
    - conventional pyro-sequencing

definition of cases/comments

- Case 3
  - Lung adenocarcinoma,
    - Predominant solid with
    - acinar and
    - papillary component
    - G3
    - activating mutation in Exon 21 - p.L858R (c.2573T>G) - present in 46% of alleles tested
  - tested by
    - next generation sequencing (Roche 454) and
    - conventional pyrosequencing

Table 1 – definition of tumors / time to report

	Case1	Case2	Case3	Comments		Comments by distributor
dates				received	report	
ID01				17.02.2012	20.02.2012	2 wd
ID02				17.02.2012	27.02.2012	7 wd
ID04				20.02.2012	28.02.2012	7 wd
ID05				20.02.2012	28.02.2012	7 wd
ID06				23.02.2012	05.03.2012	8 wd
ID07				22.02.2012	28.02.2012	9 wd
ID09				17.02.2012	24.02.2012	6 wd
ID10				14.02.2012	22.02.2012	7 wd
ID11				17.02.2012, 9h	22.02.2012	4 wd + approx.10wd
ID 12				17.02.2012, 15h	22.02.2012	4 wd
ID13				17.02.2012	29.02.2012	9 wd
ID14				17.02.2012	26.02.2012	6 wd
ID15				22.02.2012	01.03.2012	7 wd
ID16				06.03.12	20.03.2012	12 wd

Table 2 – percentage of tumor cells

percentage of tumor cells within section (as in regular report)					
ID01	>90%	>50%	>80%		Percentage ranges: Case 1 95 – 40% - mean 72% Case 2 25 – (50) 80% - mean 40% Case 3 40 – 80% - mean 64% Macrodissection by scrathing most cases; Case 1 sometimes without enrichment.
ID02	>50%	>80%	>80%	case 2 – notice error?	
ID04	>90%	30% after macrodiss	40%		
ID05	95%	50%	60%		
ID06	60%	25%	50%		
ID07					
ID09	70%	40%	50%		
ID10	70%	40% after enrichm	70% after enrichtm		
ID11					
ID 12	80%	50%	75%		
ID13	40% tumor cells	30% tumor cells	60% tumor cells		
ID14	80% without stroma	30% in tumor area	80% in tumor area		
ID15	80%	25%	70%		
ID16					

Table 3 – calculation of percentage

how was percentage calculated ?		
ID01	calculation of field	
ID02	histomorphology	
ID04		
ID05	optical morphometry	
ID06	histological evaluation	
ID07	microscopy	
ID09	estimated by 2 pathologists	
ID10	amount of tumor cells in marked area	
ID11		
ID12		
ID13		
ID14	estimated	
ID15	percentage of atypical epithelial cells in tumor area	
ID16		

Table 4 – tumor cell enrichment

Tumor cell enrichment – yes/no : method of enrichment (e.g. macrodissection by needle)				
ID01	no	yes- scratch diss.	yes- scratch diss.	
ID02	yes	yes	yes	macrodissection
ID04	no	yes – scratching	no	
ID05	macrodissection by needle			
ID06	no			
ID07	macrodissectionneedle scratching			
ID09	yes, macodissection			
ID10	no	yes, macrodiss by scratching	yes, macrodiss. by scratching	
ID11				
ID12	scratching			
ID13	yes; tissue-scratching of marked tumor-area			
ID14	macrodissection by scratching			
ID15	yes, scratching			
ID16				

Table 5 – method of DNA extraction

method of DNA extraction		
ID01	Maxwell FFPE Tissue LEV DNA	
ID02	QuiAmp DNA FFPE tissue kit Cat No 56404	
ID04	Quiagen FFPE tissue kit	
ID05	High Pure PCR Template Preparation Kit (Roche)	
ID06	Roche High Pure PCR Template Kit	
ID07	fibre glass extraction	
ID09	DNA FFPE MiniKit	
ID10	MAXWELL 16 Instrument , Fa.promega	
ID11	DNA extraction from paraffin material	
ID12	EZ 1 Automat Fa. Qiagen	
ID13	QIAmp DNA FFPE tissue kit Fa Quiagen	
ID14	bo robot Quiagen magentic beeds	
ID15	QIAamp DNA Mini Kit (Qiagen)	
ID16	Quiagen FFPE	

