Update on Squamous Cell Carcinoma Predictive Biomarker Testing

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Outline

- Overview of lung squamous cell carcinoma (SqCC)
- Predictive biomarkers and biomarker testing in SqCC
- Lung-MAP protocol

Overview of SqCC

- The second most common histologic subtype of lung cancer accounting 85,000 new cases in the US each year and over 400,000 worldwide.
- The majority of SqCC patients are current or former heavy smokers, in contrast with adenocarcinoma, where a growing proportion are never-smokers or former light smokers.

Prevalence of somatic mutations across human cancer types



Predictive Biomarkers and Biomarker Testing in SqCC

Commonly Identified Alterations in Genomic Studies of Lung SqCC

enetic pathways and alterations	Prevalence	Clinical trials
TK amplification	>30% with EGFR and FGFRI most common	EGFR mAbs, FGFR TKIs, FGFR mAbs, FGFR ligand trap
TK mutations/fusions	Rare (<10% of cases), most common in FGFR2 and FGFR3 (FGFR3-TACC3), rare DDR2 mutations	FGFR TKIs, FGFR mAbs, FGFR ligand traps, dasatinib
AS	10%–20%, most commonly loss of NF1 or RASA1, RAS mutations rare	MEK and ERK inhibitors, direct RAS inhibitors
3K	Common ~50% alterations in PIK3A, PTEN, PIK3R1	PI3K and mTOR inhibitors
P53 and CDKN2A/RB1	Genomic loss in nearly all cases, amplification of CDK4/CDK6/CCND1 in CDKN24 intact tumors	CDK inhibitors?
xidative stress regulation	Common mutation of NFE2L2/KEAP1/CUL3 (25%)	PI3K inhibitors?
ifferentiation	Common loss of NOTCH1; TP63 and SOX2 gain	2
nmune evasion	Rare HLA and B2M mutations, <10%	Immune checkpoint inhibitors, vaccines
nnune evasion	Ram HLA and B2M mutations, <0%	immune checkpoint inhibitors, vaccines



FGFR1 amplification

Prevalent Actionable Molecular Alterations and Biomarker Testing in SqCC

- *FGFR1* amplification most extensively studied to date
- PIK3CA mutation & PTEN mutation/deletion some clinical trials specific for NSCLC are ongoing
- EGFR amplification/protein overexpression predictive role of EGFR expression levels for response to cetuximab seems to emerge from retrospective analyses, but the pharmaceutical company has decided to stop the cetuximab clinical development in NSCLC
- PDGFRA amplification/mutation not much information specific for PDGFRA alterations available
- DDR2 mutation a candidate for a predictive biomarker

FGF / FGFR Signaling

- Receptors: 4 FGFRs (FGFR1-4)
- Ligands: 18 FGFs

 The receptor-ligand combinations regulate a broad spectrum of signaling during development and in adult tissue homeostasis

• FGFR amplification or mutation in 26% of examined lung SqCCs (TCGA)

- FGFR1 amplifications (majority)
- FGFR2, FGFR3, FGFR4 mutations



ESEARCH ARTICLE

LUNG CANCER

Frequent and Focal FGFR1 Amplification Associates with Therapeutically Tractable FGFR1 Dependency in Squamous Cell Lung Cancer

- 155 SqCC of the lung
- 6.0 Affimetrix 6.0 SNP arrays

t5 of 155 (9.7%) with high-level
amplification (4 or more copies)

of FGFR1 • In cell line and xenograft

models, *FGFR1* amplification was associated with tumor growth and survival that were inhibited

by a FGFR inhibitor

• FGFR inhibitors may be a

viable therapeutic option for

patients w/ FGFR1 amplification



	n	Positive rate	Criteria for positivity
Weiss (2011)	153	22% (34/153)	Average # of FGFR signals per tumor cell nucleus > 9
Schildhaus (2012)	290	20% (58/290)	• FGFR1/CEP8 ≥ 2.0 • Average # of FGFR signals per tumor cell nucleus ≥ 6 • % of tumor cells containing ≥ 15 FGFR signals or large clusters ≥ 10
Heist (2012)	226	16% (37/226)	FGFR1/CEP8 <u>></u> 2.2
		Weiss Schild Heist	s J et al. Sci Transl Med 2010;2(62):62ra93 Jhaus H-U, et al. Modern Pathol 2012;25: 1473- SR, et al, J Thorac Oncol 2012;7: 1775-1780

High- and low-level *FGFR* amplification types in SqCC

- 420 lung cancers (307 SqCC, 100 Adeno CA, 13 others)
- FGFR1 amplifications (that were often focal) found in 20% of SqCC, but not in Adeno CA using a dual color FISH
- Heterogeneous and different patterns of gene copy number gain identified – stratified as high-level and low-level amplifications
- High-level amplification found in 16% and low-level amplification in 4%
- No difference in outcome of FGFR1 amplified and nonamplified lung cancer patients

