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BRAF as a positive predictive biomarker: Focus on lung cancer and melanoma patients

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ABSTRACT

In the era of personalized medicine, *BRAF* mutational assessment is mandatory in advanced-stage melanoma and non-small cell lung cancer (NSCLC) patients. The identification of actionable mutations is crucial for the adequate management of these patients. To date various drugs have been implemented in clinical practice. Similarly, various methods may be adopted for the identification of *BRAF* mutations. Here, we briefly review the current literature on *BRAF* in melanoma and NSCLC, focusing attention in particular on the different methods and drugs adopted in these patients. In addition, an overview of the real-world practice in different Italian laboratories with high expertise in molecular predictive pathology testing is provided.

1. BRAF: an overview

Rapidly accelerated fibrosarcoma (Raf) proteins (including V-Raf Murine Sarcoma Viral Oncogene Homolog B [*BRAF*]) are involved in the Raf/extracellular signal-regulated kinase (MEK)/extracellular signal-regulated kinase (ERK) pathway (Moodie et al., 1993; Van Aelst et al., 1993; Vojtek et al., 1993; Warne et al., 1993; Zhang et al., 1993; Matallanas et al., 2011). As early as 1983, the first Raf gene, encoding

for a serine/threonine kinase protein, was described as a retroviral oncogene (Fig. 1) (Rapp et al., 1983; Moelling et al., 1984). Subsequently, the cellular homolog proto-oncogene (*c-ras*) was cloned. (Bonner et al., 1985). As far as Raf protein structure is concerned, three conserved regions (CRs) with distinct functions can be identified. The first is the CR1 site harboring the Ras-binding domain (RBD), which enables interaction with Ras proteins, and a cysteine-rich domain (CRD), which additionally allows interaction with Ras proteins and is necessary

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Fig. 1. The 3D structure of BRAF protein inhibited by dabrafenib. This figure was created by using Mol* PDB ID [Mol* (D. Sehnal, A.S. Rose, J. Kovca, S.K. Burley, S. Velankar (2018) Mol*: Towards a common library and tools for web molecular graphics MolVA/EuroVis Proceedings. doi:10.2312/molva.20181103), and RCSB PDB].

for Raf autoinhibition. (Tran et al., 2005). The second is the CR2 site displaying an inhibitory phosphorylation site able to negatively regulate interaction with Ras proteins and Raf activation (Dhillon et al., 2002). The third is the CR3 site presenting the serine/threonine kinase domain (Chong et al., 2001). The *BRAF* gene was mapped on chromosome 7 (7q34) and encodes for the BRAF protein, which is involved in the mitogen-activated protein (MAP) kinase cascade. Raf proteins play a role in the MAP kinase kinase kinase (MAP3KK) cascade (Peyssonnaux and Eychène, 2001). This is a highly conserved membrane-to-nucleus signaling pathway involved in multiple cell functions including cell growth, differentiation, proliferation, senescence and apoptosis (Peyssonnaux and Eychène, 2001; Wasylyk et al., 1989; Jamal and Ziff, 1990; Kolch et al., 1991). As far as BRAF protein activation is concerned, the phosphorylation of S446 is crucial to neutralizing the inhibitory role of the N-terminal domain and to obtaining, in association with D449, the correct three-dimensional conformation (Tran et al., 2005).

Since they were first reported in 2002 (Davies et al., 2002), *BRAF* mutations have been identified in several malignancies (Trovisco et al., 2006; De Roock et al., 2011; Nguyen-Ngoc et al., 2015; Malapelle et al., 2016; Cheng et al., 2018; Pisapia et al., 2019; Bellecine et al., 2020). More specifically, *BRAF* mutations are reported in a significant proportion of melanomas (40–60 %), papillary thyroid carcinomas (30–70 %) and colorectal cancers (5–20 %), whereas a low frequency has been reported in lung cancers (1.5–3.5 %) (Davies et al., 2002).

A classification system was adopted to better define the role of the different *BRAF* mutations (Dankner et al., 2018; Bracht et al., 2019; Yaeger and Corcoran, 2019; Frisone et al., 2020). Briefly, class I mutations (including exon 15 p.V600 mutations) enable a constitutive activation of the MAPK pathway without the need for dimerization and upstream RAS activation (Dankner et al., 2018; Bracht et al., 2019; Yaeger and Corcoran, 2019; Frisone et al., 2020). Similarly, class II mutations (p.G464E/V/R, p.G469A/V/S, p.L597Q/R/S/V and many others) are independent of upstream RAS activation, whereas dimerization is necessary in order to activate the signal transduction pathway (Dankner et al., 2018; Bracht et al., 2019; Yaeger and Corcoran, 2019; Frisone et al., 2020). Finally, class III mutations require upstream activation and involve a dimerization with wild-type CRAF (Dankner et al., 2018; Bracht et al., 2019; Yaeger and Corcoran, 2019; Frisone et al., 2020).

Here, we focus our attention on *BRAF* mutations in lung cancer and

melanoma.

2. BRAF p.V600 mutation as a positive predictive biomarker in melanoma and lung cancer

The mutational landscape of melanoma is heterogeneous. A recent study, based on the use of whole genome sequencing, reported that melanoma represents a tumor type harboring a higher mutational rate than other neoplasms (Lawrence et al., 2013). In addition, different mutational aspects have been identified among the different melanoma subtypes (Hayward et al., 2017). Overall, *BRAF* mutations occur in about 40–60 % of melanoma patients (Colombino et al., 2012). More specifically, almost all *BRAF* mutations (97 %) lie in codon 600 of exon 15 (Ihle et al., 2014). As regards exon 15 codon 600 mutations, up to 90 % displayed a transversion of T to A involving nucleotide 1799 (c.1799 T > A), resulting in the replacement of valine by glutamic acid (p.V600E) (Bradish and Cheng, 2014). Other less common substitutions within codon 600 involved lysine (p.V600 K, 8–20 %), arginine (p.V600R, 1%), methionine (p.V600 M, 0.3 %), and aspartic acid (p.V600D, 0.1 %) (Bradish and Cheng, 2014). *BRAF*-mutated melanomas seem to occur more frequently in young people and show more aggressive behavior than wild-type cases (Long et al., 2011; Hugdahl et al., 2016). Despite this, patients harboring a *BRAF* exon 15 p.V600 mutation may benefit from targeted treatment with tyrosine kinase inhibitors (TKIs), such as vemurafenib and dabrafenib, with a significant improvement in progression-free survival (PFS) and overall survival (OS) compared to standard chemotherapy (Chapman et al., 2011; Hauschild et al., 2012; McArthur et al., 2014; Trinh et al., 2014). Another therapeutic strategy for *BRAF* exon 15 p.V600-mutated patients is a BRAF inhibitor plus MEK inhibitor (trametinib and cobimetinib) combination, with a dramatic improvement in PFS and OS (Ascierto et al., 2016; Long et al., 2016; Long et al., 2017; Long et al., 2018). For all these reasons, *BRAF* exon 15 p.V600 testing is mandatory in advanced melanoma patients (Coit et al., 2019).

BRAF mutations occur very rarely in NSCLC patients (about 1.5–3.5 %) (Frisone et al., 2020; Leonetti et al., 2018). These mutations are reported more frequently in the adenocarcinoma subtype; more specifically, a micropapillary growth pattern and high expression of thyroid transcription factor 1 (TTF-1) were reported for *BRAF* exon 15 p.V600E-mutated cases (Marchetti et al., 2011). As far as epidemiological distribution is concerned, although in some experiences *BRAF* exon p.V600E mutations seem to be more frequent in females and never-smoker patients, discordant results have been obtained for p.V600E and non-p.V600E (Pisapia et al., 2019; Frisone et al., 2020; Marchetti et al., 2011; Cardarella et al., 2013; Kinno et al., 2014; Salimian et al., 2018). Discordant results have also emerged regarding the prognostic role of the different *BRAF* mutations in NSCLC patients (Marchetti et al., 2011; Paik et al., 2011; Litvak et al., 2014; Tan et al., 2019). Overall, *BRAF* exon 15 p.V600E seems to be the most commonly-reported mutation (more than 50 %) (Marchetti et al., 2011; Cardarella et al., 2013; Paik et al., 2011; Ding et al., 2017; O'Leary et al., 2019). Noteworthy, in other experiences lung cancers seem to harbor more frequently *BRAF* non-p.V600E respect to p.V600E mutations (Pisapia et al., 2019; Noeparast et al., 2016). Moreover, *BRAF* non-p.V600E mutations may coexist with other mutations, in particular Kirsten Rat Sarcoma Viral Oncogene Homolog (*KRAS*) (Pisapia et al., 2019; Salimian et al., 2018; Li et al., 2014; Smit, 2014). As far as therapeutic options are concerned, advanced NSCLC patients harboring *BRAF* p.V600E mutations may be treated with the dabrafenib plus trametinib combination (Planchard et al., 2016; Planchard et al., 2017). Anecdotal reports have shown the possibility of adopting targeted treatments for *BRAF* non-p.V600E-mutated advanced-NSCLC patients (Gautschi et al., 2015; Kotani et al., 2018; Alvarez and Otterson, 2019; Reyes et al., 2019). On the whole, the National Comprehensive Cancer Network (NCCN) (Ettinger et al., 2018), the College of American Pathologists (CAP), the International Association for the Study of Lung Cancer

(IASLC), and the Association for Molecular Pathology (AMP) (Lindeman et al., 2018), and American Society of Clinical Oncology (ASCO) (Kalemkerian et al., 2018) recommend *BRAF* mutational assessment for advanced NSCLC patients.

3. Sample management for *BRAF* mutational assessment in melanoma patients

As far as molecular tests are concerned, melanoma patients have the important advantage of high-quantity tissue material (Fig. 2). As a matter of fact, in these patients, a wide local excision of the primary lesion or metastatic sites (in particular, lymph nodes) is often available as starting material from which to extract nucleic acids for molecular tests (Hyams et al., 2019). In addition, superficial metastatic sites can be approached with fine needle aspiration (FNA) (Doubrovsky et al., 2008). The presence of melanin represents a major limitation in melanoma specimens. More specifically, melanin may determine unique challenges due to the inhibition of the polymerase chain reaction (PCR) (Petty et al., 2020). Melanin is able to form a reversible complex with the DNA

polymerase (Eckhart et al., 2000). More specifically, it seems to particularly affect large amplicons (Eckhart et al., 2000). To overcome this limitation, samples with a high melanin content may benefit from additional treatments to allow an adequate PCR analysis. Frouin et al. proposed three different pre-PCR approaches including the addition of bovine serum albumin (BSA), DNA dilution, and DNA purification using the NucleoSpin® gDNA Clean-up XS Kit (Frouin et al., 2016). Vicente et al. compared six different methods for removing melanin from genomic DNA (Agarose Gel Electrophoresis, 1 mg Chelex®-100, Chelex®-100 5%, centrifugation, OneStep™ PCR Inhibitor Removal Kit and centrifugation plus OneStep™ PCR Inhibitor Removal Kit) showing that centrifugation combined with the OneStep™ PCR Inhibitor Removal Kit was superior for obtaining adequate *BRAF* sequencing (ALSA et al., 2019). When tissue samples are not available, liquid biopsy may be a valuable tool for monitoring therapeutic response (Buder-Bakhaya et al., 2017; Gaiser et al., 2018; Lim et al., 2018). Interestingly, it has been demonstrated that an increase in plasma circulating tumor DNA (ctDNA) concentration in patients with advanced melanoma harboring a *BRAF* mutation receiving targeted therapies may predict the presence of

