Österreichische Gesellschaft für Pathologie
Österreichische Division der IAP

Ring Trial
Molecular Diagnostics

EGFR mutations in NSCLC

Series I – October 2011

results

Definition of cases/comments

- Case 1
  - Lung adenocarcinoma,
    - wild type for EGFR in exons 18-21
  - tested by
    - next generation sequencing (Roche 454) and
    - conventional pyrosequencing
- Case 2
  - Lung adenocarcinoma,
    - activating mutation L858R in Exon 21;
    - present in a minority of tumor cells: approximately 6% of tumor cells
  - tested by
    - next generation sequencing (Roche 454)
- Case 3
  - Lung adenocarcinoma,
    - activating mutation L858R in Exon 21,
    - present in approximately 45% of tumor cells
  - tested by
    - next generation sequencing (Roche 454) and
    - conventional pyrosequencing
- IMPORTANT
  - information should be provided what mutations can be detected with the method used, and what sensitivity is to be expected

Percentage of tumor cells within section

(as in regular report)

Lab-ID	Case 1-A	Case 2-B	Case3-C	Participants comment
ID01	60	30	40	
ID02	80	70	20	
ID04	40	40	30	
ID05	70	80	80	
ID06	70	60	50	
ID07	50	50	60	
ID08	60	40	25	
ID09	50	60	40	
ID10	80	60	60	
ID11	90	90	80	% Tumorzellen
ID12	45	50	60	
ID13	80	70	40	

Tumor cell enrichment – yes/no: method of enrichment

(e.g. macrodissection by needle, scratching)

Lab-ID	Case 1-A	Case 2-B	Case3-C	Comment by distributor
ID01	Macrodiss needle 80%	Macrodiss needle 70%	Macrodiss needle 70%	
ID02	Y			Method of enrichment is not stated
ID04	No			
ID05	macrodissection by scraping			
ID06	no			
ID07	macrodissection			
ID08	yes, laser-capture microdissection (LCM)			
ID09	yes: macrodissection			
ID10	Yes, macrodissection by needle			
ID11	no			
ID12	Yes scratching Tu 75%	Yes scratching Tu 75%	Yes scratching Tu 75%	
ID13	Yes, marking the tumor area by scratching			

Method of DNA extraction

Lab-ID	Case 1-A	Case 2-B	Case3-C	Comment by distributor
ID01	Maxwell FFPE tissue dev			
ID02	Quiamp DNA FFPE	Tissue Kit, cat N56404		
ID04	Quiagen FFPE tisue kit			
ID05	High Pure PCR Template Preparation Kit - Roche			
ID06	Roche DNA extr.kit			
ID07	fibre glass extraction			
ID08	Qiagen QIAamp DNA micro kit			
ID09	DNA FFPE MiniKit			
ID10	MAXWELL 16 instrument			
ID11	DNA extr. Aus Paraffinmat			
ID12	EZ1 automat Fa. Quiagen			
ID13	Quiamp FFPE kit			

Method of measurement of DNA

Lab-ID	Case 1-A	Case 2-B	Case3-C	Comment by distributor
ID01	Qubit fluorimetry			
ID02				no DNA measurement?
ID04				no DNA measurement?
ID05	spectrophotometric measurement (Nanodrop - thermo Scientific)			
ID06	Nanodrop			
ID07	UV photometry			
ID08	Nanodrop			
ID09	Nanodrop, Peqlab			
ID10	Spectralphotometer Nanodrop			
ID11	Photometr. Messung			
ID12	Nanodrop ND-1000 FA Peqlab			
ID13	Qubit			

Amount of DNA extracted

(as in regular report)

Lab-ID	Case 1-A	Case 2-B	Case3-C	Comment by distributor
ID01	43,7 ng/µl (100µl)	46,5 ng/µl (100µl	14,6 ng/µl (100µl)	
ID02				no amount of DNA used for PCR is given
ID04				no amount of DNA used for PCR is given
ID05	72,4 ng/µl	72,4 ng/µl	16,1 ng/µl	
ID06	188,9 ng/µl	330,39 ng/µl	69,79 ng/µl	
ID07	129 ng/ul	41 ng/ul	8 ng/ul	
ID08	12.0-25,9 ng/µl	14,1-21,7 ng/µl	16,6-18,2 ng/µl	
ID09	124,7 ng/µl	195,3 ng/µl	31,8 ng/µl	
ID10	130 ng/µl (60 µ)l	140 ng/µl (60 µ)l	85 ng/µl (60 µ)l	
ID11	300 ng/µl	120 ng/µl	110 ng/µl	
ID12	53,2 ng/µl	56,8 ng/µl	33,9 ng/µl	
ID13	24,1 ng/µl	50,8 ng/µl	16,5 ng/µl	