Schildhaus H-U, et al. Modern Pathol 2012;25: 1473-80

Weiss J et al. Sci Transl Med 2010;2(62):62





High- and low-level *FGFR* amplification types in SqCC

High-level amplification:

- 1. FGFR1/CEP8 ratio is \geq 2.0
- Average # of FGFR1 signals per tumor cell nucleus is <u>></u> 6
- 3. % of tumor cells containing \geq 15 FGFR1 signals is \geq 10%

Low-level amplification:

1. % of tumor cells containing > 5 FGFR1 signals is \geq 50%

In the majority of cases with high-level amplifications, at least two criteria were fulfilled, and items 1 and 2 were most consistent criteria for FGFR1 amplification

		PGPRJ signals	conductivity 2:15 PGJTD: signatio	containing 2.5 FGPRs s(grad)	
w-level amplification	1.32	4.05	2	.55	
	0.97	5.12		6.2	
	0.58	4.67		5.5	
	1.99	5.50	2.	- 63	
	1.29	4.16		- 62	
	1.83	4.89		. 50	
	1.33	4.63		-53	
	- 141	9.00			
	1.41	3.27	2	74	
	1.23	10.100			
	1.54	5.75		1.000	
	1.51	4.92	÷ .	62	
ab-level amplification []	5.17	10.25	395	395	~
	1.65	0.10	2	65	
	.231	5.30		63	÷
	4.74	9.00	11	97	6 2
	1.02	6.17	3		
	2.58	2.60		- 88	4
	1.36	0.32			~
	2.66	7.55	18	79	
	2.85	9.10	11		50
	-2.12	0.30		07	58
	4.15	2,00	-	83	ŝ
		10.00			
	1.11	9.45	11		0
	12.88	19.02			2
	5.42	9.87	17	300	
	3.32	7.82	12	95	. 0
	6.42	11.75	33	300	
	6.44	18.38	23	22	
	5.95	13.66		. 95	n°*
	2,70	0.22		64	-
	3.99	6.67	19.	2.28	
	3.32	6.72	- 1	10	
	4.88	11.88			
		14.00	20		
	1000	12.22	12		1.2
		2.42			
	0.44	11.12	-		
	2044	2.35			
	2.17	11.07	42	95	
	3,39	7.63	5	92	
	3.54	7.43	14	85	
	2.30	13.10	68	88	
	2.11	5.40		70	-
	4.58	00.92	22	300	
	1.28	2.35	10		
	2.44	0.77		1.1.178	6.0
	6.13	93.00	29	200	
	-2.50	11.25	- 12	250	10
	0.75	95.50	70.	. 330	- 6
	7.11	1.30		47	9
	2.47	3.07		100	
	334	5.62		62	15
	5.5.6	11.77			- 9
	2.25	4.95		51	

FGFR FISH Scoring at MGH

- Only score tumor cells with amplification
- Score 50 cells but review few thousand (or as many as possible) tumor cells
- Indicate focality in the report if # of amplified tumor cells is limited compared to # of the entire tumor cells
- · Positive for amplification:
 - High-level amplification: FGFR1/CEP8
 <u>></u> 5
 - Low-level amplification: FGFR1/CEP8 = 2 < 5
 - Borderline for amplification: FGFR1/CEP = 1.8 < 2

Phase II clinical trials of FGFR targeted therapies in lung SqCC

Nintedanib	Phase I/II	SQCC	165	MTD/PFS	NCT01346540
	Phase II	SQCC	67	PFS	NCT01948141
Ponatinib	Phase II	All	40	ORR	NCT01761747
Dovitinib	Phase II	SQCC	27	ORR	NCT01861197
AZD4547	Phase II	SQCC	48	ORR	NCT01795768
MTD: Maximu	m tolerated dose, PFS: pro	gression-free surviva	al, ORR: objec	tive response rate	
			Filipits	w Curr Opin Ond	01 2014;26:152-1

Selective pan-FGFR Inhibitors BGJ398 AZD4547 JNJ-42756493 IC₅₀ (nM) IC₅₀ (nM) IC₅₀ (nM) FGFR1 4.55 <1 <1 FGFR2 28.1 <1 <1 FGFR3 2.52 1.05 19.5 FGFR4 376 40.6 <1 FGFR3 (G697C) 28.8 5.25 1.90 BGJ398 AZD4547

Best Overall Response of *FGFR1* – Amplified SqCC to BGJ398 (selective pan-FGFR inhibitor)