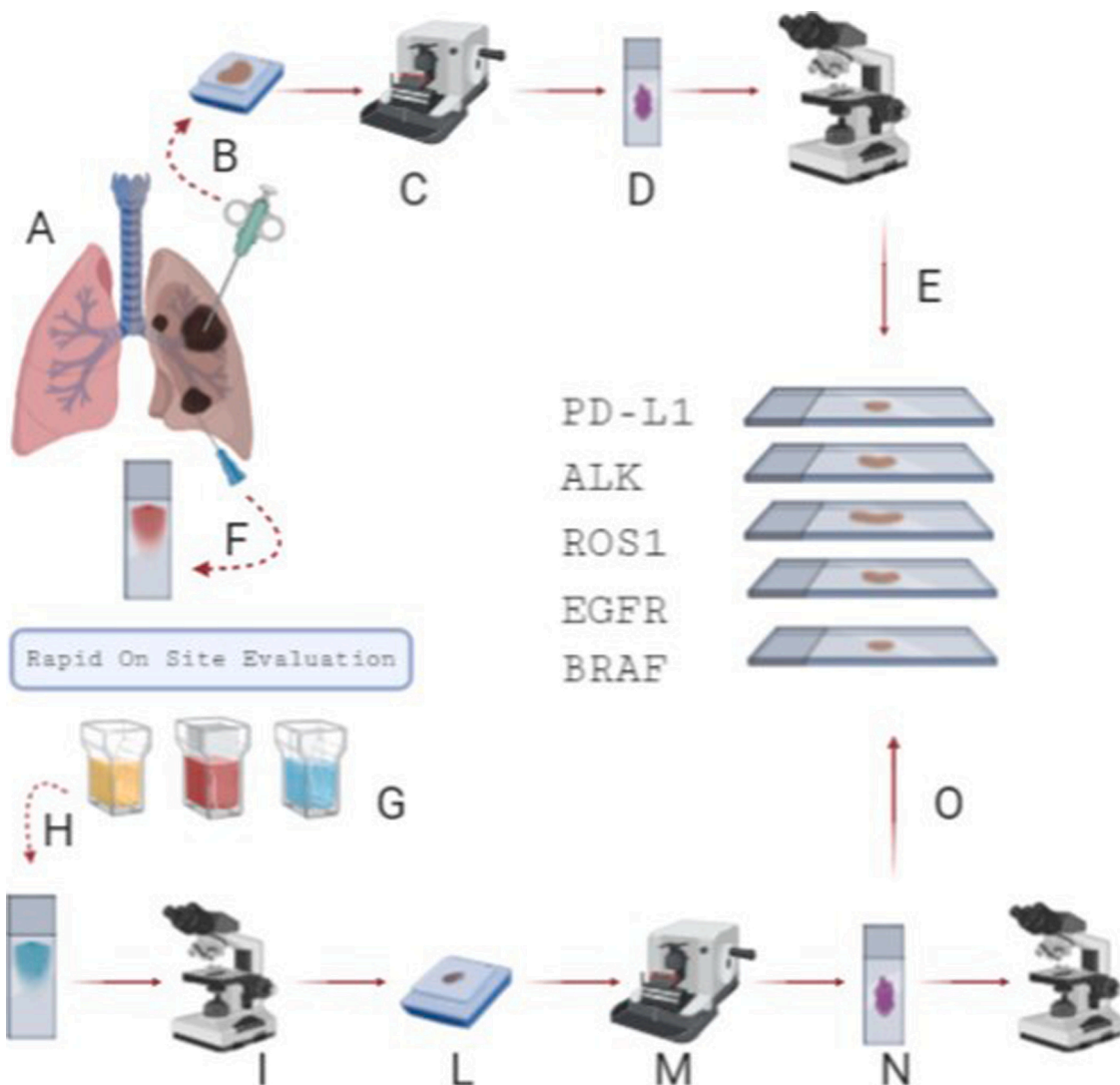


Fig. 2. NSCLC sample collection (A) and preclinical management for histological samples, starting with formalin fixation and paraffin embedding (B), tissue section production (C) and staining (D). After morphological evaluation and diagnosis (E) serial tissue sections were prepared to evaluate clinical relevant predictive biomarkers (*EGFR*, *BRAF*, *ALK*, *ROS1* and PD-L1). In the lower part of the figure, a fine needle aspiration (F) was reported and a Rapid On Site (ROSE) evaluation was schematized, from diff - quick staining (G), to slide preparation (H) for morphological evaluation (I) and, in addition, a cell - block (L) was prepared to obtain serial slides (M), that after microscopic evaluation by using an hematoxylin and hesoin staining (N), were used to clinical relevant biomarkers assessment. Credit by Biorender.com.

relapse earlier than imaging and/or clinical assessments (Gray et al., 2015).

4. Sample management for BRAF mutational assessment in non-small cell lung cancer patients

In the current era of personalized medicine, there has been a rapid increase in the number of biomarkers to be tested in advanced NSCLC patients. According to International guideline recommendations, at least Epidermal Growth Factor Receptor (*EGFR*) and *BRAF* mutations, Anaplastic Lymphoma Kinase (*ALK*) and ROS Proto-Oncogene 1 (*ROS1*) rearrangements, and the evaluation of the level of Programmed Death-Ligand 1 (PD-L1) expression must be tested for treatment decision-making (Ettinger et al., 2018; Lindeman et al., 2018; Kalemkerian et al., 2018). In addition, a plethora of novel biomarkers have emerged (Malapelle et al., 2020; Russo et al., 2020). However, due to significant delays in diagnosis, only small tissue samples (histological biopsies or cytological specimens) are available for morpho-molecular purposes (Ofiara et al., 2012; Wang et al., 2015). As a matter of fact, despite having a higher quality of nucleic acids than histological specimens, cytological samples are often characterized by a small quantity of available tissue (Clark, 2009; Aisner et al., 2016). However, it has been extensively demonstrated that cytological specimens represent a valuable starting material for molecular analysis (Malapelle et al., 2013; Barbareschi et al., 2018; Jain and Roy-Chowdhuri, 2018; Vigliar et al., 2019) (Fig. 3). In this setting, next-generation sequencing (NGS) technologies may represent a valid and fascinating way to overcome the limits associated with the small quantity of available tissue (Vigliar et al., 2015). Unfortunately, a non-negligible percentage (up to 30 %) of advanced-stage NSCLC patients do not have an available tissue specimen (Herbreteau et al., 2019). In this setting, liquid biopsy may represent a valid alternative to tissue specimens for the assessment of the molecular status of the different biomarkers (Rijavec et al., 2019; Siravegna et al., 2019). As for *EGFR*, the utility of ctDNA has also been demonstrated for *BRAF* mutational assessment (Bracht et al., 2019; Wu et al., 2019; Ortiz-Cuaran et al., 2020).

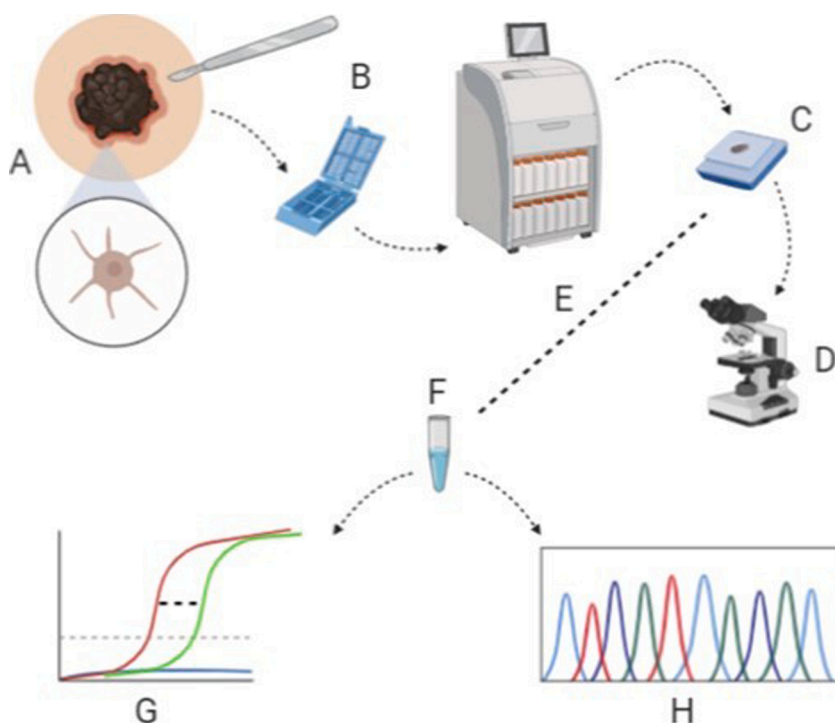


Fig. 3. Melanoma sample collection (A) and preclinical management for histological samples, starting with formalin fixation and paraffin embedding (B - C), tissue sections production and staining for morphological evaluation and diagnosis (D). To evaluate *BRAF* clinical relevant mutations, DNA was extracted (F) and analyzed by using RT - PCR (G) based approach or sequencing based assay (H). For RT - PCR based approach (G) an amplification curve swift (red to green) was schematically represented to underline the impact of melanin on DNA amplification. Credit by Biorender.com.

5. Companion diagnostic and laboratory-developed tests for the assessment of BRAF mutations

5.1. Immunohistochemistry

Immunohistochemistry (IHC) may be a reliable option for evaluating *BRAF* exon 15 p.V600E mutation (Schirosi et al., 2016). More specifically, in melanoma patients the most commonly-used antibody is the monoclonal antibody VE1, which shows cytoplasmic staining (Eriksson et al., 2015). The main advantages of IHC are associated with its simplicity, low costs, rapid turnaround time (TAT), relatively high sensitivity and specificity, and the possibility to evaluate mutant protein distribution at single-cell level (Colomba et al., 2013). Its main disadvantages are associated with the possibility of false-negative results due to heterogeneity or the low concentration of *BRAF* exon 15 p.V600E and the inability to identify *BRAF* exon 15 p.V600 K or other variants (Colomba et al., 2013). By comparing the results obtained by VE1 clone and by a PCR-based approach, an overall concordance of 88 % was achieved (Hugdahl et al., 2016). A high sensitivity and specificity (97 % and 98 %, respectively) were reported by Long et al. by comparing VE1 clone IHC with a DNA-based approach suggesting the possibility of adopting IHC to screen advanced melanoma patients (Long et al., 2013). Similar sensitivity and specificity were observed for IHC when results were compared to pyrosequencing (85 % and 100 %, respectively) and PCR-based approaches (98.6 % and 97.7 %) (Pearlstein et al., 2014; Manfredi et al., 2016).

As far as NSCLC is concerned, due to the possibility of targeting *BRAF* exon 15 p.V600E point mutation alone, IHC may represent a reliable approach. Overall, similar results to those obtained in melanoma patients were obtained for *BRAF* VE1 clone IHC, as a lung cancer screening tool. Ilie et al. demonstrated that IHC using the VE1 clone is a valid alternative to molecular biology approaches for detecting *BRAF* exon 15 p.V600E point mutation in advanced NSCLC patients, with a sensitivity of 90 % and a specificity of 100 % (Ilie et al., 2013). Similarly high sensitivity and specificity (96.6 % and 98.6 %, respectively) were obtained by Gow et al. using VE1 clone (Gow et al., 2019). In two different cohorts (retrospective and prospective) of advanced NSCLC patients, Hofman et al. evaluated the high performance of IHC testing in detecting

BRAF exon 15 p.V600E point mutation. Overall, in a retrospective series previously evaluated by NGS and/or pyrosequencing, IHC was able to correctly identify all *BRAF* exon 15 p.V600E mutated patients. Interestingly, no false positives were observed among wild-type and *BRAF* non-p.V600E patients (Hofman et al., 2020). An important advantage in terms of TAT has been reported for IHC (three days) compared to pyrosequencing (five days) and NGS (14 days) (Hofman et al., 2020). In the prospective cohort, *BRAF* IHC testing showed a positive result in 24 (3%) out of 699 tumors. Interestingly, in 20 out of 24 cases (83 %) a fully-automated real-time PCR (RT-PCR) approach (Idylla, Biocartis, Mechelen, Belgium) confirmed the *BRAF* mutational status. The Authors explain that the four negative cases may be false-negative results of RT-PCR due to a low tumor cell content (1–5%) with less than a total of 50 tumor cells observed in the tissue sections (Hofman et al., 2020).

5.2. Sanger sequencing

As a general rule, Sanger sequencing represents the gold standard technology among the various molecular biology techniques for point mutations and small variant detection, on account of its reliability, availability, reagent affordability, and relative low costs. However, its principal disadvantage is its low sensitivity (Bakker, 2006). As far as melanoma patients are concerned, Jurkowska et al. compared the results obtained by Sanger sequencing with those obtained by the cobas 4800 *BRAF* V600 mutation test (Roche, Basel, Switzerland) (Jurkowska et al., 2015). Overall, of 236 melanoma samples, a similar percentage of mutated cases was achieved for Sanger sequencing and the cobas 4800 *BRAF* V600 mutation test (60.9 % and 61.0 %, respectively); the overall agreement reported was 95.2 % (Jurkowska et al., 2015). Direct sequencing was adopted by Lopez-Rios et al. on 116 melanoma samples and the results were compared with those obtained with the cobas 4800 *BRAF* V600 mutation test. Overall, a positive percent agreement of 97.7 % and a negative percent agreement of 95.3 % were achieved with Sanger sequencing (Lopez-Rios et al., 2013). In another experience, Qu et al. showed that Sanger sequencing is better able to identify *BRAF* exon p.V600-mutated melanomas than the cobas 4800 *BRAF* V600 mutation test (43 % and 35 %, respectively), concluding that Sanger sequencing should be adopted in cases of a negative cobas 4800 *BRAF* V600 mutation test result, to increase the number of patients who may benefit from targeted therapy (Qu et al., 2013).

Sanger sequencing may be a useful approach for detecting *EGFR* sensitizing mutations in advanced NSCLC patients, as shown by Zheng et al. (2020). As far as the *EGFR* mutation rate is concerned, a 21.4 % (22/103) mutation rate was reported for 103 analyzed samples; more specifically, 20 out of 103 patients (19.5 %) had a sensitizing mutation (Zheng et al., 2020). A similar detection rate was reported by Sousa et al. (2020). Even in this large experience, Sanger sequencing was able to establish that 20.5 % (252/1225) of cases harbored at least one mutation in the *EGFR* gene (exons 18–21) (Sousa et al., 2020).

Ellison et al. observed that despite having poorer sensitivity than Amplification Refractory Mutation System PCR (ARMS-PCR), direct sequencing was able to identify mutations in regions not covered by the targeted approach (Ellison et al., 2010). Ji et al. compared the results obtained with next-generation sequencing (NGS) and Sanger sequencing for *EGFR* mutation and *ALK* fusion detection (Ji et al., 2019). Overall, NGS was able to detect a higher number of *EGFR*-mutated cases than Sanger sequencing (89 vs. 84, respectively), due to a very low abundance of mutated allele (<5%) (Ji et al., 2019). Conversely, Sanger sequencing was able to detect an *ALK*-rearranged case, which was further confirmed with ARMS PCR, that had been overlooked by NGS (Ji et al., 2019).

