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Transformation to small cell lung cancer as a mechanism of resistance to immunotherapy in non-small cell lung cancer

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ABSTRACT

Objectives: Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related death world-wide. Immune checkpoint inhibitors (ICI) have become the most promising type of treatment in oncology in general, and significantly so in NSCLC. Limited data is available about mechanisms of primary resistance. Data is lacking about mechanisms involved in acquired resistance or mixed responses in NSCLC. We aimed to identify mechanisms of resistance by studying biopsies taken from sites of secondary progression.

Materials and methods: We identified all cases of NSCLC that have received ICI for advanced disease in our institute. Of these cases, those that have demonstrated acquired resistance or mixed responses, and have underwent a biopsy from a progressive lesion were analyzed. Selected specimens were subjected to next-generation sequencing (NGS; Oncomine™ Solid Tumour Fusion Transcript Kit).

Results: Out of 664 lung cancer cases, 249 were NSCLC that have received ICI. Of these, eight cases matched our search criteria. Two of them demonstrated transformation to small cell lung cancer (SCLC; 2/8, 25%). NGS verified a common origin to a matched pre-treatment NSCLC specimen and an on-treatment progressive SCLC specimen. In two cases no tumor cells were found and in the remaining four the pathology was similar to the initial biopsy. In one of the cases of SCLC transformation platinum-etoposide chemotherapy was administered, with short-term benefit only and further disease progression.

Conclusion: Mechanisms of acquired resistance to ICI include SCLC transformation. Repeat biopsies of progressing lesions after initial response or in cases of mixed response can shed light on mechanisms of resistance.

1. Introduction

Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related death world-wide [1]. Recent years have witnessed tremendous improvements in the treatment of this devastating disease, through targeting of driver genes and the use of immunotherapeutic drugs [2]. Immunotherapy, and specifically immune-check point inhibitors (ICI), has become a new pillar among the therapeutic options in Oncology, particularly being relevant in NSCLC. Although not effective in the majority of cancer patients, immunotherapy can induce profound and prolonged tumor regressions [2,3]. Various molecular and biologic mechanisms of primary resistance are recognized, including cancer-cell intrinsic mechanisms such as expression of inhibitory co-receptors (T-cell immunoreceptor with Ig and ITIM domains (TIGIT), Lymphocyte-activation gene 3 (LAG-3), and others) or loss of antigen presentation machinery (e.g. beta-2-microglobulin (B2M)) [4]. Tumor-extrinsic,

Abbreviations: NSCLC, non-small cell lung cancer; NGS, next-generation sequencing; IO, Immunotherapy; SCLC, small cell lung cancer; C.I., confidence interval; TIGIT, T-cell immunoreceptor with Ig and ITIM domains; LAG-3, lymphocyte-activation gene 3; MANA, mutations associated neo-antigens; B2M, beta-2-microglobulin; Pt, patient; y, years; Tx, treatment; Nivo, nivolumab; Pembro, pembrolizumab; Chemo, chemotherapy; M, man; F, woman; PR, partial response; PD, progressive disease; CE, carboplatin etoposide; CG, carboplatin gemcitabin; CPem, carboplatin pemetrexed; Pem, pemetrexed; CPac, carboplatin paclitaxel; CV, cisplatin vinorelbine; Doc, docetaxel; Gef, gefitinib; Erl, erlotinib; Nint, nintedanib; Key, pembrolizumab; XRT, radiotherapy; SOB, shortness of breath; RLL, right lower lobe; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor

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micro-environmental mechanisms of resistance include infiltration of suppressor cell populations such as T-regulatory cells or type-2 macrophages, or the lack of infiltrating cytotoxic T cells within the tumor mass [5]. Various approaches are currently tested aiming to enhance ICI efficacy, mostly by combination therapies, either with chemotherapy [6] or by combining different immunotherapy drugs that target non-overlapping immune-evasion mechanisms [7].

