Assessors' report for cIQc Run 33 (Oct 2013): ALK-1 LUNG CANCER

Assessors: EE Torlakovic, C Cheung, JC Cutz, S Nielsen, SE Hellström

Assessment performed at Toronto General Hospital, Toronto

There were 22 participating laboratories in the ALK-1 lung cancer run. There were 4 laboratories that showed less than perfect agreement with the reference laboratory. Although it has been published that for breast cancer markers, agreement of > 90% for positive results and >95% for negative results are desirable, in the setting of rare disease (i.e. ALK-1 positive lung cancer), this recommendation may not apply. As such, our target is 100% agreement for both positive and negative results for this run; it is technically possible and therefore there is no reason to settle for less.

One laboratory had a kappa value of "0", which is clearly not acceptable. One laboratory had a kappa value of 0.78 (which is less than 0.80, meaning less than "nearly perfect"). Two other laboratories had kappa values >0.80, which would be considered as "pass" for the participant if we would use the same criteria as recommended for ER/PR/HER2 IHC testing. However, the criteria applied to breast cancer markers do not fully apply to lung cancer markers due to differences in underlying biology as well as the test applications.

Therefore, the expert panel suggested:

1) If the IHC test is used for screening, sensitivity of less than 100% is not acceptable (three laboratories had <100% sensitivity), and if the sensitivity is 100%, specificity should be >90% (only one laboratory had 100% sensitivity had <90% specificity);

2) If the IHC test is used for definitive stratification for targeted therapy, sensitivity and specificity <100% is not acceptable for rare disease (see below).

The results of Run 33 showed that the same four laboratories would fail irrespective if they were using the test for screening or as a definitive test. The good news is that 18/22 (82%) laboratories passed even for definitive stratification for targeted therapy.

The explanation for the above criteria is as follows:

A) Screening:
When an IHC test is used for screening (meaning that another round of testing or evaluation will subsequently be done to determine the true status of the patient; in the case of ALK-1 for lung cancer, this would be FISH), the screening test should be 100% sensitive (so that we will not miss any patients with ALK-1 expressing tumours). In this setting, about 90% specificity should be acceptable. This takes into account turn-around-time and cost. If the IHC test is used for screening and it has low specificity, many false-positive samples will be sent for FISH testing, which delays proper diagnosis and increases the cost. This is particularly important in the setting of evidence that it is entirely possible to achieve 100% sensitivity and specificity as demonstrated by >80% of participating laboratories in this run. Since the great majority of participants were able to achieve 100% sensitivity and specificity, it is recommended that all laboratories that provide this service should achieve 100% sensitivity and >90% specificity.

B) Definitive testing:

ALK-1 is a rare driver of lung cancer as published literature suggests that only about 3% or less of lung adenocarcinomas are driven by ALK-1. 82% of laboratories showed that they could achieve 100% agreement with CIQC reference results (confirmed by FISH, 100% sensitivity and 100% specificity). If IHC test is used as definitive test for patient stratification for targeted therapy in rare disease, its specificity and sensitivity should be 100%. This is based on calculations showing that missing only 2 of 600 tested patients (2/600 = 0.3% wrong results) in a general population with lung adenocarcinoma means missing 20% of positive patients.

Participants should correlate their achieved results with the manner for which the test is used at their centre(s) (i.e. whether it is a screening test or a definitive test for stratification for targeted therapy).

Figure 1. Garrattogram from expert assessment.

Lab/ Core	101	102	107	110	111	112	113	114	115	116	123	125	126	137	146	149	186	189	191	202	205	206	R1
1	U	N	N	N	U	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2	U	U	U	U	U	U	N	U	U	U	N	U	U	U	U	U	N	E	U	N	N	N	N
3	P	P	P	P	P	P	P	P	P	E	P	P	E	P	P	P	N	P	P	P	P	P	P
4	P	P	P	P	P	P	P	P	P	N	P	P	E	P	P	P	N	P	P	P	P	P	P
5	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	E	E	N	N	N	N
6	P	P	P	P	P	P	P	P	P	N	P	P	E	P	P	P	N	P	P	P	P	P	P
7	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	E	N	N	N	N	N
8	P	P	P	P	P	P	P	P	P	E	P	P	P	P	P	P	N	P	P	P	P	P	P
9	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	E	E	N	N	N	N
10	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	P
11	P	P	P	P	P	P	P	P	P	E	P	P	N	P	P	P	N	P	P	P	P	P	P
12	U	P	P	P	U	P	P	P	P	P	P	P	E	P	P	P	N	P	P	P	P	P	P
13	P	P	P	P	P	P	P	P	P	E	P	P	E	P	P	P	N	P	P	P	P	P	P
14	U	U	U	U	U	U	U	U	U	U	U	U	U	U	N	U	U	U	U	U	U	U	N
15	U	U	U	U	U	U	U	U	U	U	U	N	U	U	N	U	U	U	U	U	U	U	N
16	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	E	N	N	N	N	N
17	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	E	N	N	N	N	N
18	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	N
19	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	N
20	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	E	N	N	N	N	N
21	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	E	N	N	N	N
22	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	E	N	N	N	N	N
23	N	N	N	N	E	N	N	N	E	N	N	N	N	N	N	N	N	E	N	N	E	N	N
24	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	E	E	N	N	N	N
25	N	E	N	E	N	E	E	N	N	N	E	N	N	N	E	E	N	P	E	E	N	N	N
26	N	N	N	N	E	E	N	N	N	N	N	N	N	N	N	N	N	E	N	N	N	N	N
27	N	N	N	N	N	E	N	N	E	N	N	N	N	N	N	N	N	E	N	N	N	N	N
28	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	N
29	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
30	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	E	N	N	N	N	N
31	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	E	U	N	N	N	U
32	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	U	N	N	N	U