5.3. Pyrosequencing and matrix assisted laser desorption ionization - time of flight (MALDI – TOF)

Unlike Sanger sequencing, pyrosequencing does not use labeled

dideoxynucleotides; rather its “sequencing by synthesis” involves an enzymatic reaction that ultimately produces light (Ronaghi et al., 1998). Colomba et al. compared four different methods (IHC, pyrosequencing, RT-PCR, Sanger sequencing) to detect *BRAF* exon 15 p.V600 mutations in advanced melanoma patients (Colomba et al., 2013). Interestingly, in the 89 patients showing an adequate result for all methods, pyrosequencing was the only technique able to correctly identify all *BRAF* exon 15 p.V600 and *BRAF* exon 15 p.V600E point mutations without false-positive results (100 % sensitivity and specificity) (Colomba et al., 2013). The Authors concluded that pyrosequencing may be useful in the case of negative or uninterpretable IHC results for the detection of *BRAF* exon 15 p.V600E (Colomba et al., 2013). Pyrosequencing has demonstrated its utility in detecting *BRAF* non-p.V600E mutations, as reported by (Heinzerling et al. (2013). In this study, a higher percentage of patients harboring these rare *BRAF* mutations were detected by pyrosequencing (92.9 %, 13/14) than by the cobas 4800 *BRAF* V600 mutation test (50.0 %, 7/14) and IHC (21.4 %, 3/14) (Heinzerling et al. (2013). This highly sensitive, reliable, and time-saving approach may avoid the overlooking of rare mutations that could result in a subset of patients being excluded from targeted therapies. Ihle et al. analyzed a pre-selected cohort of 82 cancer samples (also including melanoma and lung cancer patients). Overall, pyrosequencing was able to detect 100 % of the mutations down to 5% allele frequency but only showed 90 % specificity (Ihle et al., 2014).

The potential of Matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) for DNA analysis was reported in 1995 (Tang et al., 1995). In order to use this analysis technique, nucleic acid molecules are extracted and deposited on a dedicated matrix before being irradiated by laser to induce their desorption and ionization; the ionized DNA molecules pass through a flight tube connected directly to a detector (Tang et al., 1995). Separation occurs by the time of flight, which is proportional to the mass of the individual DNA molecule (Tang et al., 1995). In recent years, MALDI-TOF has shown feasibility for single nucleotide genotyping in particular (Sauer et al., 2003). Different approaches have been implemented to improve the practicability of this technology, based on either a locus-specific PCR step followed by a primer extension, invader reaction, or hybridization steps with or without exonuclease digestion. In addition, to improve the multiplex power of MALDI – TOF, Sequenom optimized the primer extension reactions by using acyclic mass-modified base terminators that achieve at least 16-Dalton gaps between the four possible bases incorporated into a single base extension. This innovative approach led to the implementation of MALDI – TOF in routine predictive molecular pathology as high-throughput SNP-based genotype methods, representing a bridge between conventional methods, such as Sanger sequencing and RT-PCR, and NGS.

5.4. Real-time PCR

RT-PCR is a targeted method able to detect known mutations (Nollau and Wagener, 1997). As a general rule, the RT-PCR approach adopts a set of primers that specifically target *BRAF* mutations and another able to identify the wild-type sequence (Cheng et al., 2018). To date, the cobas 4800 *BRAF* V600 mutation test or THxID-*BRAF* kit have obtained Food and Drug Administration (FDA) approval in melanoma patients for the detection of *BRAF* exon 15 p.V600 mutations in order to administer TKI treatments in advanced-stage melanoma patients (Marchant et al., 2014). In the experience by Anderson et al., the cobas 4800 *BRAF* V600 mutation test was compared with Sanger sequencing in 477 melanoma samples (Anderson et al., 2012). Overall, all cases were successfully tested with the cobas 4800 *BRAF* V600 mutation test, whereas a failure rate of 9.2 % was reported for Sanger sequencing (Anderson et al., 2012). Mourah et al. compared the results obtained using the cobas 4800 *BRAF* V600 mutation test with various other techniques (including Sanger sequencing, pyrosequencing, allele-specific PCR, SNaPshot and high-resolution melting (HRM) analysis) (Mourah et al., 2015). Overall, the frequency of *BRAF* exon 15 p.V600 mutations detected was slightly

higher for the other techniques than for the cobas 4800 BRAF V600 mutation test (35.7 % vs. 34.0 %, respectively), whereas wild-type cases were detected with a higher frequency by the cobas 4800 BRAF V600 mutation test (63.3 % vs. 62.9 %) (Mourah et al., 2015). These data confirm the high clinical efficacy of the cobas 4800 BRAF V600 mutation test. Marchant et al. used the THxID BRAF diagnostic test on 113 melanoma samples (Marchant et al., 2014). Overall, just one inadequate case (0.9 %) was reported. Interestingly, complete concordance (100 %) between Sanger sequencing and the THxID BRAF test was observed, whereas between the THxID BRAF test and HRM the concordance rate was 97.3 % (Marchant et al., 2014).

As for melanoma, RT-PCR using Therascreen (Qiagen, Hilden, Germany) is an FDA-approved real-time PCR approach for detecting 21 or 29 *EGFR* mutations in exons 18, 19, 20, and 21 (Allegrini et al., 2012). In the IPASS clinical trial, the DxS *EGFR* mutation test kit was used to evaluate 29 *EGFR* mutations (19 deletions in exon 19, exon 21 p.L858R, exon 20 p.T790 M, exon 21 p.L861Q, exon 18 p.G719S/A/C, exon 20 p.S768I, and three insertions in exon 20), in order to administer the first-generation TKI gefitinib (Mok et al., 2009). Overall, 59.7 % of tested patients showed an *EGFR* mutation (Mok et al., 2009). In the IFUM phase IV clinical trial on gefitinib, an *EGFR* mutational rate of 13.7 % was reported using the Therascreen *EGFR* 29 kit (Douillard et al., 2014). In 2009, The cobas *EGFR* mutation test obtained FDA approval as a companion diagnostic for erlotinib in advanced NSCLC patients (Malapelle et al., 2017). In a retrospective analysis of the EURTAC clinical trial, an overall concordance of 96 % and a faster turnaround time (TAT) were reported for the cobas *EGFR* mutation test than a laboratory-developed RT-PCR test (Benlloch et al., 2014). The cobas *EGFR* mutation test v2 is a RT-PCR method able to detect 42 different *EGFR* gene mutations in exons 18, 19, 20, and 21 and has been approved by the FDA for the liquid biopsy setting (Malapelle et al., 2017). RT-PCR is also suitable for *EGFR* analysis on cytological samples, as reported by Malapelle et al. (TaqMan assay) and De Luca et al (Idylla, Biocartis, Mechelen, Belgium) (Malapelle et al., 2013; De Luca et al., 2017; De Luca et al., 2019).

5.5. Next-generation sequencing

NGS is a fascinating technology able to analyze several mutations for different patients, simultaneously (Rothberg et al., 2011). Briefly, although different platforms are currently commercially available, the NGS workflow is characterized by four sequential phases: library generation, clonal amplification, massive parallel sequencing, and data analysis (Vigliar et al., 2015).

Due to its higher costs and longer TAT, Zhu et al. suggested adopting NGS for advanced melanomas showing a negative result with allele-specific *BRAF* exon 15 p.V600E/K PCR (Zhu et al., 2018). The Authors highlighted the higher sensitivity of the NGS approach compared to the allele-specific PCR approach, enabling the identification of 16 actionable mutations in 24 negative advanced melanoma patients (Zhu et al., 2018). However, the costs and TAT of NGS may be more favorable when other genes are analyzed. In the study by de Unamuno Bustos et al., about 85 % of analyzed melanomas harbored at least one mutation (de Unamuno Bustos et al., 2017). More specifically, as expected, 50 % of samples showed a *BRAF* mutation (the vast majority were exon 15 p.V600E (80 %; 40/50) (de Unamuno Bustos et al., 2017). A similar result in terms of *BRAF* mutation rate was reported by Lokhandwala et al. (2019). Overall, using an NGS approach 42 % of cases were reported as being *BRAF*-mutated cases (of which 76 % in the exon 15 codon 600) Lokhandwala et al. (2019). NGS may also be useful for detecting rare actionable mutations that are overlooked by targeted methods. As reported by Proietti et al., NGS was able to detect a rare variant of *BRAF* exon 15 p.V600E (c.1799_1800TG > AA) that was overlooked by an RT-PCR approach (Proietti et al., 2020).

As far as NSCLC patients are concerned, using the AmpliSeq Cancer Hotspot Panel v2 (ThermoFisher Scientifics, Waltham, MA), Salimian

et al. were able to identify 36 *BRAF*-mutated lung adenocarcinomas (Salimian et al., 2018). Interestingly, ten harbored a *BRAF* exon 15 p.V600E (Salimian et al., 2018). Similar results were obtained using a narrow custom NGS panel (SiRe®). Pepe et al. and Pisapia et al. were able to detect 14 (4.8 %) *BRAF*-mutated cases among 294 analyzed advanced NSCLC patients on tissue specimens (histological and cytological) (Pisapia et al., 2019; Pepe et al., 2019). In particular, eight cases showed a targetable *BRAF* exon 15 p.V600E, without any other driver mutations (Pisapia et al., 2019; Pepe et al., 2019). In the study by Fumagalli et al., a large number of advanced NSCLC patients (n = 535) were evaluated using a 22-gene panel on the Ion Torrent Personal Genome Machine (PGM, ThermoFisher Scientifics) platform (Fumagalli et al., 2018). Overall, 23 (4.3 %) of the 535 tumors harbored a *BRAF* mutation. Interestingly, 14 cases harbored a *BRAF* exon 15 alteration, but a p.V600E point mutation was only observed in six cases (Fumagalli et al., 2018). In a large-scale experience, Lin et al. adopted Capture-based NGS sequencing on either plasma or tissue samples obtained from 8405 Chinese stage I-IV NSCLC patients (Lin et al., 2019). Overall, *BRAF* mutations were reported in 238 (2.8 %) patients (Lin et al., 2019). Of these, the most common mutations were identified in codon 600 (32 %) (Lin et al., 2019). As far as the liquid biopsy setting is concerned, Bracht et al. used a targeted ctDNA NGS assay (Guardant360®, Guardant Health Inc., Redwood City, CA), and results were correlated with patient outcome. Overall, the Authors were able to identify 17 (9.2 %) *BRAF*-mutated cases amongst the 185 patients analyzed, of which four (23.5 %) were *BRAF* exon 15 p.V600E (Bracht et al., 2019).

6. Integrated clinical report for BRAF mutational evaluation: from morphology to molecular biology

Molecular reports play a key role in the molecular predictive pathology workflow and should exhaustively report all relevant information to ensure the best treatment option for cancer patients (Cree et al., 2014; Li et al., 2017). To this end, in order to avoid any misinterpretation, effective communication between the different professionals involved in this field is essential. As a matter of fact, reports that are incomplete or difficult to understand may contribute to errors in cancer patient management. Molecular reports should therefore be short, easy to interpret and contain the salient information that could be of interest for the administration of the best treatment option. Firstly, molecular reports should contain patients' unique identifiers (name, surname, date of birth, and identification number). Additional relevant information regards the ward or service, the date, the type of sample, and the name of clinician requesting the molecular analysis (Cree et al., 2014). The body of the report should contain the pre-analytical information (e.g. percentage of neoplastic cells, fixation problems, presence of contaminants that could influence the analysis) and the mutational status of the analyzed biomarkers (in both nucleotide and aminoacidic forms) (Cree et al., 2014; Li et al., 2017). Other important information includes the variant allele frequency and coverage of detected alterations. The report should contain a clinical interpretation of the detected variants in order to guide clinicians in the choice of the best treatment for their patients, information that may be supported by literature citations. Methodological data, regarding the type of test adopted, the reference range, the limit of detection [LOD], and the NGS parameters run should be provided at the end of the report (Li et al., 2017).

7. Treatment decision-making

7.1. Melanoma

As discussed previously, despite its highly aggressive behavior, patients with *BRAF*-mutated melanoma may be treated with targeted therapies (Luther et al., 2019). Vemurafenib was the first *BRAF* inhibitor to be approved by the FDA for the treatment of advanced *BRAF* exon 15

p.V600E-mutated melanoma patients. In a large phase III clinical trial, vemurafenib (BRAF inhibitor) demonstrated a significant increase in PFS and OS in advanced-stage BRAF exon 15 p.V600E-mutated and previously untreated melanoma patients compared to dacarbazine (Chapman et al., 2011). Subsequently, in an update study of the BRIM-3 clinical trial, with an extended follow-up for the entire population, in the vemurafenib group an increase in survival was observed compared to dacarbazine, not only in BRAF exon 15 p.V600E, but also in BRAF exon 15 p.V600 K previously-untreated melanoma patients (McArthur et al., 2014). Similar results compared to standard chemotherapy (dacarbazine) were reported in BRAF-mutated advanced melanoma patients (exon 15 p.V600E and exon 15 p.V600 K) treated with another BRAF inhibitor (dabrafenib) as monotherapy (Hauschild et al., 2012; Hauschild et al., 2020). Interesting results, in terms of OS and PFS in patients with BRAF exon 15 p.V600-mutant advanced melanoma, were obtained for BRAF inhibitor plus MEK inhibitor combinations. In previously-untreated unresectable or metastatic melanoma patients harboring a BRAF exon 15 p.V600E/K mutation, Long et al. reported a higher PFS (9.3 vs. 8.8 months), OS (93 % vs. 85 %) and overall response rate (ORR) (67 % vs. 51 %) when patients were treated with the dabrafenib plus trametinib combination than with dabrafenib alone (Long et al., 2016; Long et al., 2017; Long et al., 2018; Long et al., 2014). In the coBRIM clinical trial, previously-untreated advanced BRAF exon 15 p.V600-mutated melanoma patients were randomized to receive vemurafenib (BRAF inhibitor) plus cobimetinib (MEK inhibitor) or vemurafenib plus placebo. Overall, higher median PFS (9.9 vs. 6.2 months), complete or partial responses (68 % vs. 45 %; more specifically, complete responses were 10 % vs. 4%), and OS (9-month survival rate of 81 % vs. 73 %) were observed in the combination group (vemurafenib plus cobimetinib) than in the group administered vemurafenib as a single agent (Larkin et al., 2014). In addition, the combination was not associated with a significantly increase of adverse events of grade 3 or higher respect to vemurafenib alone (65 % vs. 59 %) (Larkin et al., 2014). More recently, in pre-clinical models, it has been highlighted a correlation between the resistance of melanoma cells to BRAF and MEK inhibitors with an increased immunogenicity to natural killer (NK) cells (Frazao et al., 2020). However, due to the possibility of tumor escaping from NK cells activity, a boosting of NK cells may be required in association with targeted therapies to improve antitumor activity (Frazao et al., 2020).