Although ICI responders benefit from sustained responses, with a significantly long duration of response, many of them eventually progress. Acquired resistance is heterogeneous and not well characterized [8]. Mixed responses, a situation where some of the tumor masses shrink under treatment while other parts grow, is another clinical scenario with minimal detailed analyses being reported. The mechanisms of acquired resistance or mixed responses are poorly documented, probably partly overlapping with primary resistance. Lack of targets for immune cells may be the cause of initial resistance to ICI in cancers with a low mutation burden [9,10]. A study analyzing mutations associated neo-antigens (MANA) in secondary resistance samples of NSCLC identified loss of MANA as a probable mechanism of acquired resistance in some patients [11]. Somatic loss of the B2M gene leading to impaired antigen presentation, a recognized ICI primary resistance mechanism in melanoma [12], was found in a NSCLC with acquired resistance to ICI [13]. At the moment, the approach to acquired resistance of NSCLC is based on analyses of very small sets of data.

We speculated that histologic analysis of acquired resistance or mixed response NSCLC cases can provide valuable information regarding mechanisms of acquired resistance and suggest possible ways to treat these conditions. We aimed to focus on scenarios where a clear benefit of immunotherapy was either seen previously or is concurrently seen in other parts of the tumor. Thus, retrospectively analyzed biopsies included in this study were either from acquired resistance cases or mixed responses to immunotherapy. The approach taken to identify the cases of interest included detailed examination of a single institute entire clinical working dataset, thus assuring valid representation of the relevant patient population.

2. Materials and methods

The Thoracic Oncology Unit at the Sheba Institute of Oncology has a working database in which all evaluated patients are routinely registered along with demographics, pathologic diagnosis, stage and treatment. Cases for this study were identified from this database for patients diagnosed and treated from April 2015, when immunotherapy entered routine clinical practice, till the end of December 2017 (data cutoff for this analysis; Fig. 1). Data was retrieved from clinical charts and pathology reports by a manual chart review, covering initially all NSCLC patients treated with immunotherapy. Response assessment was based on treating physicians' evaluation of clinical and radiological data, as documented in the charts. The inclusion criteria were NSCLC patients treated by immunotherapy, that developed either acquired resistance or a mixed response to immunotherapy and the availability of a pathology evaluation of a biopsy from a progressing lesion during or shortly after immunotherapy treatments. A review of the imaging studies was performed. Cutoff of clinical data update was 1st February 2018. As for an exploratory cross-sectional study, no prior sample size calculation was performed.

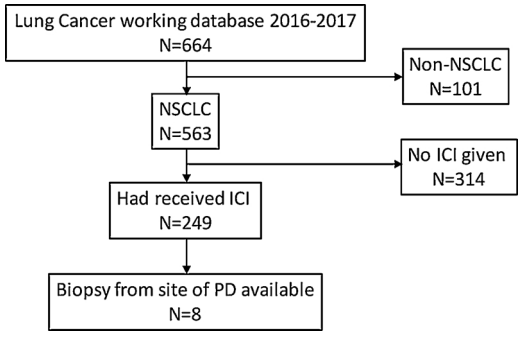

Fig. 1. Consort diagram of the patients included in this study.

Table 1
Patients' characteristics, regarding the cohort of NSCLC patients treated with immunotherapy.

Total number of patients	249
Age (years) - median (range)	66 (35-89)
Sex - men N (%)	159 (63.9)
Smoking N (%)	203 (81.5)
Histology N (%)	
NSCLC - Adenocarcinoma	164 (65.9)
NSCLC - Squamous cell carcinoma	59 (23.7)
NSCLC - NOS	24 (9.6)
NSCLC – Adeno-Squamous cell carcinoma	2 (0.8)
Immunotherapy treatment N (%)	
Nivolumab	160 (64.3)
Pembrolizumab	78 (31.3)
Atezolizumab	6 (2.4)
CPI plus CPI*	3 (1.2)
Durvalumab	2 (0.8)
Treatment line of ICI N(%)	
First	83 (33.3)
Second	134 (53.8)
Third or higher	32 (12.9)
Best response N(%)	
Progressive disease	134 (53.8)
Stable disease	58 (23.3)
Partial response	57 (22.9)

NOS: non otherwise specified, ICI: immune checkpoint inhibitor.
* Clinical trials including anti-PD-L1/PD-1 with anti-CTLA-4/placebo treatments.

