Molecular profiling of non-small cell lung cancer by histologic subtype

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Abstract

Background: A substantial proportion of NSCLC has been shown to harbour specific molecular alterations affecting tumour proliferation and resulting in sensitivity to inhibition of the corresponding activated oncogenic pathway by targeted therapies. Comprehensive tumor profiling can diagnose such alterations and may identify new alterations opening additional treatment options for all distinct NSCLC subtypes.

Methods: Over 6,700 non-small cell lung cancer cases referred to Caris Life Sciences between 2009 and 2014 were evaluated; clinical diagnoses and detailed tumor pathology were collected from referring physicians. Specific profiling was performed per physician request and included a combination of sequencing (Sanger, NGS or pyrosequencing), protein expression (IHC), gene amplification/rearrangement (CISH or FISH), and/or RNA fragment analysis within potential cancer-related genes and pathways.

Results: Patients were grouped into cohorts according to histological subtype – adenocarcinoma (AD) (n=4,286), squamous cell carcinoma (SCC) (n=1,280), large cell carcinoma (LCC) (n=153) and lepidic predominant adenocarcinoma (LPA) (previously described as bronchioalveolar carcinoma (BAC)) (n=94). Protein overexpression of cMET (>2+ in >50% cells) was higher in AD (35.9%) compared to other subgroups (12-20%) while RRM1 and TOP2A levels were lower in AD. ALK or ROS1 were rearranged in 5.3% of patients with AD compared to 3.7% of patients with LCC and 1.2% of patients with SCC. EGFR mutations were found at low prevalence in both the LCC (0%) and SCC cohorts (2.8%) compared to 21% in AD. Similar lower rates of BRAF mutations were observed in the LCC and SCC cohorts compared to AD (0%, 1.1% and 5.1%). Pathway analysis showed activating mutations in the ERK pathway in 40% of patients with AD. Only 10-12% of patients with LCC or SCC had activating mutations in the ERK pathway.

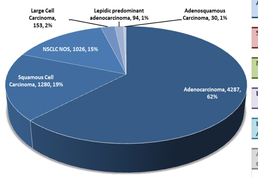
Conclusions: Despite the limitations of this retrospective series, we report comprehensive profiling of the largest cohort of NSCLC. Tumor profiling reveals that ADs may be more addicted to the ERK pathway than other histological subtypes. Drugs which target cMET may also have most utility in AD. Full analysis by histological subtype and additional correlative data on protein expression, gene copy number and mutations will be presented.

Methods

- 6870 NSCLC patients have been profiled using Caris Molecular Intelligence between 2009 and 2014. Tests performed were at the discretion of the physician and reflected the state of the art tumor profiling available at the time of ordering. This included a combination of sequencing (Sanger, NGS or pyrosequencing), protein expression (IHC), gene amplification (CISH or FISH), and/or RNA fragment analysis.
- IHC analysis was performed on formalin-fixed paraffin-embedded tumor samples using commercially available detection kits, automated staining techniques (Benchmark XT, Ventana, and AutostainerLink 48, Dako), and commercially available antibodies.
- Fluorescent in-situ hybridization (FISH) was used for evaluation of the HER-2/neu [HER-2/CEP17 probe], EGFR [EGFR/CEP7 probe], and cMET [cMET/CEP7 probe] (Abbott Molecular/Vysis). HER-2/neu and cMET status were evaluated by chromogenic in-situ hybridization (INFORM HER-2 Dual ISH DNA Probe Cocktail; commercially available cMET and chromosome 7 DIG probe; Ventana). The same scoring system was applied as for FISH.
- Direct sequence analysis was performed on genomic DNA isolated from formalin-fixed paraffin-embedded tumor samples using the Illumina MiSeq platform. Specific regions of 45 genes of the genome were amplified using the Illumina TruSeq Amplicon Cancer Hotspot panel.
- Mutation analysis by Sanger sequencing included selected regions of BRAF, KRAS, c-KIT, EGFR, and PIK3CA genes and was performed by using M13-linked PCR primers designed to amplify targeted sequences.
- Caris Molecular Intelligence™ (CMI) reports were provided to the ordering physician after comprehensive tumor profiling to contribute to biomarker-driven treatment decisions.
- Statistical analysis (unpaired t-tests used to compare biomarker expression across histologic subtypes) performed using GraphPad™.

