Evaluation Of Small Cell Undifferentiated Lung Cancer By Next Generation Sequencing Reveals Frequent Consistent Genomic Alterations

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Abstract

Background: Although the response rate to standard chemotherapy for small cell undifferentiated lung carcinoma (SCLC) is high, the clinical outcome is poor with a 5 year overall survival rate of only 5%. As opposed to other types of primary lung cancer, most notably lung adenocarcinoma, well defined genomic alterations and opportunities for targeted therapy for SCLC have not, to date been identified. We hypothesized that comprehensive genomic profiling of clinical SCLC samples by NGS could identify genomic-derived drug targets of therapy for patients diagnosed with this aggressive malignancy in a single diagnostic test.

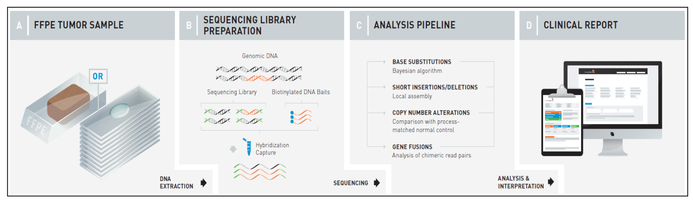
Methods: Hybridization capture of 3,769 exons from 236 cancer-related genes and 47 introns of 19 genes commonly rearranged in cancer (current version of the test) was applied to ≥ 50ng of DNA extracted from 49 SCLC FFPE tumor specimens and sequenced to high, uniform coverage. Genomic alterations (base substitutions, small indels, rearrangements, copy number alterations) were determined and then reported for these patient samples. Actionable GA were defined as those identifying anti-cancer drugs on the market or in registered clinical trials (CT).

Results: There were 31 female and 18 male SCLC patients with a median age 57.5 years (range 32-83 years). All tumors were high grade, and 21 tumors were stage IV, 24 stage III, 2 stage II and 2) stage 1 at time of sequencing. All 49 (100%) of SCLC had GA on NGS with A total of 149 GA were identified for an average of 3.04 GA per tumor with 100% of SCLC cases harboring at least one alteration. The most common non-actionable genomic alterations were alterations in *TP53* (71%), *RB1* (67%) and *MLL2* (16%). Twenty one (43%) of cases harbored at least 1 actionable GA including including mutation, amplification or homozygous deletion in *EGFR* (6%), *PIK3CA* (6%), *PTEN* (6%), *RICTOR* (6%), *TSC1* (6%) and *NOTCH1* (4%), *BRAF (2%)*, *BRCA2* (2%), *CCND1* (2%), *CCND3* (2%), *CCNE1* (2%), *CDKN2A* (2%), *CDK4* (2%), *FGFR1* (2%), *KRAS* (2%), *NF1* (2%) and *TSC2* (2%).

Conclusions: More than third of the patients harbored at least one actionable GA. The long tail of altered genes and multiple mechanisms of alteration necessitate broad diagnostic assays from limited biopsy material to maximize targeted therapeutic options in an individual patient Given the limited treatment options and poor prognosis of patients with SCLC, comprehensive NGS-based genomic profiling has the potential to identify new treatment paradigms and meet an unmet clinical need for this disease.

Design and Methods

- Hybridization capture of 3,769 exons from 236 cancer-related genes and 47 introns of 19 genes commonly rearranged in cancer
- ≥ 50ng of DNA extracted from 49 SCLC FFPE specimens
- Samples were sequenced to high (average 833X), uniform coverage
- Genomic alterations (base substitutions, small indels, rearrangements and copy number alterations) were determined and then reported for these patient samples
- Actionable GA were defined as those identifying anti-cancer drugs on the market or in registered clinical trials (CT).