	100 mg (n=2)	125 mg (n=21)	150 mg (n=3)	≥ 100 mg (n=26)
Partial Response	1 (50.0)	3 (14.3)	0	4 (15.4)
Stable Disease	1 (50.0)	6 (28.6)	2 (66.7)	9 (34.6)
Progressive Disease	0	5 (23.8)	1 (33.3)	6 (23.1)
Unknown	0	7 (33.3)	0	7 (26.9)
40- 40- 40- 40- 40- 40- 40- 40-	Ill response of adv	vanced ALK-rearra Complete +	Inged NSCLC to partial response: 5 Stable disease: 3	crizotinib 7% (47/82) 3% (27/82)
-40- -80- -100	20 3	2 40 50		0 79

Response to pan-FGFR inhibitors is not as good as we would hope

- Concurrent alterations of other genes growth and survival driven by the other genes
- · Focality of amplification
- Lack of standardization of FGFR FISH scoring
- Amplification may not lead to protein (over-)expression





PI3K/Akt/mTOR signaling

PI3K/Akt/mTOR signaling

- Play a diverse role in normal physiologic and oncogenic processes
- Upon activated via receptor tyrosine kinases, PI3K activates AKT and downstream signaling via phosphorylation of PIP2, generating PIP3
- One of most frequently activated signaling pathways in cancer
- PTEN is an important tumor suppressor that antagonizes PI3K function, and loss of PTEN results in unrestrained signaling by the PI3K pathway



Alterations in PI3K/Akt/mTOR signaling are prevalent, but may be inclusive with other pathway alterations in SqCC EGFR ERBB2 ERBB3 FGFR1 FGFR2 FGFR3 9% 4% 2% 7% 3% 2% RASA1 4% HRAS NRAS STK11 2% AKT2 4% AKT3 16% KRAS NF1 11% 1 TSC1 3% TSC2 AMPK BRAF 4% MTOR Cases (%) 50 0 50 nactivated Activated Т Pn cell survival, tra Activation Inhibition Alteration patt RTK 269 RAS 249 PI(3)K 479 e 2012:489:519-2

Agents targeting the PI3K/AKT/mTOR pathway in phase II studies for NSCLC

Pan-P13K inhibitors		
Juparlisib (BKM120)	Oral pan-PI3K inhibitor that targets all 4 isoforms of Class I PI3K $(\alpha, \mu, \gamma, \delta)$	NCT01487285 Phase UII trial of erlotinib and buparlisib in patients with advanced NSCLC previously sensitive to erlotinib
		A phase II, open-label, 2-stage study of orally administered buparlisib in
		patients with metastatic NSCLC with activated PI3K pathway
Notilisib (GDC-0941)		NCT01483843 A phase II, double-blind, placebo-controlled, randomized study evaluating the safety and efficacy of carboplatin/pacitaxel and carboplatin/pacitaxel/ bevaciumsb with and without GDC-0041 in patients with previously untreated advanced or recurrent NSCLC
PX-866	An orally available nanomolar	NCT01204099
	pan-isoform wortmannin analog PUK inhibitor	 Phase [II] study of PX-866 and docetaxel in patients with sona tumors (phase II portion to determine the antitumor activity and safety of PX-866 in combination with docetaxel versus docetaxel alone in patients with NSCLC or head and neck squamous cell carcinomas [SCCHN])
Isoform-specific PDK inhibitors		
JNL719	An x isoform-selective inhibitor that inhibits wild-type, and the most common somatic mutants, of p110x	NCT01708161 • A phase lb/ll study of the combination of 8YL719 plus AMG 479 (ganitumab) in adult patients with selected solid tumors
ART inhibitors	20.007.000	
MK-2206	An aBosteric pan-AKT inhibitor	NCT01294306 • Phase II trial of the ART inhibitor MIK-2206 plus erlotinilb (OSE-774) in patients with advanced NSCLC who have progressed after previous response (including stable disease) with erlotinilb therapy
mTOR inhibitors		
Everolimus	A rapamycin derivative that targets mTOR	NCT01427946 • A phase I/II study of retaspimycin HCI (IPI-504) in combination with everol- imus in patients with KRMS-mutant NSCC
sirolimus -	An allosteric inhibitor of the mTORC1 complex	NCT01737302 • A phase II trial of combined protein kinase C iota (PKCiota) and mTOR inhi- bitions are maintenance therapy for patients with stage IV squamous histology NSCLC without progression following at least 4 cycles of first-line platinum
	·	containing CT
Jemsirolimus i	A rapamycin analog targeting mTOR	 A phase II study of neratinib and neratinib plus temsirolimus in patients with NSCLC carrying known HER2-activating mutations