Demographics

- No clinical data on disease stage, recurrence or prior treatment history was collected for these samples.
- The distribution of histological subtypes reflects the ordering patterns of the physicians and need for further treatment options rather than the reported epidemiology of NSCLC.



	n	Male (n, %)	Female (n, %)	Average Age	Median Age	Age Range
Adenocarcinoma	4287	1884 (44%)	2403 (56%)	66	67	20-99
Squamous Cell Cancer	1280	756 (59%)	524 (41%)	67	68	21-93
NSCLC NOS	1026	559 (54%)	467 (46%)	65	66	24-91
Large Cell Carcinoma	153	91 (59%)	62 (41%)	65	65	38-90
Lepidic Predominant Adenocarcinoma	94	41 (44%)	53 (56%)	68	71	27-84
Adenosquamous Carcinoma	30	8 (27%)	22 (73%)	62	61	39-85
TOTAL	6870	3339 (49%)	3531 (51%)	66	67	20-99

	Lung & Bronchus	Lymph Nodes	Pleura	Connective & Soft Tissue	Liver	Brain	Bone	Other
Adenocarcinoma	2564 (59.8%)	574 (13.4%)	369 (8.6%)	151 (3.5%)	140 (3.3%)	142 (3.3%)	115 (2.7%)	232 (5.4%)
Squamous Cell Carcinoma	959 (74.9%)	146 (11.4%)	17 (1.3%)	44 (3.4%)	28 (2.2%)	20 (1.6%)	16 (1.3%)	50 (3.9%)
NSCLC NOS	413 (40.3%)	191 (18.6%)	69 (6.7%)	83 (8.1%)	71 (6.9%)	64 (6.2%)	39 (3.8%)	96 (9.4%)
Large Cell Carcinoma	86 (56.2%)	31 (20.3%)	4 (2.6%)	11 (7.2%)	8 (5.2%)	4 (2.6%)	2 (1.3%)	7 (4.6%)
Lepidic Predominant Adenocarcinoma	88 (93.6%)	0 (0%)	0 (0%)	1 (1.1%)	2 (2.1%)	1	0 (0%)	2 (2.1%)
Adenosquamous Carcinoma	24 (80.0%)	3 (10%)	0 (0%)	1 (3.3%)	2 (6.7%)	0 (0%)	0 (0%)	0 (0%)
TOTAL	4134 (60.1%)	945 (13.8%)	459 (6.7%)	291 (4.2%)	251 (3.7%)	231 (3.4%)	172 (2.5%)	387 (5.6%)

Other includes Abdomen NOS (17), Abdominal wall (2), Adrenal glands (101), Anal canal (1); Appendix (1), Bladder (1), Blood & bone marrow (13), Breast (9), Chest NOS (39), Colon (11), Fundus of stomach (1), Gum & mouth (1), Head, face or neck NOS (1), Heart mediastinum and pleura (7), Hypopharynx (1), Inguinal region NOS (1), Kidney (10), Omentum (7), Orbit & lacrimal gland (1), Other endocrine (1), Other nervous system (1), Ovary (4), Pancreas (6), Pelvis NOS (13), Pericardium (22), Peritoneal fluid (2), (4), Posterior cranial fossa (1), Rectum (2), Retroperitoneum & Peritoneum (19), Salivary gland (2), Skin (32), Small Bowel NOS (1), Small intestine (14), Spleen (2), Stomach (2), Thyroid Gland (5), Trachea (10), Unknown (7), Urinary Bladder (1), Visceral pleura (1), Vulva (10)