Results

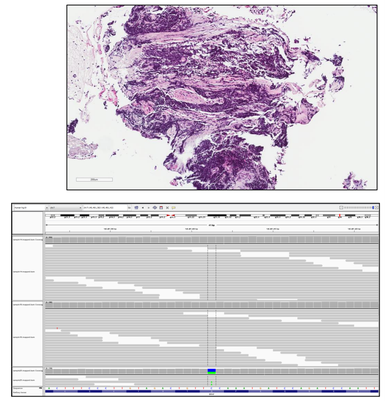
- 31 female and 18 male SCLC patients
- Median age 57.5 years (range 32-83 years)
- All tumors were high grade
- 21 tumors were stage IV, 24 stage III, 2 stage II and 2) stage 1 at time of sequencing
- All 49 (100%) of SCLC had GA on NGS with A total of 149 GA were identified for an average of 3.04 GA per tumor
- 100% of SCLC cases harboring at least one alteration
- The most common non-actionable genomic alterations were alterations in *TP53* (71%), *RB1* (67%) and *MLL2* (16%)
- 21(43%) of cases harbored at least 1 actionable GA including including mutation, amplification or homozygous deletion in *EGFR* (6%), *PIK3CA* (6%), *PTEN* (6%), *RICTOR* (6%), *TSC1* (6%) and *NOTCH1* (4%), *BRAF (2%)*, *BRCA2* (2%), *CCND1* (2%), *CCND3* (2%), *CCNE1* (2%), *CDKN2A* (2%), *CDK4* (2%), *FGFR1* (2%), *KRAS* (2%), *NF1* (2%) and *TSC2* (2%)

Genomic Alterations in 49 Cases of Small Cell Undifferentiated Lung Cancer

Case Number	Gender	Age	Specimen used for NGS	Tumor Stage at time of NGS	Total Genomic Alterations	Therapies with potential benefits	Therapies with lack of response	Clinical trials	Genomic Alterations
1	m	70	Lung	III	3	0	0	2	MYC; RB1; TP53
2	m	55	Lymph node	III	2	0	0	0	MEN1; RB1
3	f	0	Lymph node	III	4	0	0	6	CCNE1; CDKN2A; RB1; TP53
4	m	58	Lung	III	2	2	0	2	PTEN; RB1
5	f	0	Lymph node	III	1	0	0	0	TP53
6	m	67	Liver	IV	4	4	0	2	EFGR; PTPRD; RB1; TP53
7	m	0	Lung	III	4	0	0	4	MYC; RB1; RICTOR; TP53
8	f	49	Lung	III	1	0	0	0	RB1
9	f	59	Lung	III	2	2	0	2	NF1; RB1
10	f	55	Mediastinum	III	3	0	0	4	RB1; SOX2; TP53;
11	m	83	Lymph node	III	2	0	0	0	RB1; TP53
12	f		Mediastinum	III	3	0	0	2	BRCA2;
13	m	49	Lymph node	III	2	0	0	0	RB1; TP53
14	f	73	Liver	IV	3	2	0	2	ARID1A; PTEN; TP53
15	m	37	Lymph node	III	2	0	0	0	MEN1; RB1
16	f	54	Brain	IV	1	0	0	0	TP53
17	m	75	Lung	III	4	0	0	1	KDM6A; MLL2; RB1; TP53
18	f	71	Lymph node	IV	4	0	0	3	MYCL1; RB1; SPEN; TP53
19	f	54	Lung	III	1	0	0	1	TP53
20	f	68	Liver	IV	9	5	0	7	FGF10; FGFR1; NFEL2; RB1; RICTOR; TP53; TSC2; ZNF703
21	f	51	Lymph node	III	5	7	0	2	CDKN2A; EGFR; PIK3CA; RB1; TP53
22	f	50	Pleural fluid	IV	1	0	0	2	MYCL1
23	m	68	Lung	I	2	0	0	0	MLL2; RB1
24	f	32	Chest wall	IV	1	0	0	0	RB1
25	m	57	Mediastinum	III	1	0	0	0	MEN1
26	f	52	Lymph node	III	2	0	0	2	RB1; SPEN
27	m	52	Trachea	III	1	0	0	0	RB1
28	f	68	Lymph node	IV	3	0	0	2	CSF1R; RB1; TP53
29	m	68	Lung	IV	3	0	0	1	LRP1B; RB1; TP53
30	m	60	Bone	IV	3	0	0	4	ARID2; CCND1; CDK4
31	f	57	Liver	IV	2	0	0	1	CDS1R; TP53
32	f	66	Pleural biopsy	IV	4	3	0	3	BRAF; MLL2; RB1; TP53
33	m	78	Small Intestine	IV	3	2	0	5	MCL1; PTEN; TP53
34	m	69	Lung	IV	3	0	0	1	APC; MYST3; TP53
35	f	82	Lung	IV	4	0	0	5	BRCA2; NOTCH1; RICTOR; TP53
36	m	46	Lung	III	3	2	0	3	RB1; TP53; TSC1
37	f	33	Brain	IV	3	1	0	4	BRCA2; KRAS; MLL2
38	f	52	Lung	III	3	0	0	1	ATRX; MLL@; TP53
39	f	55	Brain	IV	2	0	0	1	RB1; TP53
40	f	71	Pleura	IV	9	8	0	13	BRIP1; CCND3; EGFR; IRS2; MYC; PIK3CA; RB1; SOX2; TP53
41	f	51	Lymph node	III	5	0	0	3	MLL2; MYCN; RB1; STAG2; TP53
42	m	58	Lumph node	III	1	0	0	1	TP53
43	f	66	Lymph node	IV	3	0	0	1	MAP3K; RB1; TP53
44	f	69	Lymph node	III	4	0	0	1	MLL2; RB1; SPEN; TP53
45	f	46	Lung	II	2	0	0	1	RB1; TP53
46	f	68	Lung	IV	4	8	0	7	MYC; MYCN; TP53; VHL
47	f	61	Lung	I	4	0	0	1	EPHA3; MLL2; RB1; TP53
48	f	47	Lung	II	5	2	0	3	CREBBP; RB1; TP53; TSC1
49	f	59	Lung	IV	6	2	0	5	EPHA3; EPHB1; NOTCH1; PIK3CA; SOX2; TP53