Table 6 – measurement of DNA

method of measurement of DNA		
ID01	Qubit fluorimetry	
ID02	Bio spec nano Shimatsu biotech	
ID04	Bio spec nano	
ID05	Nanodrop	
ID06	Nanodrop spectrophotometer	
ID07	UV photometry	
ID09	Nanodrop Peqlab	
ID10	spectralphotometer Nanodrop	
ID11	photometer measurement	
ID12	Nanodrop ND-1000 Fa. Peqlab	
ID13	Quant-iT ds DNA BR Assay Kit, Fa Invitrogen(Qubit)	
ID14	photometry bioquant 1300	
ID15	NanoDrop ND-100 Spectrophotometer	
ID16		

Table 7 – amount of extracted DNA

Amount of DNA extracted (as in regular report)				
ID01	67,45 µg/ml	28, 50 µg/ml	68,50 µg/ml	
ID02	56,18 µg/ml	32 µg/ml	26,67 µg/ml	
ID04	35 µg/ml diluted 1:7	12,2 µg/ml dilut..1:2	83,5 µg/ml dilut.1:15	
ID05	88,4 ng/µl	50,2 ng/µl	275,9 ng/µl	
ID06	102.78 ng/ul in 50 ul	89.79 ng/ul in 50 ul	101.41 ng/ul in 50 ul	
ID07	18 ng/µl	9 ng/µl	15 ng/µl	
ID09	196,5 ng/µl	84 ng/µl	360,5 ng/µl	
ID10	96 ng/µl (60 µl)	15 ng/µl (60µl)	85 ng/µl (60 µ)l	
ID11	150 ng/µl	198 ng/µl	100 ng/µl	
ID12	35,2 bg/µll	20,3 ng/µl	37,7 ng/µl	
ID13	54,5 µg/ml	9,22 µg/ml	64,7 µg/ml	The amount is documented in a lab-report, not in the patients report
ID14	42 ng/µl	20 ng/µl	33 ng/µl	
ID15	30,9 ng/µl (V_{total} = 50 µl)	28,7 ng/µl (V_{total} = 50 µl)	36,4 ng/µl (V_{total} = 50 µl)	
ID16				

Table 8 – quality of DNA

qualitiy of DNA				
ID01				
ID02	good	good	good	ß globin
ID04	2,15	0,69	2,06	
ID05	1,92	1,91	1,91	
ID06	good	good	good	
ID07	1,7	1,65	1,8	
ID09	1,97 260 nm/280nm	1,99 260nm/280nm	1,93 260nm/280nm	
ID10	ratio 1,7	1,9	1,8	
ID11	150 ng/µl	189 ng/µl	100 ng/µl	
ID12	260/280 1,86	260/280 1,82	260/280 1,84	
ID13	good	good	good	
ID14	260/280 1,9	2,2	2,1	
ID15	$A_{260/280}$= 2,08; $A_{260/230}$= 4,01	$A_{260/280}$= 1,92; $A_{260/230}$= 3,36	$A_{260/280}$= 2,10; $A_{260/230}$= 3,36	
ID16				

Table 9 – evaluation of DNA quality

evaluation of DNA qualitiy		
ID01		
ID02		
ID04	OD 260/280	
ID05	good	
ID06	260/280 nm ratio, 260/230 nm ratio	
ID07	sufficient for mutation testing	
ID09	conrol PCR	
ID10	ratio 260/290 (pure DNA 1,7 – 1,8)	
ID11		
ID12	2% Agarosegel u. Kontrolle	
ID13	Estimation in relation to peak height of the program and in relation to the control DNA	
ID14	exon control mix (kit) assay + inhibitor control (kit)	
ID15	NanoDrop ND-100 Spectrophotometer measurement + β-globin control PCR	
ID16		