Qualitiy of DNA

Lab-ID	Case 1-A	Case 2-B	Case3-C	Comment	Comment by distributor
ID01	Ok	Ok	ok		Please use measurement data not just OK
ID02	Ok	Ok	Ok	ß-globin PCR	Please use measurement data not just OK
ID04	CpWert 25,74	CpWert 23,95	CpWert 26,61	CpWert in allen 3 Proben ausreichend für IVD workflow	
ID05	1,9	1,98	2,02	(260/280)	
ID06	260/280 1,69	260/280 1, 64	260/280 1,69		
ID07	1,7	1.7	1.7		
ID08	Good	Good	good		
ID09	1,86 260 nm/280nm	1,91 260nm/280nm	1,82 260nm/280nm		
ID10	1,8	1,82	1,83		
ID11	Ok	Ok	Ok		Please use measurement data not just OK
ID12	Ok	Ok	Ok		Please use measurement data not just OK
ID13	Ok	Ok	Ok		Please use measurement data not just OK

Method of sequencing

(as in regular report)

Lab-ID	Case 1-A	Case 2-B	Case3-C	Comment by distributor
ID01				method of sequencing is not stated (This is important, because it will tell the oncologist about limitations of sensitivity
ID02				method of sequencing is not stated (This is important, because it will tell the oncologist about limitations of sensitivity
ID04	Light cycler 4890 realtime PCR	EGFR RGQ PCR kit24, V1		
ID05	Direct sequencing (Applied Biosystems 3130 Genetic Analyzer, Applied Biosystems) of purified (QIAquick Gel Extraction Kit -Qiagen, Valencia, CA, USA) target-specific PCR product (exon 18,19,20,21)			
ID06	Sanger ABI 310			
ID07	Dye Terminator			
ID08	capillary sequencing, genome sequencing			
ID09	Pyrosequencing, Allel-specific PCR			
ID10	Therascreen EGFR.Pyro-kit QUIAGEN			
ID11	Therascreen EGFR + light cycler480			
ID12	Sanger Sequenzierung ABI			
ID13	Thera screen KRAS PARO Kit			

Results (as in regular report)

Lab-ID	Case 1-A	Case 2-B	Case3-C	Comm.	Comment by distributor
ID01	Negative	L858R	L858R		Results are correct, but several other informations are not provided – see above
ID02	WT	L858R	L858R		results all correct, but percentage of tumor cells carrying the mutation not given – may be due tot he method used
ID04	Im vorliegenden Untersiuchungsmaterial ist eine Mutation des EGFR-Gens nicht nachweisbar (WildtyPp)	Im vorliegenden Untersuchungsmaterial ist eine Mutation des EGFR-Gens im exon 21 (L858R) in weniger als 1 % der Tumorzellen nachweisbar	Im vorliegenden Untersuchungsmaterial ist eine aktivierende Mutation des EGFR-Gen im exon 21 (L7858R) nachweisbar		correct
ID05	Wild type exon 18,19,20,21 of EGFR	Wild type exon 18,19,20,21 of EGFR	Wild type exon 18,19,20 Heterozygous point mutation in exon 21: **c.2573** T>G **p.L858R** (Cosmic ID6224)		Mutation L858R Exon 21 in case B was not detected
ID06	Exon 1819,21 wild type	Exon 1819,21 wild type	Exon 21858R		In case B the point mutation was not detected
ID07	wt	mut – pL858R (5%)	mut – pL858R (8%)		results are correct in all cases; the percentage of mutated tumor cell is higher in case C
ID08	no mutations in exons 19 and 21 of EGFR	no mutations in exons 19 and 21 of EGFR	no mutations in exons 19 and 21 of EGFR		all cases were reported as wild type; both mutated cases were missed. searching for a reason why the mutations in cases B+C were not detected I can only think of LCM microdissection as a probably reason; one possibility could be that an inexperienced technician microdissects the cells and collects many stroma cells or alike; or another reason could be that the laser beam setting is on too high energy, causing fragmentation of DNA; I had once such problems in CGH analysis and on controls with different settings of the laser beam could overcome these problems. I would suggest performing a control study with cases and using different methods of tumor cell enrichment
ID09	No Mutation detecable	Detecable mutation: p.L858R (c.2573T>G)	Detecable mutation: p.L858R (c.2573T>G)		has got all results correctly
ID10	No additional peaks detectable	Exon 21 L858R 5 %	Exon 21 L858R 21 %		has got all results correctly
ID11	Negative	Positive L858R, exon 21	Positive L8585R, exon 21		has got all results correctly
ID12	Keine Veränderung	Keine Veränderung	c.2573 T>G p.L858R	Auf die Angabe von stillen Mutationen wurde verzichtet	Point mutation in exon 21 was not detected in case B
ID13	No mutation	No mutation	p.L858R		Point mutation in exon 21 was not detected in case B