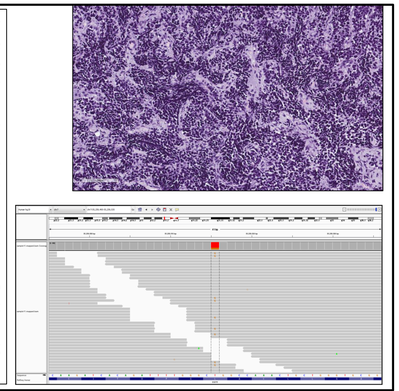
Case Examples

Case 32. Pleural biopsy of SCLC in a 66 year old female with alterations in *BRAF*; *MLL2*; *RB1*; *TP53*. Activating Braf mutations such as G469A and G469E exhibit increased Braf kinase activity, although less so than the increased kinase activity exhibited by Braf V600E, causing hyperactivation of the downstream MEK/ERK/MAPK pathway and may confer sensitivity to Braf inhibitors, including sorafenib and regorafenib, and/or MEK inhibitors (Flaherty et al. 2012).



BRAF G469A mutation (Integrated Genomics Viewer, Broad Institute Cambridge, MA

Case 40. SCLC invading the pleura in a 71 year old woman. In addition to the L858R base substitution in the EGFR gene there were alterations in *BRIP1; CCND3; IRS2; MYC; PIK3CA; RB1; SOX2;* and *TP53. EGFR* L858R is a missense mutation within the protein kinase domain of the EGFR protein and has been shown to activate the tyrosine kinase activity which can confer sensitivity to tyrosine kinase inhibitors such as erlotinib and gefitinib (Lynch et al 2004). Activating mutations in *E GFR* have been reported in 2-5% of small cell lung carcinomas (SCLCs) (Shiao et al.2011). However, in a study of 19 patients with SCLC, gefitinib treatment was not found to have a benefit (Moore et al.2006). Second generation, irreversible EGFR inhibitors are currently being tested in clinical trials for *EGFR* mutated SCLC.



Conclusions

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- The long tail of altered genes and multiple mechanisms of alteration necessitate broad diagnostic assays from limited biopsy material to maximize targeted therapeutic options in an individual patient
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References

Frampton GM, Fichtenholtz A, Otto GA et al. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. Nat Biotechnol. 2013;31(11):1023-31.