Genetic testing

Oncomine™ Solid Tumour Fusion Transcript Kit (ThermoFisher scientific, Catalog number AB-A26762) was used as per manufacturer's protocol, read on Ion torrent S5 Sequencer.

3. Results

3.1. Identification and characterization of cases

A total of 249 NSCLC cases that received ICI were identified from our working database for patients diagnosed during 2015–2017 (Fig. 1). Table 1 demonstrates the main characteristics of this cohort. Most patients received nivolumab, most commonly as second line. The most common best response was progressive disease. Median progression free survival of this cohort was 3.0 months (95% confidence interval (C.I.) 2.4–3.6), median overall survival was 8.0 months (95% C.I. 5.3–10.7). Manual chart review of these 249 cases led to the identification of eight cases that fit our inclusion criteria. Patients' characteristics, treatment details and biopsy results are outlined for each of these eight patients in Fig. 2 and in Table 2.

In eight all cases, the indication for the biopsy procedure was to direct therapeutic decisions regarding continuing immunotherapy or switching to a different treatment line. All eight cases had received prior immunotherapy as the treatment line immediately prior to the resistant-lesion biopsy which is the focus of this study. All but one of the cases had received also chemotherapy as a previous treatment line. Two patients were on clinical trials testing combination chemotherapy with immunotherapy. One of these two (#4) was on a non-blinded assignment to chemotherapy alone (Carboplatin and pemetrexed), prior to starting second-line nivolumab (Bristol-Myers Squibb, NY, NY, USA). The other (#8) was on a double-blind study, thus might have been on

