Pathology & Oncology Research (2020) 26:2795–2800
https://doi.org/10.1007/s12253-019-00713-1

SHORT COMMUNICATION

Clinicopathological and Molecular Study of Peritoneal Carcinomatosis Associated with Non-Small Cell Lung Carcinoma

Hussein Nassereddine[1,2] · Aurélie Sannier[1,3] · Solenn Brosseau[3,4] · Jean-Michel Rodier[5] · Antoine Khalil[3,6] · Simon Msika[3,7] · Claire Danel[1,3] · Anne Couvelard[1,3] · Nathalie Théou-Anton[8] · Aurélie Cazes[1,3]

Received: 13 June 2019 / Accepted: 6 August 2019 / Published online: 12 August 2019

Abstract

To retrospectively characterize the molecular features of Non-Small Cell Lung Carcinomas (NSCLC) with peritoneal carcinomatosis (PC), clinicopathological data of 12 patients diagnosed with NSCLC and PC between 2007 and 2016 were collected. Immunohistochemistry and Next Generation Sequencing (NGS) were performed on cases with available material. PC was the initial presentation of NSCLC in 17% of the cases. Overall, patients with PC displayed a poor median survival of 12 weeks. Histology was adenocarcinoma in 11 cases. 37.5% of cases showed PD-L1 immunostaining positivity (50% cut-off). ALK and ROS1 immunostainings were negative. Using NGS, we identified 17 molecular alterations in 9 genes (TP53, KRAS, STK11, BRAF, EGFR, DDR2, ERBB4, SMAD4, CTNNB1) in 88.9% of adenocarcinomas. To the best of our knowledge, 5 of these variants are not referenced in the literature. In conclusion, PC might be the initial presentation of NSCLC. Molecular profiling of our cases did not find any effective targetable alteration, except from high PD-L1 expression.

Keywords Peritoneal carcinomatosis · Non-small cell lung carcinoma · NSCLC · Massively-parallel sequencing · Molecular pathology

Introduction

Lung cancer is currently the most common cause of cancer death worldwide [1]. Peritoneal carcinomatosis (PC) is considered a rare clinical event in patients with stage IV lung cancer, with a reported incidence of 1.2% to 16% mainly in autopsy studies [2, 3]. It appears to be highly associated with malignant pleural extension [3]. The diagnosis of PC is generally made by the use of imaging techniques and/or the presence of malignant cells in the ascitic fluid [4].

As the outcome of lung cancer patients after diagnosis of PC is very poor, identification of potential therapeutic targets is a key concern. There are limited data on genomic profiles of peritoneal carcinomatosis associated with non small cell lung carcinoma (PC-NSCLC). Some authors reported EGFR and KRAS mutations, ALK rearrangements and rarely MET

Hussein Nassereddine and Aurélie Sannier contributed equally to this work.

✉ Hussein Nassereddine
hussein.nassereddine@hotmail.com

[1] Assistance Publique – Hôpitaux de Paris, département de pathologie, Hôpital Bichat-Claude Bernard, Paris, France

[2] Department of pathology, AP-HP, Bichat- Hospital, 46 rue Henri Huchard 75877, 18 Paris Cedex, France

[3] Université de Paris, Paris, France

[4] APHP, service d'Oncologie Thoracique, Hôpital Bichat-Claude Bernard, Paris, France

[5] APHP, service d'Oncologie, Hôpital Bichat-Claude Bernard, Paris, France

[6] APHP, service de Radiologie, Hôpital Bichat-Claude Bernard, Paris, France

[7] APHP, service de Chirurgie Générale et Digestive, Hôpital Bichat-Claude Bernard, Paris, France

[8] APHP, laboratoire de Génétique, Hôpital Bichat-Claude Bernard, Paris, France

mutations [3, 4]. Another study showed response to epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor without knowledge of their EGFR status. This underlines a critical need for a better classification of the patients according to gene mutations [5]. Thus the purpose of this study was to describe the main clinicopathological features of a series of PC-NSCLC and to describe their molecular profiles.

Materials and Methods

Patients Clinicopathological data of patients diagnosed with NSCLC and PC between 2007 and 2016 in Bichat University Hospital (Paris, France) were retrospectively retrieved. Hematoxylin-Eosin and immunohistochemistry slides were assessed by two pathologists.