Table 9 – method of sequencing

Method of sequencing (as in regular report)		
ID01	Quiagen EGFR RGQ PCR Kit TheraScreen 870 111	all
ID02		
ID04	COBAS 4800	
ID05	Sanger, ABI 3130 Genetic Analyser	
ID06	direct sequencing	
ID07	Dye terminator	
ID09	allele specific PCR	Thera Screen and/or PyroMark Quiagen
ID10	Thera Screen EGFR Pyro kit Quiagen	
ID11	therascreen EGFR PCR kit	
ID12	Sanger Sequencing ABI 3500xL Dx	
ID13	Method used: DNA-extraction with Quiagen QIAmp FFPE Kit, sequencing with Therascreen EGFR Pyro Kit (Quiagen, Hilden, Germany)	
ID14	thera screen EGFR RGQ PCR kit Quiagen (real time PCR)	
ID15	7500 Real Time PCR System + TaqMan Mutation Detection Assays, Applied Biosystems Dideoxy sequencing (Applied Biosystems, 3100-Avant)	
ID16		

Table 10 – types of mutations detectable

What types of mutations can be detected by your method? Sensitivity of your method, if known?			
ID01	T790M , deletion, L585R, L861Q, G719X, S786 insertions, reported as to be 5 %		
ID02	s. COBAS EGFRmutation test		
ID04	T790M, L858R, L861Q, L768I, G719(A,S,C), insertions exon20, deletions exon 19		
ID05	exon 18-21, all nucleotide changes, sensitivity 20% (cell line testing)		
ID06	all types of mutation can be detected, approx 10%		
ID07			
ID09	29 mutations detected by the TheraScreen EGFR29 Mutation Kit. Sensitivity according to the manufacturer: 15%		
ID10	exon 18 mutations on codon 719: G719X (X-S,C,A,D) exon 19: all deletions within codons 746-750 exon 20:mutation codon 768 (S768I), insertion codon 770 771, 774 mutation codon 790: T790M exon 21: mutation codon 858 and 861: L858R and L:861Q		
ID11	deletions exon 19 : G719X, exon 18, exon 21:L858R, S761I exon 20, L861Q on 21, insertions exon 20		
ID12	all types (del, ins, sub, dup)		
ID13	Point mutations codons 719, 768, 790, 858-861; Deletions Exon 19, Sensitivity 5-10% mt in wildtype		
ID14	19 deletions in exon 19, T790M,L858R. L861Q, G719X, S763I, 3 insertions in exon 20; sensitivity 1-10% mutated DNA		
ID15	Exon 18: G719A, G719S, G719C Exon 19: 19 deletions (L747_T751>S, L747_E749del, E746_S752>D, E746_A750del (2235_2249del15), E746_A750del (2236_2250del15), L747_T751del, L747_T752del, E746_S752>A, L747_T751del, L747_P753>S, L747_A750>P, L747_A751>P, E746_S752>V, L747_P753>Q, L747_T751>Q, L747_A750>P, E746_T751>A, E746_T751del and E746_T751>I) Exon 20: T790M, S768I, V769_D770insASV, H773_V774insH and D770_N771insG Exon 21: L858R and L861Q TaqMan Mutation Detection Assays can detect 0,1% mutated DNA in a background of wild type DNA.		
ID16	deletions Exon 19, Insertions Exon 20, L858R, L861Q, S768I, G719A, G719S, G719C Sensitivity: 1% Mutations in Wildtype-background		