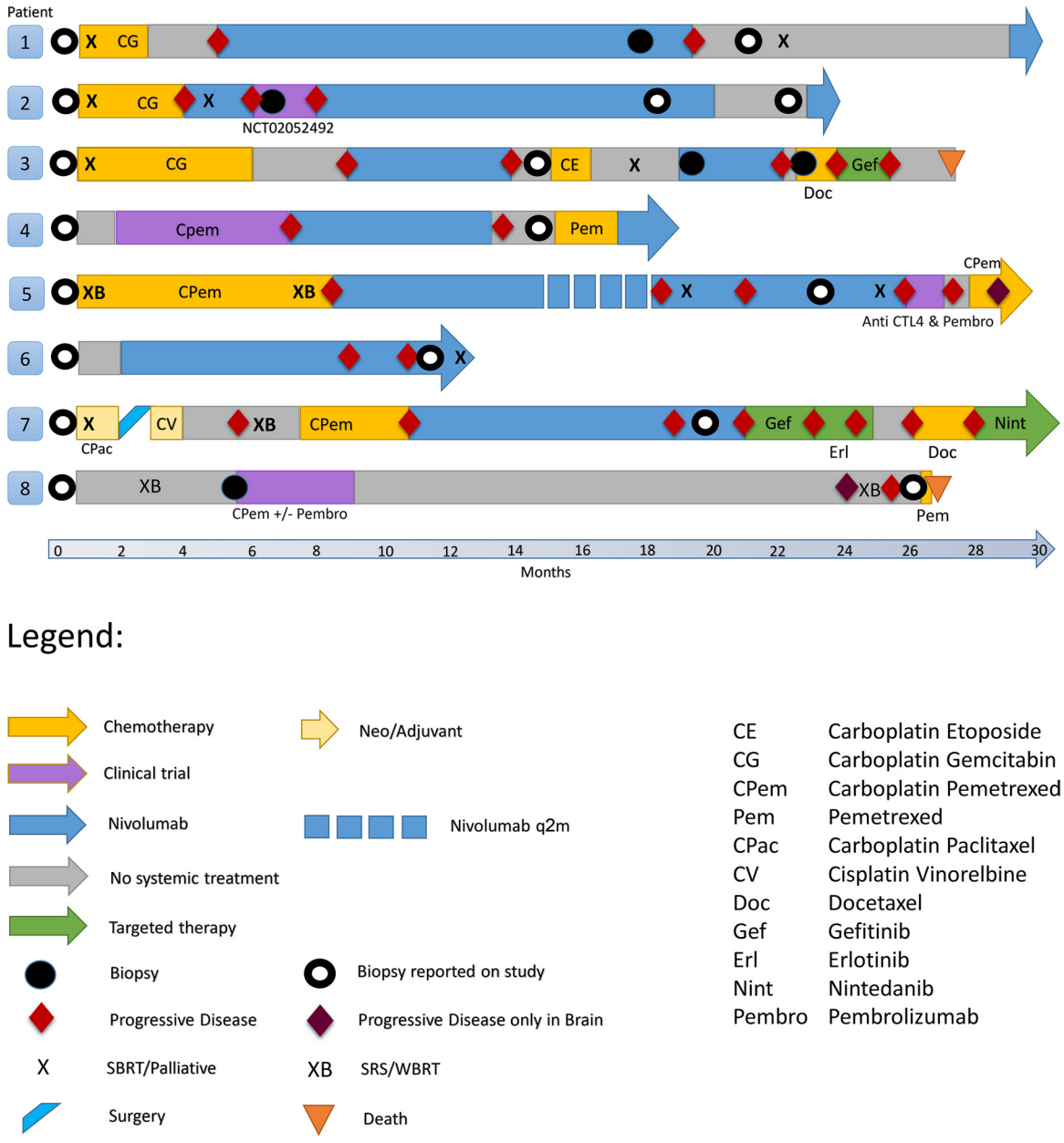

Fig. 2. Timeline of disease course and treatment of the eight patients that underwent repeat biopsy.

placebo with chemotherapy alone. However, this patient developed on the trial grade 3 nephritis that responded to high-dose steroid treatment, highly suggestive of ICI toxicity. One patient (#2, details below), who was one of the two patients that demonstrated SCLC transformation, was also on a phase I single arm clinical trial testing modified Vitamin D binding protein macrophage activator as immunotherapy (NCT02052492).

Surprisingly, two of the eight cases of this study (25%) demonstrated transformation to small cell lung cancer. In two additional cases (25%) no tumor cells were found, suggesting these were examples of the recognized although rare pseudo-progression phenomenon [14]. In the rest the cases (four of eight, 50%), pathology of the progressing lesion was essentially similar to the initial biopsy, besides one of these cases where the histologic description changed from adenocarcinoma to undifferentiated tumor (compatible with carcinoma). The detailed clinical course of both cases of SCLC transformation as well as molecular profiling of the different tumor specimens are described below.

3.2. SCLC transformation cases description

Patient #2: 70-year-old women, diagnosed in July 2015 with metastatic squamous NSCLC. Pathology from a left lung biopsy was of small to moderate sized cells summarized as compatible with squamous cell carcinoma (positive for p40 and CK5/6, negative for TTF1, focally positive for CK56, KI67 positive in 70–80% of cells). Medical history was notable for breast cancer (infiltrating ductal carcinoma, T1, N0, M0) resected at 2013, treated with adjuvant radiotherapy (XRT) and letrozole.

The patient presented with a left lung mass, left adrenal mass and a lytic lesion in vertebra D5. She received palliative XRT to the D5 lesion in July 2015. Following, she was treated with carboplatin-gemcitabine combination starting in August 2015, with progressive disease (PD) seen after 5 cycles. In December 2015 she started nivolumab treatment. At the end of January 2016, after 3 cycles of nivolumab, presented to emergency room with shortness of breath (SOB), imaging demonstrated PD at several sites. She received a single dose of XRT to left lung hilum

Table 2
Patients with acquired resistance or mixed response to immunotherapy.