Informed consent was obtained from all individual participants included in the study to complete theranostic work-up of their biological samples.

Immunohistochemistry Immunohistochemical study was performed on 4-μm sections with an automated staining system (BOND-MAX, Leica-Biosystems, Buffalo Grove, IL). Monoclonal antibodies against ALK (clone 5A4–Abcam, Cambridge, UK), ROS1 (clone D4D6–Genemed-Biotechnologies, San Francisco, CA), and PD-L1 (clone E1L3N–Cell-Signaling-Technology, Danvers, MA) were used.

Molecular Biology Maxwell® 16 (FFPE-Plus LEV DNA-kit, Promega, Fitchburg, Wisconsin) was used for DNA extraction. DNA quality and quantity was assessed with Qubit® 2.0 Fluorometer (Life-Technologies, Thermo-Fisher Scientific, Saint-Aubin, France). DNA was amplified using primers from Oncomine tumor solid DNA kit and OST+ complementary panel (Life Technologies, Thermo-Fisher Scientific). These panels target >500 hotspot mutations in 25 lung and colon cancer-associated genes (ALK, AKT, BRAF, CTNNB1, DDR2, EGFR, ERBB2, ERBB4, FBXW7, FGFR1, FGFR2, FGFR3, HRAS, KIT, KRAS, MAP2K1, MET, NOTCH1, NRAS, PDGFRA, PIK3CA, PTEN, SMAD4, STK11, TP53). 20 ng of DNA/sample were used for multiplex barcoded library. Sequencing was carried out on the S5XL (Ion-torrent, Thermo-Fisher-scientific). Sequences were aligned to the hg19 human reference genome and data was analyzed by variant calling using the Torrent suit software and Ion reporter software for annotations of variants (Thermo-Fisher-Scientific). Potential mutations were retained if allelic frequency was ≥3% and the coverage was superior to 500x. Mutations were referred to the COSMIC database (GRCh37, COSMIC-v84). Pathogenicity prediction was studied using SIFT (v6.2.0), Mutation Taster (v2013), PolyPhen-2 (v2.2.2r398), UMD (http://umd-predictor.eu/analysis.php), SNP and Go (http://snps.biofold.org/snps-and-go/pages/contact.html) and FATHMM (http://fathmm.biocompute.org.uk) pathogenicity prediction software.

Results

Clinicopathological Characteristics We identified 12 patients with NSCLC and PC (Table 1). Median age at diagnosis of PC was 58 years (range, 45–83). Nine patients (75%) were male. All patients were smokers. Lung cancer histology was adenocarcinoma (ADC) in 11 cases (92%) and squamous cell carcinoma (SCC) in 1 case (8%). In 8 patients (66.6%), PC was synchronous to lung cancer diagnosis and was the initial revealing presentation in 2 patients (bowel obstruction and intestinal transit disorders) (Fig. 1a, b). Bowel obstruction occurred in 2 of the remaining 10 patients. One of these patients had a total colectomy (Fig. 1c, d).

All patients had advanced malignancy with nodal metastasis at the time of diagnosis of PC. 92% of patients had one or more extra-nodal metastatic localizations. 50% of patients had at least 3 extra-nodal and extra-peritoneal metastatic localizations. The most common extra-nodal sites of metastasis were pleura (67%), bone (58%), adrenal glands (42%), and subcutis/liver/colon (25%). Other rare metastatic sites were: muscle, pancreas, spleen, ovary, central nervous system, and kidney.

Median and mean survival time from the diagnosis of lung cancer were respectively 51 and 45 weeks, and from the diagnosis of PC respectively 12 and 32 weeks (range, 2.1–137.1 weeks) (Fig. 2).

Immunohistochemistry Overall, 5/8 tumors expressed PD-L1 in more than 1% of tumor cells, among which 3 of high level. ALK and ROS1 immunostainings were negative (Table 2).