Interpretation of results

(as in regular report)

Lab-ID	Case 1-A	Case 2-B	Case3-C	Comment	Comment by distributor	Date rec	Date rep
ID01						11.10.11	17.10.11
ID02	wildtype	sensitivity to EGFR-TKI treatment to be expected	sensitivity to EGFR-TKI treatment to be expected		report was ready within 3 days from receipt of samples	10.10.11	13.10.11
ID04					no interpretation of results are given dates are missing therefore the time of receipt and issue of report cannot be calculated		
ID05	This tumor can be treated with the EGFR inhibitor Erlotinib, aspecially if it contains wt K-RAS. Accordingly a K-RAS test may be needed before prescription	This tumor can be treated with the EGFR inhibitor Erlotinib, aspecially if it contains wt K-RAS. Accordingly a K-RAS test may be needed before prescription	This adenocarcinoma is eligible for EGFR TK inhibitor therapy registered for EGFR-mutated NSCLC. Meanwhile the mutated population represents only a small fraction of the entire tumor population estimated to be in the range of 1-20%		the interpretation is unusual and will be confusing to oncologists: „This tumor can be treated with the EGFR inhibitor Erlotinib, especially if it contains wt K-RAS. Accordingly a K-RAS test may be needed before prescription“ : this statement appears in both cases identified as wild type; this is incorrect: a wild type case is not eligible for first line TKI treatment, and this is our duty by doing EGFR mutation analysis; for second or third line treatment with tyrosine kinase inhibitors no mutation analysis is required! „This adenocarcinoma is eligible for EGFR TK inhibitor therapy registered for EGFR-mutated NSCLC. Meanwhile the mutated population represents only a small fraction of the entire tumor population estimated to be in the range of 1-20%“; this statement should be restructured: the first part is essential, since it enables the oncologist to start with TKI therapy; the second part oft he statement is a commentary, and might alert the clinician for the event of an early resistance.		
	Exon 20: c. G2361A p.Q787Q (rs1050171) Homozygous	Exon21: c.C2508T p.R836R (rs17290559) Heterozygous	Exon 20: c. G2361A p.Q787Q (rs1050171) Homozygous	SNPs with unknown clinical significance	SNPs can be reported, but should be kept within the result section and not within interpretation or diagnosis, again to avoid confusion; dates are missing therefore the time of receipt and issue of report cannot be calculated		

Interpretation of results

(as in regular report)

Lab-ID	Case 1-A		Case 2-B	Case3-C	Comment	Comment by distributor	Date rec	Date rep
ID06	No activating mutation for TK inhibitor treatment		No activating mutation for TK inhibitor treatment	Exon 21 mutation sensitize for TK inhibitor treatment		Interpretation is correct, but case B was missed, therefore false negative; dates are missing therefore the time of receipt and issue of report cannot be calculated		
ID07	no benefit from anti-EGFR therapy expected		anti-EGFR therapy indicated	anti-EGFR therapy indicated		interpretation is correct 8 days from receipt of samples until release of report is almost too long: 7 week days/5 working days should be our personal limit	12.10.11	20.10.11
ID08	regarding the current criteria of drug prescription not recommended for gefitinib therapy		regarding the current criteria of drug prescription not recommended for gefitinib therapy	regarding the current criteria of drug prescription not recommended for gefitinib therapy		13 days elapsed from receipt of samples until report: this is too long, oncologists will start with conventional therapy in such a situation	12.10.11	25.10.11
ID09						no interpretation of results are given the time sequence between receipt of samples and reported results cannot be calculated, a date is missing		21.10.11
ID10	No mutation present in the areas tested for		Activating mutation, possibly not present in all tumor cells	Activating mutation		interpretation correct, all information is given; report was reported on the 5th day from receipt of samples	12.10.11	17.10.11
ID11	Keine Mutation im Therascreen EGFR-PCR kit		Befund spricht für Mutation im exon 21 L858R	Befund spricht für Mutation im exon 21 L858R		10 days for the report is too long	14.10.11	24.10.11
ID12				Datenlage unterstützt Einsatz von Iressa		No detailed interpretation of results; 10 days for the report is too long	17.10.11	27.10.11
ID13	ID13				s. Anhang SOP Befundung . Der Molpath-Befund ist ein Zusatzbefund zum Histo-Befund	No detailed interpretation of results; 12 days for the report is too long	15.10.11	27.10.11