Pt	Age	Sex	Smoking status	First Biopsy	Tx lines before ICI	ICI	Initial response to ICI	Time on ICI till Biopsy (Mo)	Site of PD biopsied	2nd biopsy	Change Tx due to biopsy
1	69	M	Current	SCCa	1	Nivo	PR	11	Lung	SCCa	No
2	70	F	Current	SCCa	1	Nivo	Pseudo PD	16	Adrenal	SCLC (negative for SCCa /basaloid markers,CD56+, TTF1 +)	No
3	75	M	Past (> 10 y)	SCCa	1	Nivo	PR	6	Lung	SCLC (CD56+, synaptophysin+, focally chromogranin +, TTF1 +, KI67 80%)	Yes
4	68	M	Past (1-10 y)	AdenoCa	1	Nivo	Mixed Response	7	Lung	Loose fibrotic tissue with prominent chronic inflammatory infiltrate. No tumor identified	No
5	43	F	Never	AdenoCa	1	Nivo	PR	18	Rib	Fat necrosis	No
6	61	M	Current	AdenoCa	0	Pembro	PR	8	Lung	Necrotizing undifferentiated tumor composed sheets of medium to large sized cells with pleomorphic hyperchromatic nuclei, morphologically compatible with Ca (TTF1-, CK5/6-, and MNF116-)	Yes
7	63	M	Past (1-10 y)	AdenoCa	2	Nivo	PR	9	Adrenal	AdenoCa of lung (CK7 +, TTF1 +, napsin +, CK20-, p40-, GATA3- and CK5/6-)	Yes
8	74	F	Current	AdenoCa	1	Pembro/ placebo &, Chemo	PR	20	Breast	AdenoCa with pleomorphic tumor cells. (TTF1 +, Napsin A + focal weak. GCDFP15-)	Yes

& Patient #8 was on a clinical trial, see text for details. Pt: patient, ICI: immune checkpoint inhibitor, Tx: treatment, Ca: carcinoma. AdenoCa: adenocarcinoma. SCCa: squamous cell carcinoma. M: man, F: woman, Nivo: nivolumab, Pembro: pembrolizumab, PR: partial response, PD: progressive disease.

Fig. 3. Representative imaging of patients with SCLC transformation. A: planar scan of FDG uptake of patient #2 at the time of left adrenal progressive disease on nivolumab. B: Axial CT scan of patient #3 demonstrating the RLL mass that was growing (upper panels) while all other lesions (lower panels demonstrate shrinking mediastinal nodes) were responding to nivolumab treatment. Arrows indicate the lesions of interest.

followed by gradual resolution of SOB. The patient started treatment on clinical trial (NCT02052492). Biopsy at study entry demonstrated fibrotic-inflammatory tissue, while the scan at the biopsy procedure demonstrated shrinkage of the adrenal mass. In June 2016 PD in left adrenal and neck nodes was observed and the patient restarted nivolumab. Imaging revealed SD and treatment with nivolumab continued. CT-PET in April 2017 demonstrated uptake only in left adrenal (Fig. 3a). Biopsy from that site demonstrated SCLC (Fig. 4a). In June 2017 nivolumab treatment was stopped. In July 2017 evidence of pneumonitis lead to high dose steroids treatment with improvement. In November 2017 the patient underwent left adrenalectomy. Pathology of resected adrenal demonstrated mixed neuroendocrine and squamous carcinoma, involved margins. In December 2017 CT demonstrated evidence of local recurrence, the patient started nivolumab again, in parallel to steroid treatment. Last follow up and imaging in January 2018 revealed stable disease. The patient was lost to follow-up afterwards.