Results of Tumor Profiling by Histological Subtype

- ADC tumors had significantly more cMET overexpression (p<0.0001) and amplification (p=0.0223), more high ER expression (p<0.0001), more ALK fusions (p=0.0051) and ROS1 rearrangements (p=0.0331), higher BRAF (p=0.0218) and EGFR (p<0.0001) mutations prevalence than SCC tumors.
- Similar significant alterations between ADCs and LCCs (except for ALK, BRAF and ROS1) were observed.
- SCC tumors had significantly less loss of RRM1 and TS, more loss of TUBB3 and more overexpression of TLE3, TOPO1 and TOP2A overexpression (all p<0.0001 except TOPO1, p=0.0018) compared to ADC. Similar significant changes compared to LPA were observed.
- SCC tumors also had a significantly higher percentage of tumors that had a EGFR H-score > 200 compared to ADC (p<0.0001) and all other tumor types.
- SCC also had less cKIT overexpression and more ERCC1 loss than any other histological subtypes, although more cKIT mutations than ADC (p=0.0044).
- ADCs and LPAs had significantly more KRAS mutations than SCC tumors (p<0.0001 and p=0.0028 respectively).
- In contrast, SCCs had a significantly higher prevalence of PIK3CA (p=0.0027) and PTEN (p=0.0299) mutations. SCCs and LCCs also had a significantly higher number of TP53 mutations than ADCs (p<0.0001 and 0.0272 respectively).
- No other significant observations in mutations were observed between tumor types.
- PTEN loss was not significantly different between any of the histological subtypes.

ADC	SCC	ASQ	LPA	LCC	Biomarker	Platform	Thresholds
3.1	2.3	13*	1.4	4.2	AR	IHC	*Negative: <0+ or <10%; Positive: ≥1+ and ≥10%*
17.1	8.3	18.8*	21.5	29.1	cKIT	IHC	*Negative: <0+ and <100%; Positive: >2+ and ≥30%*
35.9	19.5	44.4*	50*	12	cMET	IHC	*Negative: <50% or <2+; Positive: ≥2+ and ≥50%*
40.6	69	33.3*	20*	22.9*	EGFR (H-score)	IHC	*Negative: <200; Positive: ≥200 (H score)*
8.6	3.2	0*	14.3	1.7	ER	IHC	*Negative: <0+ or <10%; Positive: ≥1+ and ≥10%*
71.4	57.8	87.5*	76.4	76.4	ERCC1 Loss	IHC	*Negative: <2+ or ≤3+ and <10% or <2+ and <50%; Positive: ≥3+ and ≥10% or 2+ and ≥50%*
1.1	1.2	0*	1.4	2.6	HER2	IHC	*Negative: ≤1+ or <2+ and ≤10%; Positive ≥3+ and >10%*
45	44.7	43.5*	29.6	63.3	MGMT Loss	IHC	*Negative: <0+ or ≤35%; Positive ≥1+ and >35%*
24	8.9	9.1*	36.2	13.1	PGP	IHC	*Negative: <0+ or <10%; Positive ≥1+ and ≥10%*