Table 11 – results

result as in regular report				
	Case 1	Case 2	Case 3	
ID01	T790M + L858R	L858R	L858R	
ID02	L858R + T790M	L858R	L858R	
ID04	L858R- T790M	L858R	L858R	
ID05	Exon20: c.2369 C>T T790M Exon21: c.2573 T>G L858R	Exon21: c.2573 T>G L858R	Exon21: c.2573 T>G L858R	
ID06	Mutation of exon 21: c.2573T>G, p.L858R	Mutation of exon 21: c.2573T>G, p.L858R	Mutation of exon 21: c.2573T>G, p.L858R	
ID07	exon 19: wt exon 21: p.Leu858Arg (ca. 72%)	exon 19: wt exon 21: p.Leu858Arg (ca. 42%)	exon 19: wt exon 21: p.Leu858Arg (ca. 43%)	
ID09	Detectable mutations: p.T790M (c.2369C>T) and p.L858R (c.2573T>G)	Detecable mutation: p.L858R (c.2573T>G)	Detecable mutation: p.L858R (c.2573T>G)	
ID10	ex20: T790M 71% and ex 21: L858R 45%	exon 21: L858R, 30%	exon 21: L858R, 49%	
ID11	Mutation L858R in exon 21+ Mutation T790M in exon 20	Mutation L858R exon21	Mutation L858R exon21858R	
ID12	EGFR Exon 20: **c.2369C>T p.T790M** Exon 21: **c.2573T>G p.L858R**	EGFR Exon 21: **c.2573T>G p.L858R**	EGFR Exon 21: **c.2573T>G p.L858R**	auf Angabe von stillen Mutat-tionen (Exon20)und Varianten (Intron) wurde verzichtet
ID13	Mutation in Codon 858 and in Codon 270 of EGFR-gene: p. L858R and p.T790M	mutation in Codon 858 of EGFR gene: p. L858R	mutation in Codon 858 of EGFR gene: p. L858R	
ID14	T790M+, L858R +	L858R +	L858R +	
ID15	positive T790M + L858R mutations	positive L858R mutation	positive L858R mutation	Mutation Q787Q (silent mutation) was also detected in Case 1 and 3
ID16	L858R	L858R	L858R	

Table 12 – interpretation

interpretation of result as in regular report				
ID01	s.a.	s.a	s.a.	
ID02	TKI resistant	TKI sensitive	TKI sensitive	
ID04	activating and resistance-mutation in tissue	activating mutation in tissue	activating mutation in tissue	
ID05	EGFR activating mutations in exon 20 and 21.	classical EGFR activating mutation in exon 21	classical EGFR activating mutation in exon 21	
ID06	mutant	mutant	mutant	
ID07				
ID09	Resistant to EGFR TKIs	Confers sensitivity to EGFR TKIs	Confers sensitivity to EGFR TKIs	
ID10	*combination of activating mutation on exon 21 and resistance mutation on exon 20. This patient should not be treated with conventional TKI*	activating mutation	activating mutation	
ID11	adenocarcinoma positive mutation exon 21 L858R	adenocarcinoma positive mutation exon 21 L858R	adenocarcinoma positive mutation exon 21 L858R	
ID12				
ID13				
ID14	no interpretation of results or additional results ,,,, (s. below)			
ID15	Sample is positive for activating L858R mutation and also for inactivating T790M mutation.	Sample is positive for activating L858R mutation.	Sample is positive for activating L858R mutation.	
ID16				

Table 13 – additional comments

Additional comments to oncology department or recommendations					
ID01				not done in our institute	
ID02					
ID04	*personal contact with therapist*	therapy with TKI indicated	therapy with TKI indicated		
ID05	Exon 21 mutation renders tumors sensitive to EGFR TK inhibitors. However the tumor contains cells with EGFR exon 20 mutation causing resistance to TK inhibitors.	This mutation renders tumors sensitive to TK inhibitors	This mutation renders tumors sensitive to TK inhibitors		
ID06	The L858R activating mutation is known to be associated with sensitivity for drugs targeting EGFR.	The L858R activating mutation is known to be associated with sensitivity for drugs targeting EGFR.	The L858R activating mutation is known to be associated with sensitivity for drugs targeting EGFR.		
ID07	anti EGFR therapy indicated	anti EGFR therapy indicated	anti EGFR therapy indicated		
ID09	Previous exposure to EGFR TKI? EGFR mutations and EML4-ALK1 alterations are considered mutually exclusive.	EGFR mutations and EML4-ALK1 alterations are considered mutually exclusive.	EGFR mutations and EML4-ALK1 alterations are considered mutually exclusive.		
ID10	the patient might be treated with second generation TKI , in case of uncertainty your phione call is welcome	in case of uncertainty your phone call is welcome	in case of uncertainty your phone call is welcome		
id!!					