Molecular Biology One or more protein altering mutations were found only in the 8 adenocarcinoma cases tested (88.9%) (Table 3). No genomic variants were identified in the SCC sample. We identified 17 molecular alterations in 9 genes with 4 recurrent mutations in two patients. All identified variants were predicted pathogenic. TP53 molecular alterations were the most prevalent mutation (55.5%) and were concurrent with mutations in other genes (KRAS, STK11, BRAF, SMAD4, CTNNB1). We also identified point mutations in genes coding for tyrosine kinase receptors: EGFR, DDR2, and ERBB4 ($n = 1$, each). Other molecular alterations involved the MAPK pathway: 3 KRAS point mutations (one of which was recurrent) lead to a gain of function while one recurrent non-p.V600E point mutation in BRAF gene was identified in two patients and leads to a loss of function. We found molecular alterations in other pathways such as PI3K/mTOR pathway (two frameshift variants in STK11), Transforming Growth Factor-beta (TGF-β) pathway (one

Table 1 Clinico-pathological features of the 12 cases

Case	Age (Y)	Sex	Smoking Status, P-Y	Loc.	Initial diagnosis	Pathology	PC	Dx	Medication since PC (Tx line)	Initial therapy (Tx line)	Survival since PC diagnosis (days)
1	58	M	Current, 40	RSL	Bronchial biopsy Pleural cytology	ADC	Syn	Rx	Cis-Pem(1)	/	16
2	65	M	Former, 50	RIL	Lung surgical resection Pleural biopsy	ADC	Meta	Path	Pem(3)	Carbo-Gem(1), Carbo-Ptx(2)	79
3	63	M	Former, 35	LSL	Pleural cytology	ADC	Syn	Rx	Carbo-Ptx-Beva(1)	/	35
4	55	F	Former, 15	RIL	Lung surgical resection	ADC –micropapillary	Meta	Rx	Carbo-Ptx(2)	Carbo-Gem(1)	56
5	45	F	Current, 40	LSL	Skin biopsy	ADC	Syn	Rx	Carbo-Ptx(1), Pem(2)	/	83
6	66	M	Current, 40	MF	Pleural biopsy/cytology	ADC	Syn	Rx	Carbo-Gem(1), Pem(2)	/	417
7	64	M	Current, 80	LSL	Pleural cytology	ADC	Meta	Rx	Pem(2)	Carbo-Gem-Beva(1)	372
8	58	M	Current, 50	LSL	Colon biopsy	ADC	Syn *Revealing*	Path	/	/	15
9	55	M	Current, 45	LSL	Bronchial biopsy	ADC	Syn	Rx	Cis-Pem(1)	/	43
10	57	M	Former, 30	LSL	Colectomy	ADC	Syn	Path	Pem(1), Carbo-Gem-Beva(2)	/	960
11	46	M	Current, 20	RL	Peural biopsy Peritoneal cytology	ADC	Meta	Path	Nivo(2), Gem(3)	Carbo-Pem(1)	270
12	83	F	Former, 50	RIL	Intestinal resection	SCC	Syn *Revealing*	Path	/	/	357

M Male, *F* Female, *Y* years, *P-Y* pack-years, *Loc.* localization, *MF* Multifocal, *RIL* Right inferior lobe, *RL* Right lobe, *RSL* Right superior lobe, *LSL* Left superior lobe, *PC* peritoneal carcinomatosis, *Dx* Diagnosis, *ADC* Adenocarcinoma, *SCC* squamous cell carcinoma, *Syn* Synchronous, *Meta* metachronous, *Rx* Radiological, *Path* Pathology, *Tx* treatment. *Cis* cisplatin, *Pem* Pemetrexed, *Gem* gemcitabine, *Carbo* carboplatin, *Beva* bevacizumab, *Ptx* Paclitaxel, *Nivo* nivolumab

point mutation in SMAD4 gene), and Beta-catenin (one point mutation in CTNNB1 gene). To the best of our knowledge, 5 of these variants are not referenced in the published databases: TP53 c.402_403del; (p.Phe134Leufs*14), TP53 c.209dup; (p.Pro71Serfs*78), DDR2 c.2291 T > C; (p.Phe764Ser), SMAD 4 c.1093G > C; (p.Gly365Arg) and ERBB4 c.1026G > T; (p.Leu342Phe).

Discussion

Our study reviewed 12 cases of patients diagnosed with PC secondary to primitive lung carcinoma, a rare clinical event [2, 3, 6]. Satoh et al. found PC in only 12 of 1041 lung cancer patients during a 26-years period [2]. However, our study highlights the fact that PC might be the revealing mode of NSCLC, a challenging diagnosis for clinicians and pathologists.