As far as immunotherapy is concerned, various studies have evaluated the efficacy of a targeted therapy plus immunotherapy combination approach. The rationale behind this association was that in preclinical murine models, it was observed that BRAF inhibitors may increase tumor infiltration by adoptively-transferred T cells (Liu et al., 2013). The first study conducted to evaluate the efficacy of a combination approach was carried out by Ribas et al. (2013). In this phase I clinical trial, vemurafenib plus ipilimumab (an anti-Cytotoxic T-Lymphocyte Antigen 4 [CTLA-4] monoclonal antibody) were administered to previously-untreated patients with BRAF or MEK inhibitors, and anti-CTLA4 (Ribas et al. (2013). Interestingly, the study was closed due to severe liver toxicity (Liu et al., 2013). A similar fate befell the dabrafenib, trametinib, and ipilimumab triple combination in metastatic BRAF exon 15 p.V600E/K-mutated melanoma patients due to grade 3 colitis complicated by perforation (Minor et al., 2015). Conversely, the dabrafenib plus ipilimumab combination was seen to be well tolerated (Minor et al., 2015). Different results in terms of tolerability emerged for anti-Programmed death 1 (PD-1) and anti-PD-L1 monoclonal antibodies. Promising results have been reported for the durvalumab or pembrolizumab plus dabrafenib plus trametinib combination (ORR 69 % and 67 %, respectively) (Pavlick et al., 2019). In a phase Ib clinical trial, Sullivan et al. reported promising anti-tumor activity with important but manageable toxicity for the atezolizumab plus cobimetinib plus vemurafenib triplet in BRAF exon 15 p.V600-mutated advanced melanoma patients (objective response rate was 71.8 %) (Sullivan et al., 2019). More recently, the efficacy of the combination of immunotherapy (atezolizumab) and targeted therapy (vemurafenib and cobimetinib) in

BRAF p.V600-mutated advanced melanoma patients was reported in a phase III clinical trial (IMspire150) (Gutzmer et al., 2020). In this study, the combination showed a significant increase in PFS respect to the association vemurafenib and cobimetinib (15.1 vs. 10.6 months) with acceptable safety and tolerability (Gutzmer et al., 2020). Following these interesting results, the FDA approved the combination for the treatment of advanced melanoma patients harboring BRAF p.V600 mutations (FDA Approves Genentech's Tecentriq plus Cotellic and Zelboraf for People With Advanced Melanoma [news release], 2020)].

Either in target treatment or immunotherapy, ¹⁸F-fluorodeoxyglucose positron emission tomography/computed tomography (¹⁸F-FDG PET/CT) seems to be a more valuable option than CT for predicting and monitoring therapy response and toxicity (Olthof et al., 2020; Bischof et al., 2020).

7.2. Lung Cancer

A number of different experiences have been reported regarding treatment for BRAF-mutated advanced NSCLC patients (Giopanou and Pintzas, 2020). Cardarella et al. reported that advanced NSCLC patients with BRAF mutations (p.V600E or non-p.V600E) treated with the platinum-based combination approach did not show a significant difference in PFS and OS to BRAF wild-type patients (Cardarella et al., 2013). Interestingly, BRAF exon 15 p.V600E-mutated patients showed a shorter PFS than non-p.V600E-mutated patients (4.1 vs. 8.9 months; p = 0.297) (Cardarella et al., 2013). Similar results were reported by Ding et al. (2017). Conversely, Dagogo-Jack et al. reported shorter PFS and OS for class II and class III mutations (non-p.V600) when treated with chemotherapy (Dagogo-Jack et al., 2019). As far as the targeted treatment approach is concerned, advanced NSCLC patients harboring BRAF exon 15 p.V600E- or non-p.V600E-mutations receive a different treatment schedule. With regard to targeted treatment, Gautschi et al. reported the efficacy of targeted therapy (vemurafenib, dabrafenib, sorafenib) in advanced BRAF-mutated NSCLC patients (Gautschi et al., 2015). Interestingly, OS was higher for patients with BRAF exon 15 p.V600E mutations than non-p.V600E mutations (25.3 vs. 11.8 months) (Gautschi et al., 2015). More recently, Mazieres et al. confirmed the greater efficacy of vemurafenib in the BRAF p.V600 group than in non-p.V600 patients (Mazieres et al., 2020). As reported for melanoma patients, BRAF exon 15 p.V600E-mutated patients may benefit from a BRAF inhibitor (dabrafenib) plus MEK inhibitor (trametinib) combination (Planchard et al., 2016; Planchard et al., 2017). Overall, in a phase II clinical trial, the combination (dabrafenib plus trametinib) demonstrated promising results in terms of overall response, prolonged duration of response, and manageable toxicity in previously-treated (Planchard et al., 2016) and untreated (Planchard et al., 2017) advanced NSCLC patients harboring the BRAF exon 15 p.V600E mutation. Less evidence is available to suggest the role of targeted treatments in non-p.V600E-mutated cases. In a cell line setting, Kotani et al. showed that the inhibition of the MAPK pathway by MEK inhibitor monotherapy (trametinib) was insufficient in lung cancer cell lines harboring BRAF class II and III mutations (Kotani et al., 2018). This was attributed to ERK activation through the receptor tyrosine kinase (RTK) pathway (e.g. EGFR) with the activation of RAS and CRAF (Kotani et al., 2018). The Authors hypothesized the possibility of adopting the MEK inhibitors plus TKI combination for BRAF non-p.V600E patients (Kotani et al., 2018). Two case reports showed the efficacy of sorafenib (a multi-target kinase inhibitor able to block CRAF, BRAF, KIT Proto-Oncogene, Receptor Tyrosine Kinase [c-KIT], Fms Related Receptor Tyrosine Kinase 3 [FLT-3], Rearranged During Transfection [RET], vascular endothelial growth factor receptor 2 [VEGFR-2], VEGFR-3 and platelet-derived growth factor receptor alpha [PDGFRA]) in BRAF non-p.V600-mutated patients (exon 11 p.G469R and exon 11 p.G469 V) (Serenio et al., 2015; Casadei Gardini et al., 2016).

Another possible approach in BRAF-mutated patients could be ERK inhibitors (e.g. ulixertinib, an ERK1/2 kinase inhibitor) (Sullivan et al.,

2018) and pan-RAF inhibitors (e.g. LY3009120) (Sullivan et al., 2020).

As far as immunotherapy is concerned, Dudnik et al. reported favorable activity in both *BRAF* exon 15 p.V600E- and *BRAF* non-p.V600E-mutated advanced NSCLC patients (Dudnik et al., 2018). Conversely, Tan et al., in a small study highlighted the greater efficacy of chemotherapy over immunotherapy in *BRAF*-mutated patients (Tan et al., 2020). Guisier et al. observed an objective response rate among *BRAF* mutated NSCLC patients treated with immunotherapy of 30 % (33 % non-p.V600E, 26 % exon 15 p.V600E), similar to the results obtained in unselected pretreated NSCLC patients (Guisier et al., 2020).

8. BRAF mutations in lung cancer and melanoma: Italian real-world perspectives

In order to evaluate the real-world data on *BRAF* mutation assessment in both melanoma and NSCLC patients, we carried out an online survey involving nine laboratories with specialized expertise in this field, by asking the different centers to retrieve molecular data for the 2018–2020 period. All information regarding human material were managed using anonymous numerical codes, and all samples were handled in compliance with the Declaration of Helsinki (<http://www.wma.net/en/30publications/10policies/b3/>).

As far as *BRAF* analysis in NSCLC patients is concerned, a total of 3102 cases were retrieved from the digital archives. Overall, *BRAF* mutational status was assessed by NGS in the majority (5/9, 55.6 %) of the laboratories, ensuring an optimization of the starting material for the analysis of all clinically-relevant biomarkers, simultaneously. A MALDI-TOF-based approach was adopted by one institution (11.1 %). Both commercially-available and custom panels were adopted for the analysis. After highly-multiplexed approaches, the RT-PCR method was the second most frequently-adopted technique (3/9, 33.3 %). This approach may be useful for *BRAF* mutational status evaluation in this patient setting, due to need to identify *BRAF* exon 15 p.V600E alone for targeted treatment purposes (Fig. 4). As expected, only a minimal percentage of cases (167/3102, 5.4 %) harbored a *BRAF* mutation. Focusing the attention on the targetable *BRAF* exon 15 p.V600E, only a very small number of patients (71/3102, 2.3 %) may be suitable for dabrafenib plus trametinib combination therapy (Fig. 4).

Of the nine laboratories involved in the study, just four (44.4 %) performed and shared the results regarding *BRAF* mutational status in their routine practice. In the 2018–2020 period, a total of 801 melanoma cases underwent *BRAF* mutational status assessment. As regards the platform adopted, two laboratories (50.0 %) prefer an RT-PCR approach, whereas the other two laboratories adopted NGS or MOLDI-TOF approaches. Overall, 380 patients (47.4 %) harbored a *BRAF* mutation. *BRAF* exon 15 p.V600 actionable mutations were the mutations most commonly detected (368/801, 45.9 %), with a higher representation of *BRAF* exon 15 p.V600E (312/801, 39.0 %), followed by p.V600

K (48/801, 6.0 %), p.V600R (6/801, 0.7 %), p.V600D (1/801, 0.1 %) and p.V600 M (1/801, 0.1 %) (Fig. 5).

9. Conclusion

BRAF mutations play a key role in the management of both advanced melanoma and advanced NSCLC patients. Several clinical trials have demonstrated the efficacy of different targeted therapies (*BRAF* inhibitors, MEK inhibitors, combination therapies, immunotherapy) in *BRAF*-mutated patients. For these reasons, *BRAF* mutational assessment is critical for adequate management of advanced melanoma and NSCLC patients. In our real-world experience, a total of 3102 advanced NSCLC and 801 melanoma patients were assessed for *BRAF* mutational status. Amongst the NSCLC patients, as expected, only a minimal percentage of cases (5.4 %) harbored a *BRAF* mutation. Considering *BRAF* exon 15 p.V600E, a total of 71 patients (71/3102, 2.3 %) may be eligible for treatment with the dabrafenib plus trametinib combination. Of the total of 801 tested melanoma patients, 45.9 % (368/801) of patients harbored a *BRAF* exon p.V600 mutation and were potentially eligible for targeted therapy. As reported in literature and in our experience, several methods can be adopted to assess *BRAF* mutational status. IHC may be a reliable tool due to its simplicity, low costs, rapid TAT, high sensitivity and specificity, and its ability to identify mutant protein distribution at single-cell level. However, it is important to bear in mind the fact that false-negative results are possible due to heterogeneity or low concentrations of *BRAF* exon 15 p.V600E and the inability to identify other clinical relevant variants. Direct sequencing still represents the gold standard for the detection of point mutations and small variant detection, due to the technique's reliability, availability, reagent affordability, and relatively low costs. However, its poor sensitivity constitutes an important limitation. Despite having a higher sensitivity than direct Sequencing, the RT-PCR approach showed a limited reference range. NGS may be a reliable approach for overcoming these limits. This fascinating technology enables the analysis of different hotspot mutations for different patients, simultaneously. However, its higher costs, longer TAT and the need for highly-skilled personnel and careful validation precludes its availability in some predictive molecular pathology laboratories.

To conclude, careful attention should be paid to the detection of *BRAF* mutations due to the considerable efficacy of targeted treatment in *BRAF*-mutated cancer patients. Due to the high specificity of the different methods adopted and to avoid the risk and under-treatment of false-negative cases, careful attention should also be dedicated to confirming those cases with a negative result, especially when NGS methods are adopted.

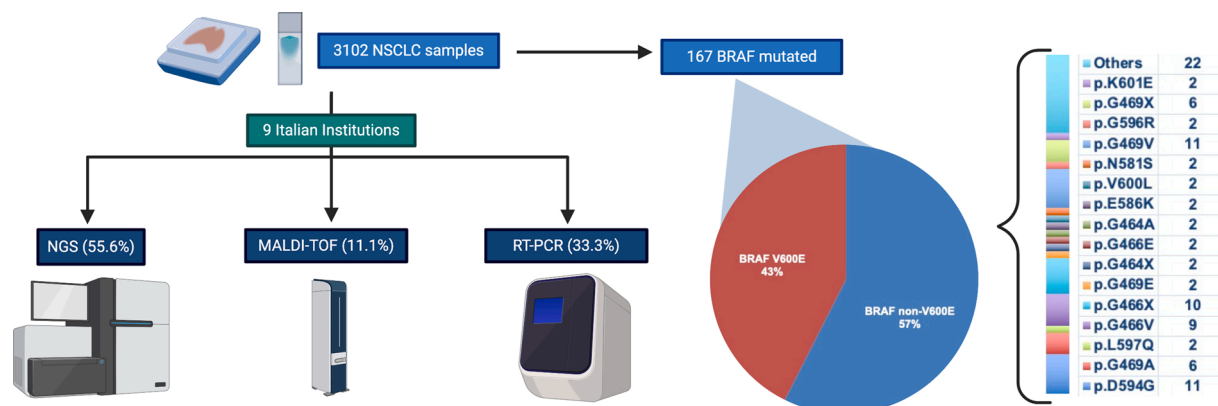


Fig. 4. Italian real word *BRAF* mutations distribution in NSCLC patients. Credit by Biorender.com.