Table 13a – additional comments

Additional comments to oncology department or recommendations					
ID12	Derzeit keine unterstützende Datenlage zum Einsatz von IRESSA (T790M = Resistenzmutation)	Datenlage unterstützt Einsatz von IRESSA	Datenlage unterstützt Einsatz von IRESSA		
ID13	Good response to anti EGFR therapy probable. Mutation in codon 790 could indicate resistance	Good response to anti EGFR therapy probable	Good response to anti EGFR therapy probable		
ID14	… because our oncologists know what the results mean				
ID15	Presence of the activating L858R mutation is associated with EGFR TKI sensitivity. Presence of the inactivating T790M mutation is associated with EGFR TKI resistance and can be secondary, following therapy. Reference: Travis W. D. et al. International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society: international multidisciplinary classification of lung adenocarcinoma: executive summary. Proc Am Thorac Soc. 2011 Sep;8(5):381-5.	Presence of the activating L858R mutation is associated with EGFR TKI sensitivity: EGFR TKI therapy is recommended. Reference: Travis W. D. et al. International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society: international multidisciplinary classification of lung adenocarcinoma: executive summary. Proc Am Thorac Soc. 2011 Sep;8(5):381-5.	Presence of the activating L858R mutation is associated with EGFR TKI sensitivity: EGFR TKI therapy is recommended. Reference: Travis W. D. et al. International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society: international multidisciplinary classification of lung adenocarcinoma: executive summary. Proc Am Thorac Soc. 2011 Sep;8(5):381-5.		
ID16					

table 14 - diagnosis

Diagnosis of case as done in regular reports					
ID01	AC, G2-3, predom papillary + acinar	acinar AC with large nonmucinous in situ component (G1,2) 5 %?	large cell variant of AC G3 (papillary, acinar, solid) L1		
ID02	Adeno G2	bronchiolo alveolar	Adeno G3		
ID04					
ID05	EGFR mutant micropapillary adenocarcinoma	EGFR mutant lepidic predominant adenocarcinoma	EGFR mutant adenocarcinoma		
ID06	Adenocarcinoma of lung with EGFR activating mutation	Adenocarcinoma of lung with EGFR activating mutation	Adenocarcinoma of lung with EGFR activating mutation		
ID07	EGFR (exon21) activating mutation detected	EGFR (exon21) activating mutation detected	EGFR (exon21) activating mutation detected		
ID09	Adenocarcinoma, predominantly acinary, tumor grade: 2. Detectable mutations: p.T790M (c.2369C>T)and p.L858R (c.2573T>G)	Adenocarcinoma of the lung, predominantly acinary, tumor grade: 2. Detecable mutation: p.L858R (c.2573T>G)	Adenocarcinoma of the lung, predominantly solid, tumor grade: 3. Detecable mutation: p.L858R (c.2573T>G)		
ID10	acinar predominant adenocarcinoma with minor micropapillary component	acinar adenocarcinoma with areas of in situ adenocarcinoma	predominant acinar adenocarcinoma		
ID11	favours existence of mutation L858R in exon 21 of tumor cells and resistance mutation T790M in exon 20	favours existence of mutation L858R in exon 21 of tumor cells	favours existence of mutation L858R in exon 21 of tumor cells		
ID12					
ID13	EGFR positive	EGFR positive	EGFR positive		
ID14	micropapillary adenoca GII	atypical adenomatous hyperplasia plus adenocarcinoma in situ (formerly BAC) with focal transition to adenocarcinoma GII	predominantly papillary adenocarcinoma GII		
ID15	Adenocarcinoma with micropapillary pattern	Adenocarcinoma with lepidic pattern	Adenocarcinoma with acinar pattern		
ID16					

discussion

- diagnosis
  - quantification of different tumor patterns in 5% discriminating steps
  - grading
- documentation
  - inclusion of mutation in histological diagnostic report
  - mutation specification

discussion

- selection of DNA
  - estimation of tumor-cell content (table 3)

- method
  - sensitivity