N Negative P Positive U Unsatisfactory E Equivocal

nb sites 125,191, 205 did not submit slides for expert assessment and results entered are from the self assessment.

Figure 2. Garrattogram from submitted ALK-1 ISH results.

Labs/ Core	115	123	125	126	137	146	171	186	191	202	205	206	R1
8	P	P	U	P	P	U	P	P	P	P	P	P	P
9	N	N	N	N	N	U	N	N	N	N	N	N	N

Table 1. Descriptive statistics generated from expert assessment.

Lab ID	Total n	% Scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	PPV (positive predictive value)	NPV (negative predictive value)	Cohen's kappa
101	32	71.88	21	21/21 (100%)	1	1	1	1	1
102	32	78.13	23	23/23 (100%)	1	1	1	1	1
107	32	78.13	23	23/23 (100%)	1	1	1	1	1
110	32	78.13	23	23/23 (100%)	1	1	1	1	1
111	32	71.88	21	21/21 (100%)	1	1	1	1	1
112	32	78.13	23	23/23 (100%)	1	1	1	1	1
113	32	81.25	24	24/24 (100%)	1	1	1	1	1
114	32	78.13	23	23/23 (100%)	1	1	1	1	1
115	32	78.13	23	23/23 (100%)	1	1	1	1	1
116	32	78.13	23	21/23 (91%)	0.71	1	1	0.89	0.78
123	32	81.25	24	24/24 (100%)	1	1	1	1	1
125	32	81.25	24	24/24 (100%)	1	1	1	1	1
126	32	78.13	23	22/23 (96%)	0.86	1	1	0.94	0.89
137	32	78.13	23	23/23 (100%)	1	1	1	1	1
146	32	84.38	25	25/25 (100%)	1	1	1	1	1
149	32	78.13	23	23/23 (100%)	1	1	1	1	1
186	32	81.25	24	17/24 (71%)	0	1	-	0.71	0
189	32	81.25	24	22/24 (92%)	1	0.88	0.78	1	0.81
191	32	71.88	23	23/23 (100%)	1	1	1	1	1
202	32	81.25	24	24/24 (100%)	1	1	1	1	1
205	32	81.25	24	24/24 (100%)	1	1	1	1	1
206	32	81.25	24	24/24 (100%)	1	1	1	1	1

Table 2. Descriptive statistics generated from submitted self-assessments.

Lab ID	Total n	% Scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	PPV (positive predictive value)	NPV (negative predictive value)	Cohen's kappa
101	32	65.63	21	21/21 (100%)	1	1	1	1	1
102	32	81.25	24	24/24 (100%)	1	1	1	1	1
107	32	78.13	23	23/23 (100%)	1	1	1	1	1
110	32	87.5	26	26/26 (100%)	1	1	1	1	1
111	32	84.38	25	24/25 (96%)	0.86	1	1	0.95	0.9
112	32	87.5	26	26/26 (100%)	1	1	1	1	1
113	32	84.38	25	25/25 (100%)	1	1	1	1	1
114	32	81.25	26	26/26 (100%)	1	1	1	1	1
115	32	93.75	28	28/28 (100%)	1	1	1	1	1
116	32	90.63	27	25/27 (93%)	0.71	1	1	0.91	0.79
123	32	78.13	23	23/23 (100%)	1	1	1	1	1
125	32	81.25	24	24/24 (100%)	1	1	1	1	1
126	32	78.13	23	23/23 (100%)	1	1	1	1	1
137	32	78.13	23	23/23 (100%)	1	1	1	1	1
146	32	78.13	23	23/23 (100%)	1	1	1	1	1
149	32	81.25	24	24/24 (100%)	1	1	1	1	1
186	32	78.13	23	17/23 (74%)	0	1	-	0.74	0
189	32	78.13	23	23/23 (100%)	1	1	1	1	1
191	32	71.88	23	23/23 (100%)	1	1	1	1	1
202	32	84.38	25	25/25 (100%)	1	1	1	1	1
205	32	81.25	24	24/24 (100%)	1	1	1	1	1
206	32	81.25	24	24/24 (100%)	1	1	1	1	1

Table 3. Reported IHC staining protocols.