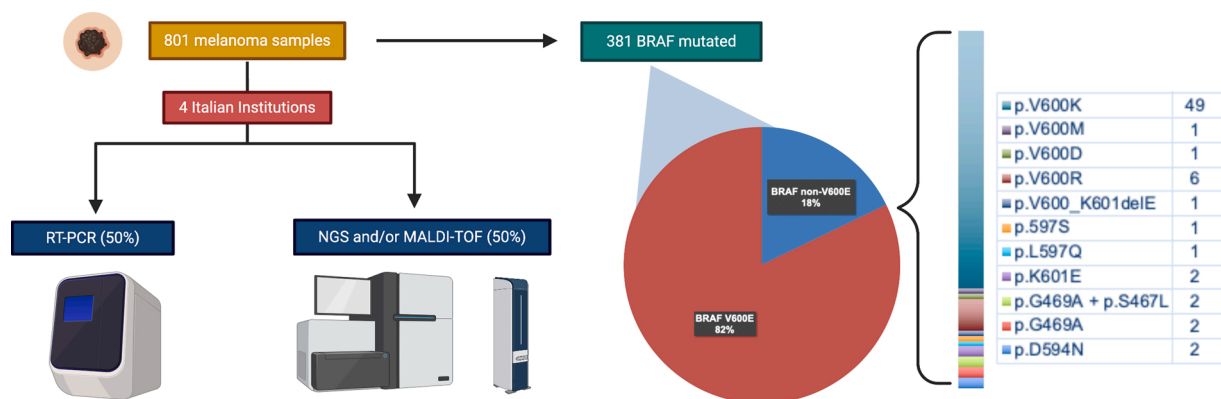


Fig. 5. Italian real word *BRAF* mutations distribution in melanoma patients. Credit by Biorender.com.

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Umberto Malapelle: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization, Project administration, Funding acquisition. **Giulio Rossi:** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - review & editing, Visualization, Project administration, Funding acquisition. **Pasquale Pisapia:** Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization, Project administration, Funding acquisition. **Massimo Barberis:** Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - review & editing, Visualization, Project administration, Funding acquisition. **Fiamma Buttitta:** Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - review & editing, Visualization, Project administration, Funding acquisition. **Antonio Maria Grimaldi:** Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - review & editing, Visualization, Project administration, Funding acquisition. **Antonino Iaccarino:** Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - review & editing, Visualization, Project administration, Funding acquisition. **Antonio Marchetti:** Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - review & editing, Visualization, Project administration, Funding acquisition. **Daniela Massi:** Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - review & editing, Visualization, Project administration, Funding acquisition. **Daniela Medicina:** Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - review & editing, Visualization, Project administration, Funding acquisition. **Fabio Mele:** Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - review & editing, Visualization, Project administration, Funding acquisition. **Roberta Minari:** Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - review & editing,

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Declaration of Competing Interest

Umberto Malapelle reports personal fees (as speaker bureau or advisor) from Boehringer Ingelheim, AstraZeneca, Roche, MSD, Amgen and Merck, unrelated to the current work. Massimo Barberis reports personal fees (as speaker bureau or advisor) from AstraZeneca, Roche, MSD, Pfizer, Biocartis, Illumina unrelated to the current work. Antonio Maria Grimaldi received honoraria from Bristol Myers Squibb, Novartis, MSD, Roche Genetech and Merck Serono, Travel Grant from Bristol Myers Squibb, MSD and Merck Serono, and had advisory/consultant role for Bristol Myers Squibb, MSD and Novartis, unrelated to the current work. Daniela Massi has received honoraria for professional services and consultancy for Novartis, Bayer HealthCare Pharmaceuticals Inc., Pierre-Fabre, Sanofi Genzyme, MSD Italia S.r.l., Roche, unrelated to the current work. Fabio Pagni reports personal fees (for service in the speaker bureau and as an advisor) from Boehringer Ingelheim, AstraZeneca, Roche, MSD, Amgen, outside the submitted work. Giuseppe Palmieri has/had advisory role for Bristol Myers Squibb (BMS), Incyte, Merck Sharp & Dohme (MSD), Novartis, Pierre Fabre, and Roche-Genetech, unrelated to the current work. Giancarlo Troncone reports personal fees (as speaker bureau or advisor) from Roche, MSD, Pfizer and Bayer, unrelated to the current work. These companies had no role

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The other authors declare no potential conflicts of interest.

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References

- Aisner, D.L., Rumery, M.D., Merrick, D.T., Kondo, K.L., Nijmeh, H., Linderman, D.J., Doebele, R.C., Thomas, N., Chesnut, P.C., Varella-Garcia, M., Franklin, W.A., Camidge, D.R., 2016. Do more with less: tips and techniques for maximizing small biopsy and cytology specimens for molecular and ancillary testing: the university of colorado experience. *Arch. Pathol. Lab. Med.* 140, 1206–1220.
- Allegrini, S., Antona, J., Mezzapelle, R., Miglio, U., Paganotti, A., Veggiani, C., Frattini, M., Monga, G., Balbo, P., Boldorini, R., 2012. Epidermal growth factor receptor gene analysis with a highly sensitive molecular assay in routine cytologic specimens of lung adenocarcinoma. *Am. J. Clin. Pathol.* 138, 377–381.
- ALSA, Vicente, Bianchini, R.A., Laus, A.C., Macedo, G., Reis, R.M., Vazquez, V.L., 2019. Comparison of protocols for removal of melanin from genomic DNA to optimize PCR amplification of DNA purified from highly pigmented lesions. *Histol. Histopathol.* 34, 1089–1096.
- Alvarez, J.G.B., Otterson, G.A., 2019. Agents to treat BRAF-mutant lung cancer. *Drugs Context* 8, 212566.
- Anderson, S., Bloom, K.J., Vallera, D.U., Rueschoff, J., Meldrum, C., Schilling, R., Kovach, B., Lee, J.R., Ochoa, P., Langland, R., Halait, H., Lawrence, H.J., Dugan, M. C., 2012. Multisite analytic performance studies of a real-time polymerase chain reaction assay for the detection of BRAF V600E mutations in formalin-fixed, paraffin-embedded tissue specimens of malignant melanoma. *Arch. Pathol. Lab. Med.* 136, 1385–1391.
- Ascierto, P.A., McArthur, G.A., Dréno, B., Atkinson, V., Liskay, G., Di Giacomo, A.M., Mandalà, M., Demidov, L., Stroyakovskiy, D., Thomas, L., de la Cruz-Merino, L., Dutriaux, C., Garbe, C., Yan, Y., Wongchenko, M., Chang, I., Hsu, J.J., Koralek, D.O., Rooney, I., Ribas, A., Larkin, J., 2016. Cobimetinib combined with vemurafenib in advanced BRAF(V600)-mutant melanoma (coBRIM): updated efficacy results from a randomised, double-blind, phase 3 trial. *Lancet Oncol.* 17, 1248–1260.
- Bakker, E., 2006. Is the DNA sequence the gold standard in genetic testing? Quality of molecular genetic tests assessed. *Clin. Chem.* 52, 557–558.
- Barbareschi, M., Barberis, M., Buttitta, F., Doglioni, C., Fiorentino, M., Fontanini, G., Franco, R., Marchetti, A., Rossi, G., Troncone, G., 2018. Predictive markers in lung cancer: a few hints for the practicing pathologist. *Pathologica* 110, 29–38.
- Bellecivine, C., Migliat�o, I., Sgariglia, R., Nacchio, M., Vigliar, E., Pisapia, P., Iaccarino, A., Bruzzese, D., Fonderico, F., Salvatore, D., Biondi, B., Masone, S., Novizio, V., Scavuzzo, F., Serino, D., De Palma, M., Chiofalo, M.G., Botti, G., Pezzullo, L., Nuzzo, V., Spiezia, S., De Chiara, G., Iorio, S., Conzo, G., Docimo, G., Faggiaro, A., Bongiovanni, M., Malapelle, U., Colao, A., Triassi, M., Troncone, G., Tiroide Network, 2020. Evaluation of BRAF, RAS, RET/PTC, and PAX8/PPARγ alterations in different Bethesda diagnostic categories: a multicentric prospective study on the validity of the 7-gene panel test in 1172 thyroid FNAs deriving from different hospitals in South Italy. *Cancer Cytopathol.* (128), 107–118.
- Benlloch, S., Botero, M.L., Beltran-Alamillo, J., Mayo, C., Gimenez-Capitań, A., de Aguirre, I., Queralt, C., Ramirez, J.L., Ramoń y Cajal, S., Klughammer, B., Schlegel, M., Bordogna, W., Chen, D., Zhang, G., Kovach, B., Shieh, F., Palma, J.F., Wu, L., Lawrence, H.J., Taron, M., 2014. Clinical validation of a PCR assay for the detection of EGFR mutations in non-small-cell lung cancer: retrospective testing of specimens from the EURTAC trial. *PLoS One* 9, e89518.
- Bisschop, C., de Heer, E.C., Brouwers, A.H., Hospers, G.A.P., Jalving, M., 2020. Rational use of 18F-FDG PET/CT in patients with advanced cutaneous melanoma: a systematic review. *Crit. Rev. Oncol. Hematol.* 153, 103044.
- Bonner, T.I., Kerby, S.B., Sutrave, P., Gunnell, M.A., Mark, G., Rapp, U.R., 1985. Structure and biological activity of human homologs of the raf/mil oncogene. *Mol. Cell. Biol.* 5, 1400–1407.
- Bracht, J.W.P., Karachaliou, N., Bivona, T., Lanman, R.B., Faull, I., Nagy, R.J., Drozdowskyj, A., Berenguer, J., Fernandez-Bruno, M., Molina-Vila, M.A., Rosell, R., 2019. BRAF mutations Classes I, II, and III in NSCLC patients included in the SLLIP Trial: the need for a new pre-clinical treatment rationale. *Cancers (Basel)* 11, 1381.
- Bradish, J.R., Cheng, L., 2014. Molecular pathology of malignant melanoma: changing the clinical practice paradigm toward a personalized approach. *Hum. Pathol.* 45, 1315–1326.
- Buder-Bakhaya, K., Machiraju, D., Hassel, J.C., 2017. Liquid biopsy: value for melanoma therapy. *Oncol. Res. Treat.* 40, 430–434.
- Cardarella, S., Ogino, A., Nishino, M., Butaney, M., Shen, J., Lydon, C., Yeap, B.Y., Sholl, L.M., Johnson, B.E., Jänne, P.A., 2013. Clinical, pathologic, and biologic features associated with BRAF mutations in non-small cell lung cancer. *Clin. Cancer Res.* 19, 4532–4540.
- Casadei Gardini, A., Chiadini, E., Faloppi, L., Marisi, G., Delmonte, A., Scartozzi, M., Loretelli, C., Lucchesi, A., Oboldi, D., Dubini, A., Frassinetti, G.L., Ulivi, P., 2016. Efficacy of sorafenib in BRAF-mutated non-small-cell lung cancer (NSCLC) and no response in synchronous BRAF wild type-hepatocellular carcinoma: a case report. *BMC Cancer* 16, 429.
- Chapman, P.B., Hauschild, A., Robert, C., Haanen, J.B., Ascierto, P., Larkin, J., Dummer, R., Garbe, C., Testori, A., Maio, M., Hogg, D., Lorigan, P., Lebbe, C., Jouary, T., Schadendorf, D., Ribas, A., O'Day, S.J., Sosman, J.A., Kirkwood, J.M., Eggermont, A.M., Dreno, B., Nolop, K., Li, J., Nelson, B., Hou, J., Lee, R.J., Flaherty, K.T., McArthur, G.A., BRIM-3 Study Group, 2011. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *N. Engl. J. Med.* 364, 2507–2516.
- Cheng, L., Lopez-Beltran, A., Massari, F., MacLennan, G.T., Montironi, R., 2018. Molecular testing for BRAF mutations to inform melanoma treatment decisions: a move toward precision medicine. *Mod. Pathol.* 31, 24–38.
- Chong, H., Lee, J., Guan, K.L., 2001. Positive and negative regulation of Raf kinase activity and function by phosphorylation. *EMBO J.* 20, 3716–3727.
- Clark, D.P., 2009. Seize the opportunity: underutilization of fine-needle aspiration biopsy to inform targeted cancer therapy decisions. *Cancer* 117, 289–297.
- Coit, D.G., Thompson, J.A., Albertini, M.R., Barker, C., Carson, W.E., Contreras, C., Daniels, G.A., DiMaio, D., Fields, R.C., Fleming, M.D., Freeman, M., Galan, A., Gastman, B., Guild, V., Johnson, D., Joseph, R.W., Lange, J.R., Nath, S., Olszanski, A. J., Ott, P., Gupta, A.P., Ross, M.I., Salama, A.K., Skitzki, J., Sosman, J., Swetter, S.M., Tanabe, K.K., Wuthrick, E., McMillian, N.R., Eng, A.M., 2019. Cutaneous melanoma, version 2.2019, NCCN clinical practice guidelines in oncology. *J. Compr. Canc. Netw.* 17, 367–402.
- Colomba, E., Hélias-Rodzewicz, Z., Von Deimling, A., Marin, C., Terrones, N., Pechaud, D., Surel, S., Côté, J.F., Peschard, F., Capper, D., Blons, H., Zimmermann, U., Clerici, T., Saïag, P., Emile, J.F., 2013. Detection of BRAF p.V600E mutations in melanomas: comparison of four methods argues for sequential use of immunohistochemistry and pyrosequencing. *J. Mol. Diagn.* 15, 94–100.
- Colombino, M., Capone, M., Lissia, A., Cossu, A., Rubino, C., De Giorgi, V., Massi, D., Fonsatti, E., Staibano, S., Nappi, O., Pagani, E., Casula, M., Manca, A., Sini, M., Franco, R., Botti, G., Caracò, C., Mozzillo, N., Ascierto, P.A., Palmieri, G., 2012. BRAF/NRAS mutation frequencies among primary tumors and metastases in patients with melanoma. *J. Clin. Oncol.* 30, 2522–2529.
- Cree, I.A., Deans, Z., Ligtner, M.J., Normanno, N., Edsjö, A., Rouleau, E., Solé, F., Thunnissen, E., Timens, W., Schuurin, E., Dequeker, E., Murray, S., Dietel, M., Groenen, P., Van Krieken, J.H., European Society of Pathology Task Force on Quality Assurance in Molecular Pathology, Royal College of Pathologists, 2014. Guidance for laboratories performing molecular pathology for cancer patients. *J. Clin. Pathol.* 67, 923–931.
- Dagogo-Jack, I., Martinez, P., Yeap, B.Y., Ambrogio, C., Ferris, L.A., Lydon, C., Nguyen, T., Jessop, N.A., Iafate, A.J., Johnson, B.E., Lennerz, J.K., Shaw, A.T., Awad, M.M., 2019. Impact of BRAF mutation class on disease characteristics and clinical outcomes in BRAF-mutant lung cancer. *Clin. Cancer Res.* 25, 158–165.
- Dankner, M., Rose, A.A.N., Rajkumar, S., Siegel, P.M., Watson, I.R., 2018. Classifying BRAF alterations in cancer: new rational therapeutic strategies for actionable mutations. *Oncogene* 37, 3183–3199.
- Davies, H., Bignell, G.R., Cox, C., Stephens, P., Edkins, S., Clegg, S., Teague, J., Woffendin, H., Garnett, M.J., Bottomley, W., Davis, N., Dicks, E., Ewing, R., Floyd, Y., Gray, K., Hall, S., Hawes, R., Hughes, J., Kosmidou, V., Menzies, A., Mould, C., Parker, A., Stevens, C., Watt, S., Hooper, S., Wilson, R., Jayatilake, H., Gusterson, B.A., Cooper, C., Shipley, J., Hargrave, D., Pritchard-Jones, K., Maitland, N., Chenevix-Trench, G., Riggins, G.J., Bigner, D.D., Palmieri, G., Cossu, A., Flanagan, A., Nicholson, A., Ho, J.W., Leung, S.Y., Yuen, S.T., Weber, B.L., Seigler, H.F., Darrow, T.L., Paterson, H., Marais, R., Marshall, C.J., Wooster, R., Stratton, M.R., Futreal, P.A., 2002. Mutations of the BRAF gene in human cancer. *Nature* 417, 949–954.
- De Luca, C., Gragnano, G., Pisapia, P., Vigliar, E., Malapelle, U., Bellecivine, C., Troncone, G., 2017. EGFR mutation detection on lung cancer cytological specimens by the novel fully automated PCR-based Idylla EGFR Mutation Assay. *J. Clin. Pathol.* 70, 295–300.
- De Luca, C., Conticelli, F., Leone, A., Gragnano, G., Salatiello, M., Galasso, P., Pisapia, P., Grillo, L.R., Iaccarino, A., Vigliar, E., Bellecivine, C., Malapelle, U., Troncone, G., 2019. Is the Idylla EGFR Mutation Assay feasible on archival stained cytological smears? A pilot study. *J. Clin. Pathol.* 72, 609–614.
- De Rook, W., De Vriendt, V., Normanno, N., Ciardiello, F., Tejpar, S., 2011. KRAS, BRAF, PIK3CA, and PTEN mutations: implications for targeted therapies in metastatic colorectal cancer. *Lancet Oncol.* 12, 594–603.
- de Unamuno Bustos, B., Murria Estal, R., Pérez Simó, G., de Juan Jimenez, I., Escutia Muñoz, B., Rodríguez Serna, M., Alegre de Miquel, V., Llavador Ros, M., Ballester Sánchez, R., Nagore Enguñadano, E., Palanca Suela, S., Botella Estrada, R., 2017. Towards personalized medicine in melanoma: implementation of a clinical next-generation sequencing panel. *Sci. Rep.* 7, 495.
- Dhillon, A.S., Meikle, S., Yazici, Z., Eulitz, M., Kolch, W., 2002. Regulation of Raf-1 activation and signalling by dephosphorylation. *EMBO J.* 21, 64–71.
- Ding, X., Zhang, Z., Jiang, T., Li, X., Zhao, C., Su, B., Zhou, C., 2017. Clinicopathologic characteristics and outcomes of Chinese patients with non-small-cell lung cancer and BRAF mutation. *Cancer Med.* 6, 555–562.
- Doubrovsky, A., Scolyer, R., Murali, R., McKenzie, P., Watson, G., Lee, C., McLeod, D.J., McCarthy, W., Uren, R., Stretch, J., Saw, R., Thompson, J.F., 2008. Diagnostic accuracy of fine needle biopsy for metastatic melanoma and its implications for patient management. *Ann. Surg. Oncol.* 15, 323–332.
- Douillard, J.Y., Ostoros, G., Cobo, M., Ciuleanu, T., McCormack, R., Webster, A., Milenkova, T., 2014. First-line gefitinib in Caucasian EGFR mutation-positive NSCLC patients: a phase-IV, open-label, single-arm study. *Br. J. Cancer* 110, 55–62.