Study	Antibody*	Scoring method	Results**
Spoerke 2012	CST clone 138G6	H scoring	H score 0: SqCC 21% (9/43), ADC 4% (2/56)
Scrima 2012	CST clone 138G6	3-tierd: 50%, 25-50%, 0-25%	0-25%: SqCC 55% (22/40), ADC 27% (14/51)
Trigka 2013	n/a	H scoring	SqCC (n=48) median 10(10-175), ADC (n=39) median 22.5 (0-180)
Camberbatch 2013	CST clone 138G6	4-tiered: 0-31)	Score 0: SqCC 34.7% (140/404), ADC 9.9% (65/656)
* CST: Cell Sigr adenocarcinom	naling Technolo na, 1) scoring ba	gy, ** SqCC: squ ased on intensity	amous cell carcinoma, ADC: and extent
oerke JM, et al. ClinCa oka EA. et al. Oncolo	ancer Res 2012;8:67 av Reports 2013:30:	71-83, ScrimaM,etal. I 623-636. Camberbatc	PLosOne2012;7:e30427 h et al. Clin Cancer Res 2013:20:595-6

DDR2 (Discoidin Domain Receptor 2)

- Interaction with its ligand collagen results in:
 - Recruitment of downstream adaptor proteins, kinases ad phosphatases including SHC, NCK1, SRC and SHP-2
 - Subsequent activation of downstream signaling pathways including MAPK and PI3K pathways
- DDR2 mutations have been reported in 3-4% of lung SqCC



ne LS, et al. J Thorac Oncol 2014;9: 900

DDR2 Mutation

- Sanger sequencing of multiple tyrosine kinases identified DDR2
 mutations in 11 (3.8%) of 290 SqCC samples (including 13 cell lines)
- SqCC cell lines harboring DDR2 mutations were selectively killed by knockdown of DDR2 by RNA interference or by treatment w/ dasatinib*
- Tumors established from a DDR2 mutant cell line were sensitive to dasatinib* in xenograft models
- Expression of mutant DDR2 led to cellular transformation that was blocked by dasatinib*

DDR2 mutation is likely a driver event in lung SqCC that can be targeted by dasatinib



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Clinical Sensitivity of *DDR2* – mutated Lung SqCC to Dasatinib

 Treatment with a combination of erlotinib and dasatinib lead to partial response in a patient with DDR2-mutated, EGFR wild type lung SqCC that had progressed despite carboplatin and paclitaxal chemotherapy







mmerman PS, et al. Cancer Discovery 2011;1:78ini V. et al. Lung Cancer 2013:82:171-2

Phase II clinical trials of DDR2 targeted therapies in lung SqCC

Dru	e g	Clinical development status	Histologic subtype	n	Primary endpoint	Trial identifier
Das	satinib	Phase II	SQCC	5	ORR	NCT01491633
		Phase II	SQCC	73	ORR	NCT01514864
		Phase II	All	37	ORR	NCT00787267
		Phase II	All	35	ORR/PFS	NCT00459342
	NCT01 adminis enrolled	491633 was halted stered at 140 mg/d d, and none of ther	d due to excess ay in the first 5 m showed DDF	s toxi 5 lung R2 m	cities of dasa sqCC paties utations in the	atinib nts e study

Filipits M Curr Opin Oncol 2014:26:152-158

Lung Master Protocol (Lung-MAP): A Biomarker Driven Protocol for Accelerating Development of Therapies for SqCC

Lung-MAP

- No approved targeted therapies specific to advanced lung SqCC currently available despite a significant number of somatic mutations/amplifications detected by TCGA project and similar studies
- Identifying and accruing biomarker-selected patients to clinical trials is challenging due to:
 - · Rarity of any putative oncogenic drivers in SqCC
 - Substantial time and tissue required for screening patients for solitary biomarker-drive studies with a low chance of enrollment
 - Impracticality of serial screening for individual biomarkers to determine eligibility for other trials in SqCC patients who have already progressed on standard therapy

Herbst RS, et al. Clin Cancer Res 2015;21:1514-24

Lung-MAP

• New strategies are essential for matching patients to therapies from which they are most likely to benefit

• The process requires efficient clinical trial designs for evaluating these therapies with rapid multibiomarker testing and accelerated drug development timelines

Herbst RS, et al. Clin Cancer Res 2015;21:1514-24



2015 PPS Biennial Meeting









Summary

- Although no established targeted therapy is currently available for patients with lung SqCC, potentially targetable molecular alterations have been identified by several studies
- Those molecular alteartions, including FGFR1 amplification, PI3K signaling pathway alterations, and DDR2 mutation, are under investigation in clinical trials
- Unfortunately, the clinical trials suffer from low response rates, likely due to significant overlap between multiple alterations and the lack of standardized biomarker testing
- Lung-MAP, a biomarker-driven multisubstudy protocol, may advance the efficient development of targeted therapies combined with predictive biomarker testing for lung SqCC