Lab ID	Clone	Dilution	Vendor	Antigen Retrieval	Detection System	Amplification	Chromagen
101	5A4	1:15	Leica	CC1 32mins	OPtiView	Non	DAB
102	5A4	1/50	NCL	DAKO FLEX HIGH pH	DAKO FLEX+	LINKER	DAB
107	5A4	1:10	Leica	cc1 90 mins	Ventana ultraView DAB	Ventana Amp Kit	DAB
110	5A4	1:50	BIOCARE	HIGH PH	DAKO FLEX+30	15 MIN LINKER	DAB
111	5A4	1/10	Leica	cc1 - 92 min	Ultraview	Yes	DAB
112	D5F3	1:100	New England Biolabs	CC1 64 mins	Optiview	no	DAB
113	5A4	1/25	Novocastra	High pH	Dako Flex	+Mouse	DAB
114	5A4	1/20	Leica	CC1-32min	Optiview Ventana	None	DAB
115	5A4	1/10	novocastra	CC1 90 min	Ultraview DAB	Cooper	DAB
116	ALK-1	1/25	Cell Marque	CC1 , 64min	Optiview DAB, Ventana	no	DAB
123	5A4	1:30	Leica	TRS High pH (Dako)	Flex+30 (Dako)	no	DAB
125	5A4	1/50	Leica/NCL	ER2-20	Bond Polymer Refine Detection	NA	DAB
126	ALK-1	1:100	Dako	Tris pH 10.0	polyer	none	DAB
137	5A4	1:30	Leica	CC1	Opti-View	no	DAB
146	5A4	1/100	Bio-Care	Flex TRS HIGH	EnVision Flex Peroxyda	None	DAB
149	5A4	1:50	Novacastra	high pH 20 min at 96 C	Dako Envision Flex	Yes (Envision Flex+Mouse Linker)	DAB
186	ALK1	1:25	CELL MARQUE	HIER 20' in EDTA	Bond refine detection kit	NONE	DAB
189	D5F3	Predilute	Ventana	CC1 92 min	OptiView DAB	OptiView Amplification	DAB
191	D5F3	RTU	Roche	CC1	optiview	yes	DAB
202	SP8	1/50	Novocastra	H2 (high pH)	Leica Refine	none	DAB
205	5A4	1/10	LEICA (NOVOCASTRA)	CC1 (92 MN)	Ultraview universal DAB	yes	DAB
206	5A4	1:10	LEICA	ULTRA CC1 92 minutes	ULTRAVIEW	YES	DAB
R1	Based on ALK FISH results						

Table 4. Reported ISH staining protocols.

Lab ID	Probe	Supplier	Instrument	Hybridization Time	Pretreatment Time	Post Hybridization Wash, Reagent, Temp, Time
115	Vysis ALK break apart	vysis intermedico	thermobrite hybridizer	15 hours	15 min	post ybridization wash buffer room temp for 8 min than at 75C for 3 min
123	Vysis LSI ALK Dual Colour	Abbott Molecular	Thermobrite	overnight	2 hours NaCitrate; 25 min pepsin	0.4XSSC/0.3%NP40, 72C, 2 min; 2xSSC/0.1%NP40, RT, 1 min
125	ALK-FISH	VYSIS-ABBOTT	THERMOBRITE	16 hrs; 37C	12 min	Buffer II 74 degrees 2 min, Buffer I room temp 1 min
126	ALK	Cymogen	MetaSystems	16h	3h	2xSSC w/0.4%NP-40, 79C, 3m
137	LSI ALK Break Apart	Abbott Molecular	Thermobrite	18 hours	2 hr sodium citrate buffer; 50 min 0.01N HCl with	0.4xSSC/0.3%NP40 at 72C for 2 minutes
146	Vysis ALK Break apart Fish probe kit	Abott Diagnostic	manual	22h00	12 min	2 min, 74C
171	Dual-probe break apart	Vysis	Thermobrite	18 hours	50 minutes	2xSSC, 0.3 NP40, 35C3 min,
186	Alk FISH	DAKO	HYBRITE FOR INCUBATION	16 HR	20 MIN	NP40 IN SSC, 73 C, 2.5 MIN
191	ALK-EML4	Abbott	in house method	overnight	20 min	2xSSC/ 0,3% NP-40, 73Â°C,2min
202	ALK 06N38-020	Abbott	Hybrite	73C for 3 min	37C for 18 hrs	2 min in WAsh Buffer of Vysis Paraffin Prtreatment IV INtermedico 01N31-005
205	Dual-probe break apart	Vysis	Thermobrite	18 hours	50 minutes	2xSSC, 0.3 NP40, 35C3 min,
206	ALK breakapart	Abbott	manual	48 hours	30 minutes	2SSC,3% Igepal rm temp 2min, then 8 min 72C, then 2SSC rm. temp 2 min