4.3	5	13*	10	6	PR	IHC	*Negative: <0+ or <10%; Positive ≥1+ and ≥10%*
55.2	64.9	64*	59.2	60.9	PTEN Loss	IHC	*Negative: <0+ or ≤50%; Positive ≥1+ and >50%*
83.4	62.7	82.6*	82.9	59.2	RRM1 Loss	IHC	*Negative: <0+ or <50%; Positive <2+ or ≥2+ and ≥50%*
31.4	32.1	30.4*	31	24.2	SPARC	IHC	*Negative: <30% or <2+; Positive: ≥2+ and ≥30%*
20.9	41.9	25*	0*	6.1*	TLE3	IHC	*Negative: <30% or <2+; Positive: ≥2+ and ≥30%*
44.8	48.1	50*	32.4	47	TOPO1	IHC	*Negative: <0+ or <30% or <2+; Positive ≥2+ and ≥30%*
51.2	80.4	90.9*	10	78.5	TOP2A	IHC	*Negative: <0+ or <10%; Positive ≥1+ and ≥10%*
73.8	65.3	51.2*	83.6	59	TS Loss	IHC	*Negative: <0+ or ≤3+ and <10%; Positive ≥1+ and ≥10%*
29.4	58.4	14.3*	40*	24.2*	TUBB3 Loss	IHC	*Negative: <30% or <2+; Positive ≥2+ and ≥30%*
3.3	1.2	7.1*	0*	3.7*	ALK	FISH	*The FISH assay looks for rearrangements involving ALK, either inversion or translocation, are observed when one of the two fusion signals separates*
5.7	2.4	0*	0*	14*	cMET	FISH	*Positivity for increased gene copy number by FISH has been defined as ≥ 5 copies in lung tumor cells. The gene copy number threshold for other tumor types has not been determined.*
4.2	4.7	0*	9.1*	4.8	HER2	FISH	*HER2/Neu:CEP17 signal ratio of ≥ 2.0 and non-amplification as <2.0 as per Ventana INFORM HER2 CISH Package insert*
2	0	0*	0*	0	ROS1	FISH	*Fluorescent in situ hybridization was performed using the Cytocell ROS1 (6q22.1) break-apart probes. 100 interphase cells were scored for this analysis.*
5.1	1.1	0*	0*	0*	BRAF	NGS	*Amino acids 419-472 and 581-614*
0.5	2.8	0*	0*	0*	cKIT	NGS	*Amino acids 42-101, 493-592, 632-745 and 806-866*
4.2	5	0*	0*	0*	cMET	NGS	*Amino acids 168-218, 366-400, 1105-1132 and 1238-1284*
21	2.8	0*	0*	0*	EGFR	NGS	*Amino acids 279-297, 590-627, 700-823, 849-875*
0.9	0	0*	0*	0*	HER2	NGS	*Amino acids 746-827, 832-833*
35	7.7	40*	50*	7.4*	KRAS	NGS	*Amino acids 1-31, 38-71, 97-150*
0.3	1.1	0*	0*	0*	NRAS	NGS	*Amino acids 1-27, 38-71*
1.1	1.1	0*	0*	0*	PDGFRA	NGS	*Amino acids 552-596, 650-719*
2.3	7.2	20*	0*	0*	PIK3CA	NGS	*Amino acids 75-118, 336-353, 418-555, 692-729, 979-1068*
2	5.1	0*	0*	7.7*	PTEN	NGS	*Amino acids 1-27, 165-267, 280-342*
0	0	0*	0*	0*	RET	NGS	*Amino acids 602-627, 629-676, 762-798*
43.5	72.5	60*	25*	66.7*	TP53	NGS	*Amino acids 1-20, 60-121, 126-307, 322-346*
0.3	0	0*	0*	0*	VHL	NGS	*Amino acids 80-114, 121-210*