- Dudnik, E., Peled, N., Nechushtan, H., Wollner, M., Onn, A., Agbarya, A., Moskovitz, M., Keren, S., Popovits-Hadari, N., Urban, D., Mishaeli, M., Zer, A., Allen, A.M., Rabinovich, N.M., Rotem, O., Kuznetsov, T., Shochat, T., Roisman, L.C., Bar, J., Israel Lung Cancer Group, 2018. BRAF mutant lung cancer: programmed death ligand 1 expression, tumor mutational burden, microsatellite instability status, and response to immune checkpoint inhibitors. *J. Thorac. Oncol.* 13, 1128–1137.
- Eckhart, L., Bach, J., Ban, J., Tschachler, E., 2000. Melanin binds reversibly to thermostable DNA polymerase and inhibits its activity. *Biochem. Biophys. Res. Commun.* 271, 726–730.
- Ellison, G., Donald, E., McWalter, G., Knight, L., Fletcher, L., Sherwood, J., Cantarini, M., Orr, M., Speake, G., 2010. A comparison of ARMS and DNA sequencing for mutation analysis in clinical biopsy samples. *J. Exp. Clin. Cancer Res.* 29, 132.
- Eriksson, H., Zebary, A., Vassilaki, I., Omholt, K., Ghaderi, M., Hansson, J., 2015. BRAFV600E protein expression in primary cutaneous malignant melanomas and paired metastases. *JAMA Dermatol.* 151, 410–416.
- Etinger, D.S., Aisner, D.L., Wood, D.E., Akerley, W., Bauman, J., Chang, J.Y., Chirieac, L.R., D'Amico, T.A., Dilling, T.J., Dobbela, M., Govindan, R., Gubens, M.A., Hennon, M., Horn, L., Lackner, R.P., Lanuti, M., Leal, T.A., Lilienbaum, R., Lin, J., Loo, B.W., Martins, R., Otterson, G.A., Patel, S.P., Reckamp, K., Riely, G.J., Schild, S.E., Shapiro, T.A., Stevenson, J., Swanson, S.J., Tauer, K., Yang, S.C., Gregory, K., 2018. NCCN guidelines insights: non-small cell lung cancer, version 5.2018. *J. Compr. Canc. Netw.* 16, 807–821.
- FDA Approves Genentech's Tecentriq plus Celastrol and Zelboraf for People With Advanced Melanoma [news release], 2020. South San Francisco, CA. Approved July 30, 2020. [gene.com/media/press-releases/14868/2020-07-30/fda-approved-genentechs-tecentriq-plus-c](https://www.fda.gov/media/press-releases/14868/2020-07-30/fda-approved-genentechs-tecentriq-plus-c). Approved July 30, 2020.
- Fraza, A., Rethacker, L., Jeudy, G., Colombo, M., Pasmant, E., Avril, M.F., Toubert, A., Moins-Teisserenc, H., Roelens, M., Dalac, S., Maubec, E., Caignard, A., 2020. BRAF inhibitor resistance of melanoma cells triggers increased susceptibility to natural killer cell-mediated lysis. *J. Immunother. Cancer* 8, e000275.
- Frison, D., Friedlaender, A., Malapelle, U., Banna, G., Addeo, A., 2020. A BRAF new world. *Crit. Rev. Oncol. Hematol.* Epub ahead of print.
- Frouin, E., Maudelonde, T., Senal, R., Larrière, X., Costes, V., Godreuil, S., Vendrell, J.A., Solassol, J., 2016. Comparative methods to improve the detection of BRAF V600 mutations in highly pigmented melanoma specimens. *PLoS One* 11, e0158698.
- Fumagalli, C., Vacirca, D., Rappa, A., Passaro, A., Guarize, J., Rafanelli Raviello, P., de Marinis, F., Spaggiari, F., Casadio, C., Viale, G., Barberis, M., Guerini-Rocco, E., 2018. The long tail of molecular alterations in non-small cell lung cancer: a single-institution experience of next-generation sequencing in clinical molecular diagnostics. *J. Clin. Pathol.* 71, 767–773.
- Gaiser, M.R., von Bubnoff, N., Gebhardt, C., Utikal, J.S., 2018. Liquid biopsy to monitor melanoma patients. *J. Dermatol. Ges.* 16, 405–414.
- Gautschi, O., Milia, J., Cabarró, B., Bluthgen, M.V., Besse, B., Smit, E.F., Wolf, J., Peters, S., Früh, M., Koeberle, D., Oulkhour, Y., Schuler, M., Curioni-Fontecedro, A., Huret, B., Kerjovan, M., Michels, S., Pall, G., Rothschild, S., Schmid-Bindert, G., Scheffler, M., Veillon, R., Wannesson, L., Diebold, J., Zalcman, G., Filleron, T., Mazières, J., 2015. Targeted therapy for patients with BRAF-mutant lung cancer: results from the European EURAF cohort. *J. Thorac. Oncol.* 10, 1451–1457.
- Giopanou, I., Pintzas, A., 2020. RAS and BRAF in the foreground for non-small cell lung cancer and colorectal cancer: similarities and main differences for prognosis and therapies. *Crit. Rev. Oncol. Hematol.* 146 (102859).
- Gow, C.H., Hsieh, M.S., Lin, Y.T., Liu, Y.N., Shih, J.Y., 2019. Validation of immunohistochemistry for the detection of BRAF V600E-Mutated lung adenocarcinomas. *Cancers (Basel)* 11, 866.
- Gray, E.S., Rizos, H., Reid, A.L., Boyd, S.C., Pereira, M.R., Lo, J., Tembe, V., Freeman, J., Lee, J.H., Scolyer, R.A., Siew, K., Lomma, C., Cooper, A., Khattak, M.A., Meniawy, T.M., Long, G.V., Carlino, M.S., Millward, M., Ziman, M., 2015. Circulating tumor DNA to monitor treatment response and detect acquired resistance in patients with metastatic melanoma. *Oncotarget* 6, 42008–42018.
- Guisier, F., Dubos-Arvic, C., Viñas, F., Dobre, H., Ricordel, C., Ropert, S., Janicot, H., Bernardi, M., Fournel, P., Lamy, R., Pérol, M., Dauba, J., Gonzales, G., Falchero, L., Decroisette, C., Assouline, P., Chouaid, C., Bylicki, O., 2020. Efficacy and safety of Anti-PD-1 immunotherapy in patients with advanced NSCLC with BRAF, HER2, or MET mutations or RET translocation: GFPC 01-2018. *J. Thorac. Oncol.* 15, 628–636.
- Gutzmer, R., Stroyakovskiy, D., Gogas, H., Robert, C., Lewis, K., Protsenko, S., Pereira, R. P., Eigentler, T., Rutkowski, P., Demidov, L., Manikhas, G.M., Yan, Y., Huang, K.C., Uyei, A., McNally, V., McArthur, G.A., Ascierto, P.A., 2020. Atezolizumab, vemurafenib, and cobimetinib as first-line treatment for unresectable advanced BRAFV600E mutation-positive melanoma (IMspire150): primary analysis of the randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 395, 1835–1844.
- Hauschild, A., Grob, J.J., Demidov, L.V., Jouary, T., Gutzmer, R., Millward, M., Rutkowski, P., Blank, C.U., Miller Jr, W.H., Kaempgen, E., Martin-Algarra, S., Karaszewska, B., Mauch, C., Chiarion-Sileni, V., Martin, A.M., Swann, S., Haney, P., Mirakhur, B., Guckert, M.E., Goodman, V., Chapman, P.B., 2012. Dabrafenib in BRAF-mutated metastatic melanoma: a multicentre, open-label, phase 3 randomised controlled trial. *Lancet* 380, 358–365.
- Hauschild, A., Ascierto, P.A., Schadendorf, D., Grob, J.J., Ribas, A., Kiecker, F., Dutriaux, C., Demidov, L.V., Lebbé, C., Rutkowski, P., Blank, C.U., Gutzmer, R., Millward, M., Kefford, R., Haas, T., D'Amelio Jr, A., Gasal, E., Mookerjee, B., Chapman, P.B., 2020. Long-term outcomes in patients with BRAF V600E-mutant metastatic melanoma receiving dabrafenib monotherapy: analysis from phase 2 and 3 clinical trials. *Eur. J. Cancer* 125, 114–120.
- Hayward, N.K., Wilmott, J.S., Waddell, N., Johansson, P.A., Field, M.A., Nones, K., Patch, A.M., Kakavand, H., Alexandrov, L.B., Burke, H., Jakrot, V., Kazakov, S., Holmes, O., Leonard, C., Sabarinathan, R., Mularoni, L., Wood, S., Xu, Q., Waddell, N., Tembe, V., Pupo, G.M., De Paoli-Iseppi, R., Vilain, R.E., Shang, P., Lau, L.M.S., Dagg, R.A., Schramm, S.J., Pritchard, A., Dutton-Regester, K., Newell, F., Fitzgerald, A., Shang, C.A., Grimmond, S.M., Pickett, H.A., Yang, J.Y., Stretch, J.R., Behren, A., Kefford, R.F., Hersey, P., Long, G.V., Cebon, J., Shackleton, M., Spillane, A.J., Saw, R.P.M., López-Bigas, N., Pearson, J.V., Thompson, J.F., Scolyer, R.A., Mann, G.J., 2017. Whole-genome landscapes of major melanoma subtypes. *Nature* 545, 175–180.
- Heinzelinger, L., Kühnapfel, S., Meckbach, D., Baiter, M., Kaempgen, E., Keikavoussi, P., Schuler, G., Agaimy, A., Bauer, J., Hartmann, A., Kiesewetter, F., Schneider-Stock, R., 2013. Rare BRAF mutations in melanoma patients: implications for molecular testing in clinical practice. *Br. J. Cancer* 108, 2164–2171.
- Herbretau, G., Vallée, A., Charpentier, S., Normanno, N., Hofman, P., Denis, M.G., 2019. Circulating free tumor DNA in non-small cell lung cancer (NSCLC): clinical application and future perspectives. *J. Thorac. Dis.* 11 (Suppl. 1), S113–S126.
- Hofman, V., Benzaquen, J., Heeke, S., Lassalle, S., Poudenx, M., Long, E., Lantéri, E., Bordone, O., Lespinet, V., Tanga, V., Bonnetaud, C., Bille, Y., Ilić, M., Marquette, C., Barlesi, F., Boutros, J., Hofman, P., 2020. Real-world assessment of the BRAF test in non-squamous cell lung carcinoma using VE1 immunohistochemistry: a single laboratory experience (LPCE, Nice, France). *Lung Cancer* 145, 58–62.
- Hugdahl, E., Kalvenes, M.B., Puntervoll, H.E., Ladstein, R.G., Akslen, L.A., 2016. BRAF-V600E expression in primary nodular melanoma is associated with aggressive tumour features and reduced survival. *Br. J. Cancer* 114, 801–808.
- Hyams, D.M., Cook, R.W., Buzaid, A.C., 2019. Identification of risk in cutaneous melanoma patients: prognostic and predictive markers. *J. Surg. Oncol.* 119, 175–186.
- Ihle, M.A., Fassunke, J., König, K., Grünwald, I., Schlaak, M., Kreuzberg, N., Tietze, L., Schildhaus, H.U., Büttner, R., Merkelbach-Bruse, S., 2014. Comparison of high resolution melting analysis, pyrosequencing, next generation sequencing and immunohistochemistry to conventional Sanger sequencing for the detection of p. V600E and non-p.V600E BRAF mutations. *BMC Cancer* 14, 13.
- Ilie, M., Long, E., Hofman, P., Dadone, B., Marquette, C.H., Mouroux, J., Vignaud, J.M., Begueret, H., Merlio, J.P., Capper, D., von Deimling, A., Emile, J.F., Hofman, P., 2013. Diagnostic value of immunohistochemistry for the detection of the BRAFV600E mutation in primary lung adenocarcinoma Caucasian patients. *Ann. Oncol.* 24, 742–748.
- Jain, D., Roy-Chowdhuri, S., 2018. Molecular pathology of lung cancer cytology specimens: a concise review. *Arch. Pathol. Lab. Med.* 142, 1127–1133.
- Jamal, S., Ziff, E., 1990. Transactivation of c-fos and beta-actin genes by raf as a step in early response to transmembrane signals. *Nature* 344, 463–466.
- Ji, X., Che, N., Lin, R., Chen, J., Wu, X., 2019. Efficient ten-generation analysis of NSCLC tissue samples by next-generation sequencing. *Pathol. Res. Pract.* 215, 1066–1070.
- Jurkowska, M., Gos, A., Ptaszyński, K., Micej, W., Tysarowski, A., Zub, R., Siedlecki, J. A., Rutkowski, P., 2015. Comparison between two widely used laboratory methods in BRAF V600 mutation detection in a large cohort of clinical samples of cutaneous melanoma metastases to the lymph nodes. *Int. J. Clin. Exp. Pathol.* 8, 8487–8493.
- Kalemkerian, G.P., Narula, N., Kennedy, E.B., Biermann, W.A., Donington, J., Leigh, N. B., Lew, M., Pantelas, J., Ramalingam, S.S., Reck, M., Saqi, A., Simoff, M., Singh, N., Sundaram, B., 2018. Molecular testing guideline for the selection of patients with lung cancer for treatment with targeted tyrosine kinase inhibitors: American Society of Clinical Oncology Endorsement of the College of American Pathologists/International Association for the Study of Lung Cancer/Association for Molecular Pathology Clinical Practice Guideline Update. *J. Clin. Oncol.* 36, 911–919.
- Kinno, T., Tsuta, K., Shiraiishi, K., Mizukami, T., Suzuki, M., Yoshida, A., Suzuki, K., Asamura, H., Furuta, K., Kohno, T., Kushima, R., 2014. Clinicopathological features of nonsmall cell lung carcinomas with BRAF mutations. *Ann. Oncol.* 25, 138–142.
- Kolch, W., Heidecker, G., Lloyd, P., Rapp, U.R., 1991. Raf-1 protein kinase is required for growth of induced NIH/3T3 cells. *Nature* 349, 426–428.
- Kotani, H., Adachi, Y., Kitai, H., Tomida, S., Bando, H., Faber, A.C., Yoshino, T., Voon, D. C., Yano, S., Ebi, H., 2018. Distinct dependencies on receptor tyrosine kinases in the regulation of MAPK signaling between BRAF V600E and non-V600E mutant lung cancers. *Oncogene* 37, 1775–1787.
- Larkin, J., Ascierto, P.A., Dréno, B., Atkinson, V., Liszky, G., Maio, M., Mandalà, M., Demidov, L., Stroyakovskiy, D., Thomas, L., de la Cruz-Merino, L., Dutriaux, C., Garbe, C., Sovak, M.A., Chang, I., Choong, N., Hack, S.P., McArthur, G.A., Ribas, A., 2014. Combined vemurafenib and cobimetinib in BRAF-mutated melanoma. *N. Engl. J. Med.* 371, 1867–1876.
- Lawrence, M.S., Stojanov, P., Polak, P., Kryukov, G.V., Cibulskis, K., Sivachenko, A., Carter, S.L., Stewart, C., Mermel, C.H., Roberts, S.A., Kiezun, A., Hammerman, P.S., McKenna, A., Drier, Y., Zou, L., Ramos, A.H., Pugh, T.J., Stransky, N., Helman, E., Kim, J., Sougnez, C., Ambrogio, L., Nickerson, E., Shefler, E., Cortés, M.L., Auclair, D., Saksena, G., Voet, D., Noble, M., DiCara, D., Lin, P., Lichtenstein, L., Heiman, D.I., Fennell, T., Imielinski, M., Hernandez, B., Hodis, E., Baca, S., Dulak, A. M., Lohr, J., Landau, D.A., Wu, C.J., Melendez-Zajgla, J., Hidalgo-Miranda, A., Koren, A., McCarroll, S.A., Mora, J., Crompton, B., Onofrio, R., Parkin, M., Winckler, W., Ardlie, K., Gabriel, S.B., Roberts, C.W.M., Biegel, J.A., Stegmaier, K., Bass, A.J., Garraway, L.A., Meyerson, M., Golub, T.R., Gordenin, D.A., Sunyaev, S., Lander, E.S., Getz, G., 2013. Mutational heterogeneity in cancer and the search for new cancer-associated genes. *Nature* 499, 214–218.
- Leonetti, A., Facchinetti, F., Rossi, G., Minari, R., Conti, A., Friboulet, L., Tiseo, M., Planchard, D., 2018. BRAF in non-small cell lung cancer (NSCLC): pickaxing another brick in the wall. *Cancer Treat. Rev.* 66, 82–94.
- Li, S., Li, L., Zhu, Y., Huang, C., Qin, Y., Liu, H., Ren-Heidenreich, L., Shi, B., Ren, H., Chu, X., Kang, J., Wang, W., Xu, J., Tang, K., Yang, H., Zheng, Y., He, J., Yu, G., Liang, N., 2014. Coexistence of EGFR with KRAS, or BRAF, or PIK3CA somatic mutations in lung cancer: a comprehensive mutation profiling from 5125 Chinese cohorts. *Br. J. Cancer* 110, 2812–2820.