Despite chemotherapy, the outcome of lung cancers with PC was very poor in our study (median survival of 12 weeks), as reported in other small series [5]. Su et al. reported in 2/4 patients a response to gefitinib therapy with an overall survival for 203 and 343 days, respectively [5]. However, the genomic profiles of these cancers were not available. Sereno et al. reported EGFR mutations in two patients diagnosed with NSCLC and PC. Only one patient responded to tyrosine kinase inhibitors [4]. Patil et al. found ALK rearrangement, and EGFR, KRAS, and MET mutations in 15%, 52%, 15%, and 3% respectively [3]. Notably, the patients characteristics were very different from our study: mainly females and never smokers [3]. In our cohort, patients were mainly males and smokers. As expected in our cases, EGFR mutations were infrequent. There was only one uncommon EGFR mutation discovered retrospectively, so the patient did not receive a tyrosine kinase inhibitor. The EGFR mutation we found has been reported only once [7]. The efficacy of EGFR tyrosine kinase inhibitors in this setting is unknown [8].

In our cohort, TP53 has been the most frequently mutated gene. As reported in the literature, these mutations were associated with poorer survival and poorer response to radiation and adjuvant cisplatin therapies [9]. Allelic frequencies of TP53 mutations were in some of our cases lower than those of other mutations. This may be a representation of tumor heterogeneity and the selection and adaptation occurring during metastatic disease progression [10].

KRAS, the most common driver mutation in patients with NSCLC, was the second most frequently mutated gene. Mutations in KRAS gene are not yet targetable and confer a poor prognosis in the metastatic setting [11, 12].

We found two different frameshift mutations in the tumor suppressor gene STK11 that were concomitant to TP53 mutations.