Molecular characterization was performed on the tumor sample taken at diagnosis and on the biopsy specimen demonstrating SCLC transformation. This analysis identified the same two TP53 mutations (Arg249Ser and Arg196Ter) in 38% and 38.7% (respectively) of the reads in the earlier biopsy, and 32% and 44% of the later biopsy. No additional genetic variants were called as significant in these

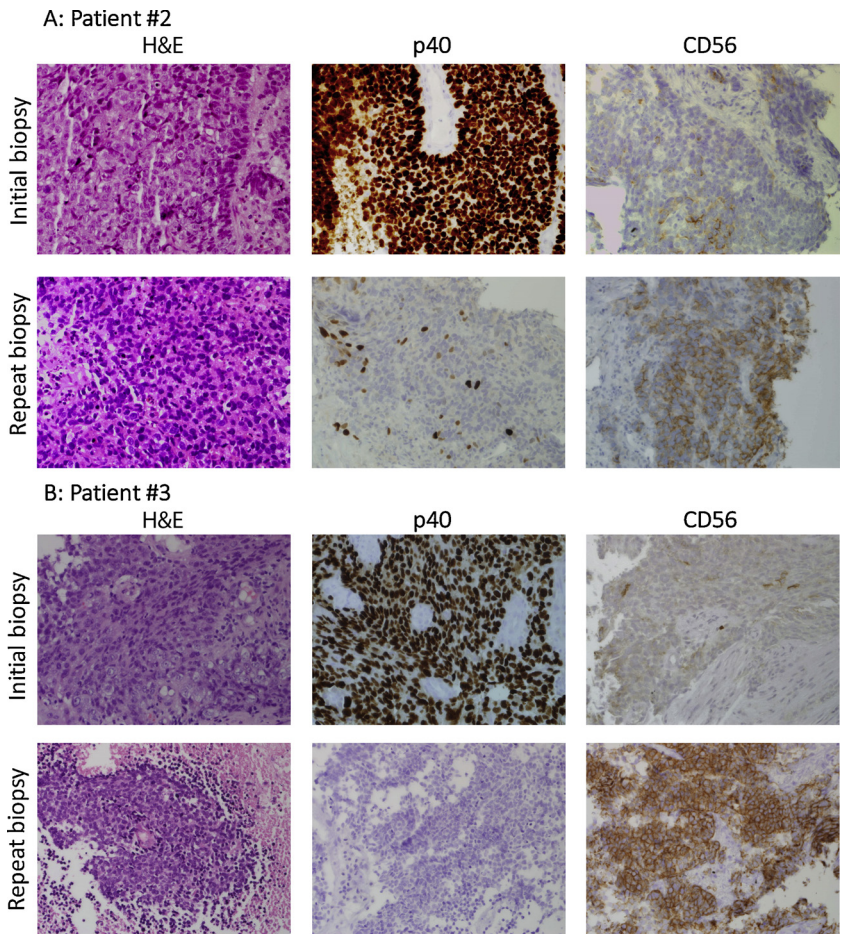

Fig. 4. Representative pathologic micrographs of A; patient #2 and B; patient #3 biopsies. For each patient the upper panel represents biopsy taken at diagnosis of the disease, the lower panel represents the biopsy taken on IO (timing specified on Fig. 2). Shown are hematoxylin and eosin (H&E), p40 and CD56 as depicted. All microphotos were taken at X400 magnification.

specimens.

Patient #3: 75-year-old man, with a squamous cell NSCLC diagnosed in April 2015. Pathology from a right lung biopsy was of small to medium sized cells, strongly positive for p63, CK903, focally weakly positive for CD56, negative for TTF1, synaptophysin and chromogranin, KI67 was positive in 80–90% of the cells, consistent with non-keratinizing squamous cell carcinoma with basaloid features. Past medical history is notable for non-muscle invasive bladder carcinoma, treated by TURT in 2012, HTN and hyperlipidemia.