- Li, M.M., Datto, M., Duncavage, E.J., Kulkarni, S., Lindeman, N.I., Roy, S., Tsimberidou, A.M., Vnencak-Jones, C.L., Wolff, D.J., Younes, A., Nikiforova, M.N., 2017. Standards and guidelines for the interpretation and reporting of sequence variants in cancer: a joint consensus recommendation of the association for molecular pathology, american society of clinical oncology, and college of american pathologists. *J. Mol. Diagn.* 19, 4–23.
- Lim, S.Y., Lee, J.H., Diefenbach, R.J., Kefford, R.F., Rizos, H., 2018. Liquid biomarkers in melanoma: detection and discovery. *Mol. Cancer* 17, 8.
- Lin, Q., Zhang, H., Ding, H., Qian, J., Lizaso, A., Lin, J., Han-Zhang, H., Xiang, J., Li, Y., Zhu, H., 2019. The association between BRAF mutation class and clinical features in BRAF-mutant Chinese non-small cell lung cancer patients. *J. Transl. Med.* 17, 298.
- Lindeman, N.I., Cagle, P.T., Aisner, D.L., Arcila, M.E., Beasley, M.B., Bernicker, E.H., Colasacco, C., Dacic, S., Hirsch, F.R., Kerr, K., Kwiatkowski, D.J., Ladanyi, M., Nowak, J.A., Sholl, L., Temple-Smolkin, R., Solomon, B., Souter, L.H., Thunnissen, E., Tsao, M.S., Ventura, C.B., Wynes, M.W., Yatabe, Y., 2018. Updated molecular testing guideline for the selection of lung cancer patients for treatment with targeted tyrosine kinase inhibitors: guideline from the college of american pathologists, the international association for the study of lung cancer, and the association for molecular pathology. *Arch. Pathol. Lab. Med.* 142, 321–346.
- Litvak, A.M., Paik, P.K., Woo, K.M., Sima, C.S., Hellmann, M.D., Arcila, M.E., Ladanyi, M., Rudin, C.M., Kris, M.G., Riely, G.J., 2014. Clinical characteristics and course of 63 patients with BRAF mutant lung cancers. *J. Thorac. Oncol.* 9, 1669–1674.
- Liu, C., Peng, W., Xu, C., Lou, Y., Zhang, M., Wargo, J.A., Chen, J.Q., Li, H.S., Watowich, S.S., Yang, Y., Tompkins Frederick, D., Cooper, Z.A., Mbofung, R.M., Whittington, M., Flaherty, K.T., Woodman, S.E., Davies, M.A., Radvanyi, L.G., Overwijk, W.W., Lizée, G., Hwu, P., 2013. BRAF inhibition increases tumor infiltration by T cells and enhances the antitumor activity of adoptive immunotherapy in mice. *Clin. Cancer Res.* 19, 393–403.
- Lokhandwala, P.M., Tseng, L.H., Rodriguez, E., Zheng, G., Pallavajjala, A., Gocke, C.D., Eshleman, J.R., Lin, M.T., 2019. Clinical mutational profiling and categorization of BRAF mutations in melanomas using next generation sequencing. *BMC Cancer* 19, 665.
- Long, G.V., Menzies, A.M., Nagrial, A.M., Haydu, L.E., Hamilton, A.L., Mann, G.J., Hughes, T.M., Thompson, J.F., Scolyer, R.A., Kefford, R.F., 2011. Prognostic and clinicopathologic associations of oncogenic BRAF in metastatic melanoma. *J. Clin. Oncol.* 29, 1239–1246.
- Long, G.V., Wilmott, J.S., Capper, D., Preusser, M., Zhang, Y.E., Thompson, J.F., Kefford, R.F., von Deimling, A., Scolyer, R.A., 2013. Immunohistochemistry is highly sensitive and specific for the detection of V600E BRAF mutation in melanoma. *Am. J. Surg. Pathol.* 37, 61–65.
- Long, G.V., Stroyakovskiy, D., Gogas, H., Levchenko, E., de Braud, F., Larkin, J., Garbe, C., Jouary, T., Hauschild, A., Grob, J.J., Chiarion-Sileni, V., Lebbe, C., Mandalà, M., Millward, M., Arance, A., Bondarenko, I., Haanen, J.B., Hansson, J., Utikal, J., Ferraresi, V., Kovalenko, N., Mohr, P., Probachai, V., Schadendorf, D., Nathan, P., Robert, C., Ribas, A., DeMarini, D.J., Irani, J.G., Casey, M., Ouellet, D., Martin, A.M., Le, N., Patel, K., Flaherty, K., 2014. Combined BRAF and MEK inhibition versus BRAF inhibition alone in melanoma. *N. Engl. J. Med.* 371, 1877–1888.
- Long, G.V., Weber, J.S., Infante, J.R., Kim, K.B., Daud, A., Gonzalez, R., Sosman, J.A., Hamid, O., Schuchter, L., Cebon, J., Kefford, R.F., Lawrence, D., Kudchadkar, R., 3rd, Burris H.A., Falchook, G.S., Algazi, A., Lewis, K., Puzanov, I., Ibrahim, N., Sun, P., Cunningham, E., Kline, A.S., Del Buono, H., McDowell, D.O., Patel, K., Flaherty, K.T., 2016. Overall survival and durable responses in patients with BRAF V600-Mutant metastatic melanoma receiving dabrafenib combined with trametinib. *J. Clin. Oncol.* 34, 871–878.
- Long, G.V., Flaherty, K.T., Stroyakovskiy, D., Gogas, H., Levchenko, E., de Braud, F., Larkin, J., Garbe, C., Jouary, T., Hauschild, A., Chiarion-Sileni, V., Lebbe, C., Mandalà, M., Millward, M., Arance, A., Bondarenko, I., JBAG, Haanen, Hansson, J., Utikal, J., Ferraresi, V., Mohr, P., Probachai, V., Schadendorf, D., Nathan, P., Robert, C., Ribas, A., Davies, M.A., Lane, S.R., Legos, J.J., Mookerjee, B., Grob, J.J., 2017. Dabrafenib plus trametinib versus dabrafenib monotherapy in patients with metastatic BRAF V600E/K-mutant melanoma: long-term survival and safety analysis of a phase 3 study. *Ann. Oncol.* 28, 1631–1639.
- Long, G.V., Eroglu, Z., Infante, J., Patel, S., Daud, A., Johnson, D.B., Gonzalez, R., Kefford, R., Hamid, O., Schuchter, L., Cebon, J., Sharfman, W., McWilliams, R., Sznol, M., Redhu, S., Gasal, E., Mookerjee, B., Weber, J., Flaherty, K.T., 2018. Long-term outcomes in patients with BRAF V600-Mutant metastatic melanoma who received dabrafenib combined with trametinib. *J. Clin. Oncol.* 36, 667–673.
- Lopez-Rios, F., Angulo, B., Gomez, B., Mair, D., Martinez, R., Conde, E., Shieh, F., Vaks, J., Langland, R., Lawrence, H.J., de Castro, D.G., 2013. Comparison of testing methods for the detection of BRAF V600E mutations in malignant melanoma: pre-approval validation study of the companion diagnostic test for vemurafenib. *PLoS One* 8, e53733.
- Luther, C., Swami, U., Zhang, J., Milhem, M., Zakharia, Y., 2019. Advanced stage melanoma therapies: detailing the present and exploring the future. *Crit. Rev. Oncol. Hematol.* 133, 99–111.
- Malapelle, U., Bellevicine, C., De Luca, C., Salatiello, M., De Stefano, A., Rocco, D., de Rosa, N., Vitiello, F., Russo, S., Pepe, F., Iaccarino, A., Micheli, P., Illiano, A., Carlomagno, C., Piantedosi, F.V., Troncone, G., 2013. EGFR mutations detected on cytology samples by a centralized laboratory reliably predict response to gefitinib in non-small cell lung carcinoma patients. *Cancer Cytopathol.* 121, 552–560.
- Malapelle, U., Pisapia, P., Sgariglia, R., Vigiari, E., Biglietto, M., Carlomagno, C., Giuffrè, G., Bellevicine, C., Troncone, G., 2016. Less frequently mutated genes in colorectal cancer: evidences from next-generation sequencing of 653 routine cases. *J. Clin. Pathol.* 69, 767–771.
- Malapelle, U., Sirera, R., Jantus-Lewintre, E., Reclusa, P., Calabuig-Fariñas, S., Blasco, A., Pisapia, P., Rolfo, C., Camps, C., 2017. Profile of the Roche cobas® EGFR mutation test v2 for non-small cell lung cancer. *Expert Rev. Mol. Diagn.* 17, 209–215.
- Malapelle, U., Muscarella, L.A., Pisapia, P., Rossi, A., 2020. Targeting emerging molecular alterations in the treatment of non-small cell lung cancer: current challenges and the way forward. *Expert Opin. Investig. Drugs* 29, 363–372.
- Manfredi, L., Meyer, N., Tournier, E., Grand, D., Uro-Coste, E., Rochaix, P., Brousset, P., Lamant, L., 2016. Highly concordant results between immunohistochemistry and molecular testing of mutated V600E BRAF in primary and metastatic melanoma. *Acta Derm. Venereol.* 96, 630–634.
- Marchant, J., Mange, A., Larriex, M., Costes, V., Solassol, J., 2014. Comparative evaluation of the new FDA approved ThxID™-BRAF test with High Resolution melting and Sanger sequencing. *BMC Cancer* 14, 519.
- Marchetti, A., Felicioni, L., Malatesta, S., Grazia Sciarrotta, M., Guetti, L., Chella, A., Viola, P., Pullara, C., Mucilli, F., Buttitta, F., 2011. Clinical features and outcome of patients with non-small-cell lung cancer harboring BRAF mutations. *J. Clin. Oncol.* 29, 3574–3579.
- Matallanas, D., Birtwistle, M., Romano, D., Zebisch, A., Rauch, J., von Kriegsheim, A., Kolch, W., 2011. Raf family kinases: old dogs have learned new tricks. *Genes Cancer* 2, 232–260.
- Mazieres, J., Cropet, C., Montané, L., Barlesi, F., Souquet, P.J., Quantin, X., Dubos-Arvis, C., Otto, J., Favier, L., Avrillon, V., Cadranet, J., Moro-Sibilot, D., Monnet, I., Westeel, V., Le Treut, J., Brain, E., Trédaniel, J., Jaffro, M., Collot, S., Ferretti, G.R., Tiffon, C., Mahier-Ait Oukhatar, C., Blay, J.Y., 2020. Vemurafenib in non-small-cell lung cancer patients with BRAFV600 and BRAFnonV600 mutations. *Ann. Oncol.* 31, 289–294.
- McArthur, G.A., Chapman, P.B., Robert, C., Larkin, J., Haanen, J.B., Dummer, R., Ribas, A., Hogg, D., Hamid, O., Ascierto, P.A., Garbe, C., Testori, A., Maio, M., Lorigan, P., Lebbé, C., Jouary, T., Schadendorf, D., O’Day, S.J., Kirkwood, J.M., Eggermont, A.M., Dréno, B., Sosman, J.A., Flaherty, K.T., Yin, M., Caro, I., Cheng, S., Trunzer, K., Hauschild, A., 2014. Safety and efficacy of vemurafenib in BRAF (V600E) and BRAF(V600K) mutation-positive melanoma (BRIM-3): extended follow-up of a phase 3, randomised, open-label study. *Lancet Oncol.* 15, 323–332.
- Minor, D.R., Puzanov, I., Callahan, M.K., Hug, B.A., Hoos, A., 2015. Severe gastrointestinal toxicity with administration of trametinib in combination with dabrafenib and ipilimumab. *Pigment Cell Melanoma Res.* 28, 611–612.
- Moelling, K., Heimann, B., Beimpling, P., Rapp, U.R., Sander, T., 1984. Serine- and threonine-specific protein kinase activities of purified gag-mil and gag-raf proteins. *Nature* 312, 558–561.
- Mok, T.S., Wu, Y.L., Thongprasert, S., Yang, C.H., Chu, D.T., Saijo, N., Sunpaweravong, P., Han, B., Margono, B., Ichinose, Y., Nishiikawa, Y., Ohe, Y., Yang, J.J., Chewaskulyong, B., Jiang, H., Duffield, E.L., Watkins, C.L., Armour, A.A., Fukuoka, M., 2009. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N. Engl. J. Med.* 361, 947–957.
- Moodie, S.A., Willumsen, B.M., Weber, M.J., Wolfman, A., 1993. Complexes of Ras-GTP with Raf-1 and mitogen-activated protein kinase kinase. *Science* 260, 1658–1661.
- Mourah, S., Denis, M.G., Narducci, F.E., Solassol, J., Merlin, J.L., Sabourin, J.C., Scoazec, J.Y., Ouafik, L., Emile, J.F., Heller, R., Souvignet, C., Bergougnou, L., Merlio, J.P., 2015. Detection of BRAF V600 mutations in melanoma: evaluation of concordance between the Cobas® 4800 BRAF V600 mutation test and the methods used in French National Cancer Institute (INCa) platforms in a real-life setting. *PLoS One* 10, e0120232.
- Nguyen-Ngoc, T., Bouchaab, H., Adjei, A.A., Peters, S., 2015. BRAF alterations as therapeutic targets in non-small-cell lung cancer. *J. Thorac. Oncol.* 10, 1396–1403.
- Noeparast, A., Teugels, E., Giron, P., Verschelden, G., De Brakeleer, S., Decoster, L., De Grève, J., 2016. Non-V600 BRAF mutations recurrently found in lung cancer predict sensitivity to the combination of Trametinib and Dabrafenib. *Oncotarget* 8, 60094–60108.
- Nollau, P., Wagener, C., 1997. Methods for detection of point mutations: performance and quality assessment. IFCC Scientific Division, Committee on Molecular Biology Techniques. *Clin. Chem.* 43, 1114–1128.
- O’Leary, C.G., Andelkovic, V., Ladwa, R., Pavlakis, N., Zhou, C., Hirsch, F., Richard, D., O’Byrne, K., 2019. Targeting BRAF mutations in non-small cell lung cancer. *Transl. Lung Cancer Res.* 8, 1119–1124.
- Ofiara, L.M., Navasakulpong, A., Ezer, N., Gonzalez, A.V., 2012. The importance of a satisfactory biopsy for the diagnosis of lung cancer in the era of personalized treatment. *Curr. Oncol.* 19 (Suppl. 1), S16–S23.
- Olthof, S.C., Krumm, P., Weichold, O., Eigentler, T., Bösmüller, H., la Fougère, C., Pfannenberger, C., Martus, P., Klumpp, B., 2020. CT texture analysis compared to Positron Emission Tomography (PET) and mutational status in resected melanoma metastases. *Eur. J. Radiol.* 131, 109242.
- Ortiz-Cuaran, S., Mezquita, L., Swaldutz, A., Aldea, M., Mazieres, J., Leonce, C., Jovelet, C., Pradines, A., Avrillon, V., Chumbi Flores, W.R., Lacroix, L., Loriot, Y., Westeel, V., Ngo-Camus, M., Tissot, C., Raynaud, C., Gervais, R., Brain, E., Monnet, I., Giroux Leprieux, E., Caramella, C., Mahier-Ait Oukhatar, C., Hoog-Labouret, N., de Kievit, F., Howarth, K., Morris, C., Green, E., Friboulet, L., Chabaud, S., Guichou, J.F., Perol, M., Besse, B., Blay, J.Y., Saintigny, P., Planchard, D., 2020. Circulating tumor DNA genomics reveals potential mechanisms of resistance to BRAF-targeted therapies in BRAF-mutant metastatic non-small cell lung cancer patients. *Clin. Cancer Res. Epub ahead of print.*
- Paik, P.K., Arcila, M.E., Fara, M., Sima, C.S., Miller, V.A., Kris, M.G., Ladanyi, M., Riely, G.J., 2011. Clinical characteristics of patients with lung adenocarcinomas harboring BRAF mutations. *J. Clin. Oncol.* 29, 2046–2051.
- Pavlick, A.C., Fecher, L., Ascierto, P.A., Sullivan, R.J., 2019. Frontline therapy for BRAF-mutated metastatic melanoma: how do you choose, and is there one correct answer? *Am. Soc. Clin. Oncol. Educ. Book* 39, 564–571.