* Test performed in ≤50 cases

Results of Tumor Profiling by Biopsy Site

- ADC biopsy material from the lung had significantly more CKIT (p<0.001), ER (p<0.0001) and more MGMT loss than material from other sites.
- In contrast, other metastatic sites expressed significantly higher EGFR H-score (p=0.0003) more ERCC1 and RRM1 loss, and more tumors overexpressing SPARC, TOPO2A or TOPO1 (p<0.001).
- ADC tumors biopsied from the lung also had significantly less TP53 mutations than those from other sites (p=0.0049).
- SCC biopsies from lung and elsewhere were more comparable, though those taken from the lung had a significantly higher percentage which overexpressed PR (p=0.0164) and had more TS loss (p=0.0173). Tumors biopsied from other sites had more RRM1 loss (p=0.0155) and more TOP2A overexpression (p=0.0155).
- SCC tumors from other sites had significantly more PTEN mutations (p=0.0464) than those taken from the lung.

ADC (n=4287)	*LUNG (n=2564)*	*OTHER (n=1723)*	SCC (n=1280)	*LUNG (n=959)*	*OTHER (n=321)*	Biomarker	Platform
3.1	3.5	2.7	2.3	2	3.1	AR	IHC
35.9	34.8	37.3	19.5	17.6	24.4	cMET	IHC
40.6	35.9	46.7	69	68.1	71.4	EGFR (H-score)	IHC
8.6	10.7	5.4	3.2	3.7	2	ER	IHC
71.4	74.9	65.9	57.8	57.3	58	ERCC1 Loss	IHC
1.1	1.1	1	1.2	1.5	0.4	HER2	IHC
45	42.8	48.3	44.7	43.1	49.1	MGMT Loss	IHC
24	23.6	24.6	8.9	8.3	10.7	PGP	IHC
4.3	4.3	4.4	5	4.1	7.9	PR	IHC
55.2	54.2	56.7	64.9	65.2	64.3	PTEN Loss	IHC
83.4	87	78.2	62.7	64.8	56.6	RRM1 Loss	IHC
31.4	28.5	35.7	32.1	31.4	34.2	SPARC	IHC
20.9	18.1	24.7	41.9	40.7	45.1	TLE3	IHC
44.8	40.1	51.6	48.1	47.3	50.4	TOPO1	IHC
51.2	42.1	64.8	80.4	78.2	86.9	TOP2A	IHC
73.8	75.2	71.8	65.3	63.3	71.3	TS Loss	IHC
29.4	31	27.4	58.4	60.1	53.8	TUBB3 Loss	IHC
3.3	3.1	3.5	1.2	1.5	0.6	ALK	FISH
5.7	5.7	5.7	2.4	3.3	0	cMET	FISH
4.2	4.1	4.2	4.7	5.3	3.3	HER2	FISH
2	1.3	2.9	0	0	0	ROS1	FISH
5.1	4.3	6.1	1.1	1.5	0	BRAF	NGS
0.5	0.3	0.7	2.8	3	2.1	cKIT	NGS
4.2	4.3	4	5	4.4	6.4	cMET	NGS
21	23.6	17.7	2.8	3	2.1	EGFR	NGS
0.9	0.8	1	0	0	0	HER2	NGS
35	37.6	31.7	7.7	6	12.8	KRAS	NGS
0.3	0.3	0.3	1.1	1.5	0	NRAS	NGS
1.1	0.8	1.4	1.1	0.8	2.1	PDGFRA	NGS
2.3	2.5	2.1	7.2	7.5	6.4	PIK3CA	NGS
2	1.7	2.4	5.1	3.1	10.6	PTEN	NGS
0	0	0	0	0	0	RET	NGS
43.5	38.3	50	72.5	72.5	72.3	TP53	NGS
0.3	0.3	0.4	0	0	0	VHL	NGS

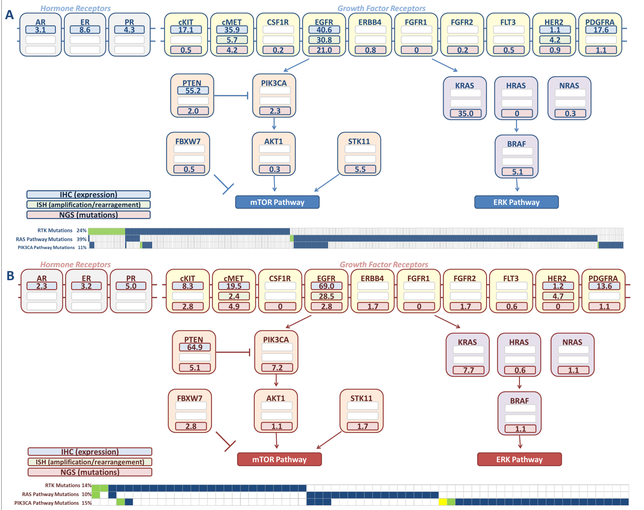
Treatment Options Not Routinely Considered in NSCLC

- On- and Off-compendium treatment options associated with benefit according to the outcome of profiling in entire NSCLC cohort highlights that off-compendium targeted therapies are rarely found in NSCLC but could be beneficial to the patient.