The patient presented with a right lung mass occluding right lower lobe (RLL) bronchus, mediastinal nodes and a cervical vertebra lesion. He received palliative XRT to the mediastinal lesion. In July 2015 started carboplatin-gemcitabine, which was stopped in December 2015. In March 2016 PD was seen, and nivolumab treatment was initiated. In June 2016 partial response was seen, with all lesions shrinking besides a single lesion in RLL that slightly grew (Fig. 3b). In August 2016 nivolumab treatment was stopped due to suspected pneumonitis and the patient started treatment with prednisone (40 mg qd). In October 2016 a CT-guided biopsy was done from the growing RLL lesion. Pathology report was of small cell carcinoma, diffusely positive for CD56 and synaptophysin, focally for chromogranin and TTF1, negative for CK5/6, KI67 positive in about 80% of cells (Fig. 4b). In November 2016 started carboplatin-etoposide was initiated. Initial shrinkage of lung mass was seen followed by fast regrowth. In January 2017 the patient received XRT to the RLL mass (50 Gray in 5 fractions). CT scan at February 2017 demonstrated near resolution of the RLL mass, but growth of lesions at right lung hilum and sub-carina nodes. Nivolumab treatment was restarted in April 2017. In April 2017 a biopsy from the RLL lesion demonstrated again small cell carcinoma. The lesion was diffusely positive for CD56, synaptophysin, NMF116 and TTF1 (weak), negative for CK5/6, CD45 and chromorgranin, KI67 was positive in 95% of cells. The patient continued nivolumab treatment. A May 2017 CT demonstrated further PD. In June 2017 a bronchoscopic biopsy was performed from the RUL, demonstrating fibrosis and inflammation. The patient started docetaxel at June 2017 with significant toxicity, stopped treatment at July. In August he started gefitinib, and in September stopped treatment due to PD. The patient died in November 2017.

Molecular analysis was performed on the diagnostic biopsy of this patient as well as on one of the biopsies showing SCLC pathology. It should be noted that molecular analysis could not be performed on the first SCLC sample, taken at October 2016 due to lack of material. Thus, the analysis was done on the April 2017 sample, which was acquired after chemotherapy and radiotherapy treatments. Unlike the results for patient #2, there was no similarity in the mutations found, although both in the earlier as well as in the later biopsy, TP53 mutations were found. Specifically, in the earlier biopsy, two TP53 mutations, at Asn131fs (in 14.4% of the reads), and at Pro177Ser TP53 mutation (2.8% of the reads) were found, as well as a mutation in FBXW7 gene (Arg441Phe site in 18.7% of the reads). In the later SCLC only a Cys238Phe mutation of TP53 (71% of the reads) was found.

4. Discussion

We report here for the first time, to the best of our knowledge, a proof of transformation to SCLC as a mechanism of NSCLC resistance to immunotherapy. The initial dataset analyzed was of considerate size of 249 ICI-treated NSCLC cases, with typical characteristics of advanced patients on second-line immunotherapy, the common scenario of ICI treatment in the period covered by this study. However, the final number of eight analyzed biopsies of acquired resistance or mixed response cases is small. This reflects the relative low response rate to ICI as 2nd line treatment, as well as the low rate of repeat biopsies

performed during the management of metastatic lung cancer patients. In two of these eight cases, histologic transformation from NSCLC to SCLC is suggested. In one of these, molecular profiling provides a definitive proof of such transformation as a mechanism of resistance to ICI. We cannot conclude from this small set the prevalence of this mechanism. In addition to our report, one case report has been published providing histologic evidence supportive of such transformation during ICI therapy, similarly to the cases we have described [15], although lacking the molecular fingerprinting proof that we have provided. Physicians should be made aware of the possibility of SCLC transformation as a mechanism of escape from ICI to allow further study of such cases.

The lung cancers that we have identified to evolve from NSCLC to SCLC under immunotherapy might have been mixed NSCLC-SCLC tumors to begin with. Indeed, the original pathology report of both cases demonstrated neuroendocrine features. Both cases were summarized by expert Lung-Pathologists (E.O. and M.P.) as compatible with NSCLC, despite morphology of small-to-medium cells. Both cases were initially compatible with squamous cell carcinomas. Resection of a large tumor specimen was performed for one of these patients (patient #2) later on, confirming a mixed neuroendocrine and squamous carcinoma. Notably, for patient #2, molecular fingerprinting prove similarity between the NSCLC and the SCLC specimens. For patient #3, the difference in the identified mutations can support the existence of two different histologies at diagnosis, although changes related to genotoxic treatment might have been the cause of the different mutations found. Notwithstanding the possibility of a mixed tumor as the cause of the apparent NSCLC to SCLC transformation, our observations remains of high value for clinicians treating NSCLC patients with immunotherapy.