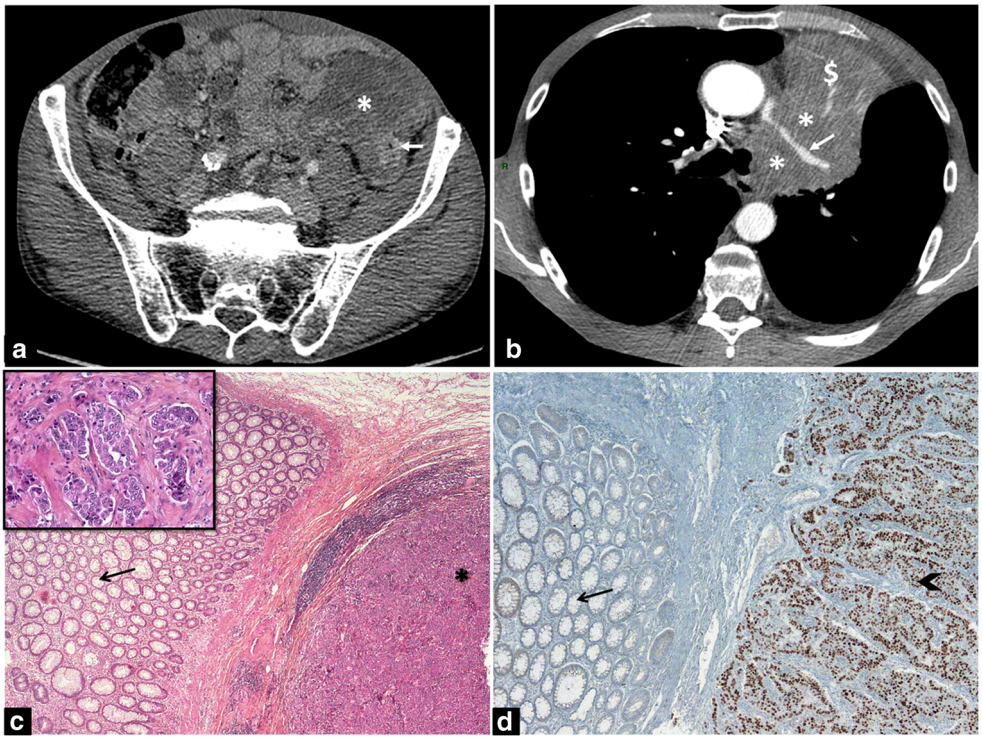

Fig. 1 *Case 8*: A 58 year-old woman was referred to the gastroenterology department with complaints of abdominal pain, bowel occlusion and pelvic mass. An enhanced abdomino-pelvic CT-scan showed: (**a**) a pelvi-peritoneal mass (asterisks) localized in the sigmoidal region (arrow showing the left colon; the mass invades the sigmoidal wall). A colonic biopsy was performed. The microscopic evaluation revealed lymphangitic carcinomatosis and the immunohistochemical analysis showed TTF-1 positivity in tumor cells consistent with the diagnosis of metastatic lung adenocarcinoma. Later, on enhanced thoracic CT-scan (**b**) a mediastino-pulmonary tumor (asterisks) was found. The tumor compressed the left pulmonary artery (arrow) with obstruction of the left superior lobar bronchus and atelectasis of the left superior pulmonary lobe ($). *Case 10*: Histological section showing a poorly differentiated adenocarcinoma (asterisk; in frame) invading the colon submucosa (arrow: colon mucosa). **c** Haematoxylin and Eosin staining, ×2 magnification, in frame ×40 magnification. **d** Immunohistochemical findings showing TTF-1 positivity in tumor cells (arrowhead). TTF-1 positivity is not detected in the colonic mucosa (arrow). Hematoxylin counterstaining, ×2 magnification

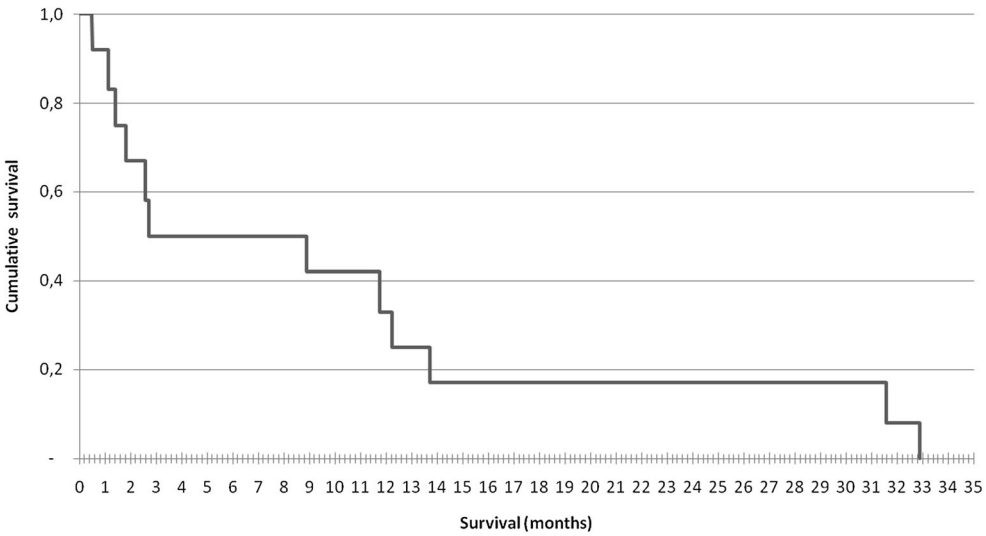

Fig. 2 Survival curve of the 12 patients after diagnosis of peritoneal carcinomatosis

Table 2 Molecular profile of NSCLC with PC by immunohistochemistry

Case	Histology	TTF1	CK7	ALK	ROS1	PD-L1
1	ADC	–	+	–	/	/
2	ADC	+	+	–	–	90%
3	ADC	+	/	/	/	/
4	ADC	+	+	–	–	<1%
5	ADC	+	+	–	–	30%
6	ADC	+	+	–	–	<1%
7	ADC	+	+	/	/	/
8	ADC	+	/	/	/	/
9	ADC	+	+	–	–	<1%
10	ADC	+	+	–	–	70%
11	ADC	+	+	–	–	80%
12	SCC	–	–	–	–	2%

ADC Adenocarcinoma, *SCC* Squamous cell carcinoma

The high frequencies of TP53, KRAS and STK11 mutations in our cohort are known to be correlated with smoking [9, 11, 13, 14].