	Associated Biomarker	Platform used	% of NSCLC patients for which this treatment associated with benefit	Number of Positive Tests/ Number of Tests performed	Potential predictive biomarker, cancer type	REF
On-Compendium Therapies						
docetaxel, paclitaxel	TLE3	IHC	25.5	457/1794	Breast, Bladder, Ovarian	1
	TUBB3 Loss	IHC	36.8	436/1185	NSCLC	2
cisplatin, carboplatin	ERCC1 Loss	IHC	67.9	2765/4070	NSCLC, Colon, Ovarian	3
gemcitabine	RRM1 Loss	IHC	76.0	3737/4914	NSCLC	4
irinotecan	TOPO1	IHC	46.7	2137/4576	Colon, Ovarian	5
pemetrexed (non-squamous only)	TS Loss	IHC	69.8	3388/4854	NSCLC, Breast, Colon	6
On-Compendium Targeted						
erlotinib, gefitinib, afatinib	EGFR mutation	SEQ	13.3	512/3849	NSCLC	7
		NGS	15.0	159/1059		
cetuximab	EGFR IHC H-Score	IHC	46.0	811/1762	NSCLC	8
crizotinib	ALK	FISH	2.9	103/3612	NSCLC	9
	ROS1	FISH	1.4	18/1296	NSCLC	10
Off-Compendium Therapies						
abiraterone, bicalutamide, flutamide	AR	IHC	3.0	135/4492	Prostate	11
hormone antagonists	ER	IHC	6.3	281/4496	Breast, Ovarian	12
	PR	IHC	4.9	221/4480	Breast, Ovarian	
temozolomide, dacarbazine	MGMT Loss	IHC	46.8	2172/4640	Melanoma, Glioblastoma, Neuroendocrine tumors, Pituitary carcinomas, Oligodendroglioma	13
nab-paclitaxel	SPARC	IHC	31.8	1499/4707	Head and Neck, Pancreatic	14
doxorubicin, liposomal-doxorubicin, epirubicin	TOP2A	IHC	62.7	2587/4140	Breast, Soft Tissue Sarcoma	15
	HER2	ISH	4.2	109/2576	Breast, Ovarian	
Off-Compendium Targeted						
trastuzumab, lapatinib, pertuzumab, TDM-1	HER2	IHC	1.1	48/4549	Breast, Gastric, Gastroesophageal, Ovarian	16
		NGS	0.8	8/1049	NSCLC, Breast	17
imatinib	cKIT	NGS	1.1	12/1058	GIST, Melanoma	18
	PDGFRA	NGS	1.0	10/1043	GIST	19
vandetanib	RET	NGS	0.0	0/1057	Medullary Thyroid Cancer	20

Overview of RAS and PI3K Pathway Alterations in ADC and SCC

- Table B shows all ADC (A) or SCC(B) patients in who a mutation in an RTK, RAS pathway or PIK3CA pathway occurred and the overlap in mutations within these patients. Dark blue is a mutation, green indicates 2 mutations present.
- RTK mutations include mutations in either cKIT, cMET, CSF1R, EGFR, ERBB4, FGFR1, FGFR2, FLT3, HER2 and PDGFRA. RAS pathway mutations have been grouped as KRAS, NRAS, HRAS and BRAF. PI3K pathway alterations include PIK3CA, PTEN, FBXW7, AKT1 and STK11.
- The majority of tumors harboring RTK mutations did not have RAS or PI3K pathway mutations.



Conclusions

- This data can help to identify new predictive biomarkers and explore potential innovative treatment strategies.
- Molecular driver alteration analysis can provide guidance for many investigational drugs, currently available in enrolling trials. Knowledge of these individual molecular alterations may provide a strong rationale for treating patients with these investigational drugs, but as observed, coexistence of seemingly independent mutations may have a synergistic or detrimental influence on the efficacy of these drugs.
- Similar significant alterations between ADCs compared to SCCs and LCCs (except for ALK, BRAF and ROS1) were observed. ADCs and LPAs had significantly more KRAS mutations than SCC tumors. In contrast, SCCs had a significantly higher prevalence of PIK3CA and PTEN mutations. SCCs and LCCs also had a significantly higher number of TP53 mutations than ADCs.
- Intriguing treatment options can be found in between 0.8-1.1% of patients where off-compendium targeted agents, such as anti-HER2 targeted agents in NSCLC harboring HER2 protein overexpression, amplification or mutation, might represent a viable treatment option based on the presence of the same molecular alteration which direct the use of these drugs in other tumor types.

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