Pathologic transformation from adenocarcinoma to neuroendocrine carcinoma is well documented as a mechanism of resistance in epidermal growth factor receptor (EGFR) mutation positive NSCLC under EGFR-tyrosine kinase inhibitor (TKI) treatment [16–19]. In addition, SCLC-transformation is recognized as a resistance-related phenomenon in hormonal-therapy treated prostate cancer [20,21]. Prostate cancer small cell or neuroendocrine transformation is mostly seen as a late mechanisms of resistance, proceeded by aberrations in the androgen signaling pathway [22]. Further studies are required to understand the implication of SCLC histology as a potential common pathway of resistance, as well to look for evidence of such transformation in larger sets of lung cancer patients on various treatments.

Transformation of adenocarcinoma type of NSCLC into SCLC has been reported also without a selective pressure of EGFR-TKIs [23] suggestive of a common lineage to these cancers. Indeed, some data suggests that alveolar type II cells, thought to be the origin of adenocarcinoma have the potential to develop to SCLC [24]. Most reports about lung cancer NSCLC transformation to SCLC relate to EGFR mutation bearing adenocarcinoma cases. However, this might be biased by the higher rate of repeat biopsies performed at progression of EGFR mutation positive NSCLC, which are almost always adenocarcinomas. Several case series report of combined SCLC-NSCLC tumors in a notable minority (2–15% in different series) of untreated or treated lung cancers [16,25,26]. The occurrence of SCLC-NSCLC combined cases support the idea of a common linage origin of these cancers. Some of these reports detail the histology of the NSCLC component of the combined cases, including both adenocarcinomas as well as squamous cell cancers. In our series, both cases of SCLC transformation originated from squamous cell carcinomas.

Considering the possibility that the cases eventually found to transform to SCLC might had a mixed histology to begin with, a differential sensitivity of NSCLC and SCLC to ICI might underlie the clinical phenomena we describe. The SCLC-transformation cases described in our series might be individual examples of a general phenomenon of loss of MANA or other tumor-cell dependent elements of the immune response as a mechanism of resistance to ICI [11,13]. From the clinical trials performed so far, the efficacy of ICI seems limited in SCLC in comparison to NSCLC [27,28], possibly supportive of SCLC transformation as a mechanism of escape from immunotherapy. The molecular mechanism underlying this difference may relate to lower PD-L1 expression levels, lower cytotoxic T cell infiltration or enhanced presence of suppressor cells [29,30]. Further studies evaluating the pathology of ICI-resistant NSCLC are critical to allow insight into the mechanism of ICI-resistance and to develop relevant treatment approaches.

5. Conclusions

Transformation of NSCLC to SCLC may be a mechanism of resistance to ICI. Awareness of this phenomenon is important, possibly mostly so regarding acquired resistance or mixed response cases. Repeat biopsies of progressing lesions after response to ICI can shed light on unexpected mechanisms of resistance to immunotherapy.

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Ethics

The study was approved by the ethics committee of the Sheba Medical Center.

Declaration of Competing Interest

In the interest of full transparency: J.B. reports research funding from MSD, Pfizer, AstraZeneca and Boehringer Ingelheim, and honoraria from AstraZeneca, MSD, Boehringer Ingelheim, Roche, BMS, Takeda, AbbVie and VBL. D.U. reports honoraria from BI, Roche, BMS, MSD, Teva, AstraZeneca and Takeda. A.O. reports research funding from Boehringer Ingelheim, Roche, Sanofi-Aventis, Xcovery, AstraZeneca, BMS and MSD, advisory fees from Boehringer Ingelheim, MSD, Takeda and AstraZeneca, and honoraria from Takeda, Roche and Boehringer Ingelheim. All reported above are unrelated to the submitted work; All other authors declare no potential conflict of interests.

Transparency document

The Transparency document associated with this article can be found in the online version.

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