The recurrent non-V600E BRAF mutation D594G we found, described in lung and colorectal carcinomas, is associated with loss of function of the protein so BRAF inhibitors are not expected to be effective [15, 16]. Recent data suggest that tumors with the D594 mutations may harbor RAS mutations and respond to MEK inhibitors [17]. BRAF mutations are reported in about 10% of lung adenocarcinomas [18]. Although limited by our small number of cases, we found a D594G BRAF mutation in 2 patients (22%).

The SMAD4 and CTNNB1 mutations we found are uncommon in lung cancer according to cosmic database.

None of our patients was eligible for treatment with ALK/ROS1 inhibitors since immunostainings were negative. This result is consistent with the predominance of male smokers in our cohort. Interestingly 3 cases expressing high level of PD-L1 (>50%) would be eligible today to immunotherapy with pembrolizumab. One patient (case 11) received a second-line therapy with nivolumab but showed progression. Immunotherapy in this setting has to be evaluated.

In conclusion, our results point out that although NSCLC related-PC is mainly discovered in advanced tumors it might be the initial presentation of NSCLC and must be considered as a potential cause of PC. Advanced lung carcinomas with peritoneal metastasis do not seem different from other NSCLC in terms of driver genes alterations but require molecular characterization and PD-L1 immunohistochemistry to guide therapeutic management.

Table 3 Molecular profile of NSCLC with PC by NGS

Gene name	Prevalence (%)	HGVSc	HGVSp	References	Prediction of Pathogenicity	Case	%TC (%)	AF (%)
TP53	5/9 (55.5%)	c.402_403del	p.Phe134Leufs*14	/	P	1	30	9.7
		c.650 T > G	p.Val217Gly	COSM44375	P	10	80	32
		c.733G > A	p.Gly245Ser	COSM6932	P	9	20	32.8
		c.472C > G	p.Arg158Gly	COSM11087	P	11	30	11
		c.209dup	p.Pro71Serfs*78	/	P	5	>50	5.8
KRAS	3/9 (33.3%)	c.37G > T (2/3)	p.Gly13Cys	COSM527	P (GOF)	1, 2	30, 20	31,20
		c.34G > A (1/3)	p.Gly12Ser	COSM517	P (GOF)	5	>50	18
STK11	2/9 (22.2%)	c.608dup	p.Phe204Valfs*62	RCV000492160.1	P	1	30	41
		c.157del	p.Asp53Thrfs*11	COSM48969	P	9	20	24.4
BRAF	2/9 (22.2%)	c.1781A > G (2/2)	p.Asp594Gly	COSM467	P (LOF)	6,9	50,20	30, 26.5
EGFR	1/9 (11.1%)	c.2267A > G	p.Asn756Ser	COSM26435	P	3	>50	27
DDR2	1/9 (11.1%)	c.2291 T > C	p.Phe764Ser	/	P	3	>50	9
ERBB4	1/9 (11.1%)	c.1026G > T	p.Leu342Phe	/	P	3	>50	9.6
SMAD4	1/9 (11.1%)	c.1093G > C	p.Gly365Arg	/	P	10	80	28
CTNNB1	1/9 (11.1%)	c.134C > T	p.Ser45Phe	COSM5667	P	11	30	4

HGVSc Human Genome Variation Society (coding sequence variation), *HGVSp* Human Genome Variation Society (protein sequence variation), *P* Pathogenic, *GOF* Gain of Function, *LOF* Loss of Function, *%TC* percentage of tumor cells, *AF* Allelic frequency

Author Contributions All authors had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design*: Nassereddine, Sannier, Danel, Couvelard, Cazes. *Acquisition, analysis, and interpretation of data*: Nassereddine, Sannier, Théou-Anton, Brosseau, Rodier, Msika, Khalil, Couvelard, Cazes. *Drafting of the manuscript*: Nassereddine, Sannier, Couvelard, Cazes. *Drafting the article or revising it critically for important intellectual content*: Brosseau, Rodier, Khalil, Msika, Danel, and Théou-Anton. Final approval of the version to be published: All authors approved this version to be published. Guarantor for the article: Cazes.

Funding Institutional funding.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

This research didn't involve any procedure/trial on living human individual.

This study was approved by our Institutional Review Board (IRB00006477-N°2018–044).

