

T. Depoilly^a, S. Garinet^b, J Siemanowski^c, S Merkelbach-Bruse^c, V Tischler^d, M-C Demes^e, H Paridaens^f, C Sibille^f, L Van Kempen^g, E Schuurin^g, V de Montpreville^h, E Rouleauⁱ, P Desmeules^j, A Bartczak^k, M Pasięka-Lis^k, R Y Wei Teo^l, K L Chuah^l, M Barbosa^m, C Quintana^m, SC Safontⁿ, B Bellosilloⁿ, M Biscuola^o, M Delgado^o, D Vacirca^p, A Rappa^p, C Ercolani^q, S Buglioni^q, M Cashmore^r, M Smith^r, P Jasionowicz^r, A Meeney^s, B Terris^a, A Mansuet-Lupo^a

^aPathology, Cochin Hospital & ^bMolecular Oncology, GPEuropean Hospital, Paris, France; ^cPathology, University Hospital Cologne, Germany; ^dPathology, University Hospital Bonn, Germany; ^ePathology, University clinics Frankfurt, Germany; ^fPathology, CHR de la Citadelle, Liège, Belgium; ^gPathology, University Medical Center Groningen, Netherlands; ^hPathology, M Lannelongue Hospital, Le Plessis-Robinson, France; ⁱGenetic, G Roussy, Villejuif, France; ^jPathology, Institut Universitaire de Québec, Canada; ^kPathology, Public Specialist Hospital of Lung Diseases, Zakopane, Poland; ^lPathology, Tan Tock Seng Hospital, Singapore; ^mPathology, Hospital do Espírito Santo de Évora, Portugal; ⁿPathology, Hospital del Mar Medical Research Institute, Barcelona, Spain; ^oPathology, Hospital Universitario Virgen del Rocío-IBIS, Seville, Spain; ^pPathology, European Institute of Oncology, Milan, Italy; ^qPathology, Regina Elena National Cancer Institute, Rome, Italy; ^rPathology, The Royal Wolverhampton NHS Trust, Wolverhampton, UK; ^sPathology, Queen Elizabeth Hospital Birmingham, UK; ^tPathology, Royal Hallamshire Hospital, Sheffield, UK

Introduction

- Incidence of lung cancer worldwide is high and most patients have a poor prognosis with a **5-year survival** rate in metastatic disease of only **5%**
- All patients with metastatic NSCLC **should be tested before the first line of therapy** for pathogenic driver mutations in *EGFR*, *BRAF*, *ERBB2*, *MET_{ex14}* and fusions in *ALK*, *ROS1*, *RET* and *NTRK1/2/3* genes
- As the available material is often scarce, **multiplex technique** is the best approach, like that developed by biocartis (**Idylla™ GeneFusion assay**)

Idylla™ GeneFusion Assay

- Detection of **ALK, ROS1, RET, NTRK1/2/3 fusions & MET_{ex14} skipping mutations**
- Currently available in Research Use Only
- Fully automated test **directly from FFPE tissue** without the need for RNA extraction
- Turnaround time: **3 hours**
- 2 detection technology types:
 - Specific fusion detection (spe)** of the most relevant gene fusions by RT-qPCR
 - Expression imbalance (imb)** by analyzing expression ratios 5'-3'



Material and Methods

- Multicenter study (18 centers)
- 313 FFPE tissue samples** from lung cancer patients with molecular data previously obtained by reference methods (FISH, RT-PCR +/- NGS) : **97 ALK, 44 ROS1, 20 RET, 3 NTRK1, 2 NTRK3, 32 MET_{ex14}** and **115 WT samples**
- 1-3 sections of 5µm FFPE with ≥ 10% tum cell content
- 3 types of results : detected, not detected and invalid
- Definition of inconclusive cases : i) if more than one fusion or MET_{ex14} skipping mutation are detected; ii) if the invalid gene is the one that has been detected as altered by the ref method

Overall results

- Valid results : 306/313 cases (98%)
- Idylla™ confirmed the alteration in 165/193 (85%) and absence of alteration in 107/113 (95%) negative samples
- Idylla™ failed to detect 23 fusions and 5 MET_{ex14}

Idylla™ vs reference method	ALK	ROS1	RET	MET	NTRK
positive percentage agreement	94% 82/87	95% 36/38	100% 17/17	100% 27/27	100% 3/3
negative percentage agreement	93% 193/205	97% 255/263	99% 289/290	98% 275/280	99% 288/290
Overall concordance	94%	97%	99%	99%	99%

Conclusion

- Idylla™ allows in **3 hours** the detection of ALK, ROS1, RET, NTRK fusions and MET_{ex14} with a **good concordance**
- All of the ALK, ROS1 and RET **specific fusion detection** identified by Idylla™ were confirmed by ref method, except for 1 ROS1
- ALK and ROS1 imbalance only should be confirmed** (8 false positive samples)
- Idylla™ GeneFusion : good method to offer, along with Idylla™ EGFR testing in metastatic NSCLC patients, **who cannot wait for treatment**

Aim of this study : to compare the results of Idylla™ GeneFusion prototype with those obtained by reference methods (FISH, RT-PCR and NGS)

Results (Gene per gene)

Idylla™	Routine reference methods		
	Alteration	No alteration	Total
Alteration	82	5	87
No alteration	12	193	205
Invalid	3	18	21
Total	97	216	313

ALK	Sensitivity	85%
	Specificity	98%
	Pos % agreement	94%
	Neg % agreement	93%

Idylla™	Routine reference methods		
	Alteration	No alteration	Total
Alteration	17	0	17
No alteration	1	289	290
Invalid	2	4	6
Total	20	293	313

RET	Sensitivity	85%
	Specificity	100%
	Pos % agreement	100%
	Neg % agreement	99%

Idylla™	Routine reference methods		
	Alteration	No alteration	Total
Alteration	27	0	27
No alteration	5	275	280
Invalid	0	6	6
Total	32	281	313

MET_{ex14}	Sensitivity	84%
	Specificity	100%
	Pos % agreement	100%
	Neg % agreement	98%

Idylla™	Routine reference methods		
	Alteration	No alteration	Total
Alteration	36	2	38
No alteration	8	255	263
Invalid	0	12	12
Total	44	269	313

ROS1	Sensitivity	82%
	Specificity	99%
	Pos % agreement	95%
	Neg % agreement	97%

Idylla™	Routine reference methods		
	Alteration	No alteration	Total
Alteration	3	0	3
No alteration	2	288	290
Invalid	0	20	20
Total	5	308	313

NTRK	Sensitivity	60%
	Specificity	100%
	Pos % agreement	100%
	Neg % agreement	99%

- Negative samples** : 107/113 cases
 - ✓ 6 false positive cases (5 ALKimb and 1 ROS1 spe/imb)
 - ✓ 2 inconclusive cases (2 alterations found by Idylla)
- Inconclusive samples** : N=7
 - 6 cases (2 alterations found by Idylla) :
 - ✓ 1 (ALKimb/spe + MET) and 1 (ALKimb/spe + NTRK) : ALK confirmed
 - ✓ 2 (ALKimb + RETimb/spe) : RET confirmed
 - ✓ 1 (ALKimb + ROS1imb) and 1 (ALKimb + MET) : negative
 - 1 ALK positive found ALKimb invalid with no ALK specific detection