References

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F (2015) Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 136(5):E359–E386
2. Satoh H, Ishikawa H, Yamashita YT, Kurishima K, Ohtsuka M, Sekizawa K (2001) Peritoneal carcinomatosis in lung cancer patients. Oncol Rep 8(6):1305–1307
3. Patil T, Aisner DL, Noonan SA, Bunn PA, Purcell WT, Carr LL, Camidge DR, Doebele RC (2016) Malignant pleural disease is highly associated with subsequent peritoneal metastasis in patients with stage IV non-small cell lung cancer independent of oncogene status. Lung Cancer 96:27–32
4. Sereno M, Rodríguez-Esteban I, Gómez-Raposo C, Merino M, López-Gómez M, Zambrana F et al (2013) Lung cancer and peritoneal carcinomatosis. Oncol Lett 6(3):705–708
5. Su H-T, Tsai C-M, Perng R-P (2008) Peritoneal carcinomatosis in lung cancer. Respirol 13(3):465–467
6. McNeill PM, Wagman LD, Neifeld JP (1987) Small bowel metastases from primary carcinoma of the lung. Cancer 59(8):1486–1489
7. Okami J, Taniguchi K, Higashiyama M, Maeda J, Oda K, Orita N, Koizumi K, Kodama K, Kato K (2007) Prognostic factors for gefitinib-treated postoperative recurrence in non-small cell lung cancer. Oncology 72(3–4):234–242
8. Klughammer B, Brugger W, Cappuzzo F, Ciuleanu T, Mok T, Reck M, Tan EH, Delmar P, Klingelschmitt G, Yin AY, Spleiss O, Wu L, Shames DS (2016) Examining treatment outcomes with Erlotinib in patients with advanced non-small cell lung cancer whose tumors harbor uncommon EGFR mutations. J Thorac Oncol 11(4):545–555
9. Shajani-Yi Z, de Abreu FB, Peterson JD, Tsongalis GJ (2018) Frequency of somatic TP53 mutations in combination with known pathogenic mutations in colon adenocarcinoma, non-small cell lung carcinoma, and gliomas as identified by next-generation sequencing. Neoplasia 20(3):256–262
10. Marte B (2013) Tumour heterogeneity. Nature 501:327
11. Bhattacharya S, Socinski MA, Burns TF (2015) KRAS mutant lung cancer: progress thus far on an elusive therapeutic target. Clin Transl Med 4(1):35
12. Del Re M, Rofi E, Restante G, Crucitta S, Arrigoni E, Fogli S et al (2017) Implications of KRAS mutations in acquired resistance to treatment in NSCLC. Oncotarget 9(5):6630–6643
13. Ding L, Getz G, Wheeler DA, Mardis ER, McLellan MD, Cibulskis K et al (2008) Somatic mutations affect key pathways in lung adenocarcinoma. Nature 455(7216):1069–1075
14. Pécuchet N, Laurent-Puig P, Mansuet-Lupo A, Legras A, Alifano M, Pallier K et al (2017) Different prognostic impact of STK11 mutations in non-squamous non-small-cell lung cancer. Oncotarget 8(14):23831–23840
15. Shinozaki E, Yoshino T, Yamazaki K, Muro K, Yamaguchi K, Nishina T et al (2017) Clinical significance of BRAF non-V600E mutations on the therapeutic effects of anti-EGFR monoclonal antibody treatment in patients with pretreated metastatic colorectal cancer: the Biomarker Research for anti-EGFR monoclonal Antibodies by Comprehensive Cancer genomics (BREAC) study. Br J Cancer 117(10):1450–1458
16. Carter J, Tseng L-H, Zheng G, Dudley J, Illei P, Gocke CD, Eshleman JR, Lin MT (2015) Non-p.V600E BRAF mutations are common using a more sensitive and broad detection tool. Am J Clin Pathol 144(4):620–628
17. Heidorn SJ, Milagre C, Whittaker S, Nourry A, Niculescu-Duvas I, Dhomen N, Hussain J, Reis-Filho JS, Springer CJ, Pritchard C, Marais R (2010) Kinase-dead BRAF and oncogenic RAS cooperate to drive tumor progression through CRAF. Cell 140(2):209–221
18. Cancer Genome Atlas Research Network (2014) Comprehensive molecular profiling of lung adenocarcinoma. Nature 511(7511):543–550