- Pearlstein, M.V., Zedek, D.C., Ollila, D.W., Treece, A., Gulley, M.L., Groben, P.A., Thomas, N.E., 2014. Validation of the VE1 immunostain for the BRAF V600E mutation in melanoma. *J. Cutan. Pathol.* 41, 724–732.
- Pepe, F., De Luca, C., Smeraglio, R., Pisapia, P., Sgariglia, R., Nacchio, M., Russo, M., Serra, N., Rocco, D., Battiloro, C., Ambrosio, F., Gragnano, G., Vigiari, E., Bellevicine, C., Troncone, G., Malapelle, U., 2019. Performance analysis of SiRe next-generation sequencing panel in diagnostic setting: focus on NSCLC routine samples. *J. Clin. Pathol.* 72, 38–45.
- Petty, D.R., Hassan, O.A., Barker, C.S., O'Neill, S.S., 2020. Rapid BRAF mutation testing in pigmented melanomas. *Am. J. Dermatopathol.* 42, 343–348.
- Peyssonaux, C., Eychène, A., 2001. The Raf/MEK/ERK pathway: new concepts of activation. *Biol. Cell* 93, 53–62.
- Pisapia, P., Pepe, F., Malapelle, U., Troncone, G., 2019. BRAF mutations in lung cancer. *Acta Cytol.* 63, 247–250.
- Planchard, D., Besse, B., Groen, H.J.M., Souquet, P.J., Quoix, E., Baik, C.S., Barlesi, F., Kim, T.M., Mazieres, J., Novello, S., Rigas, J.R., Upalawanna, A., D'Amelio Jr, A.M., Zhang, P., Mookerjee, B., Johnson, B.E., 2016. Dabrafenib plus trametinib in patients with previously treated BRAF(V600E)-mutant metastatic non-small cell lung cancer: an open-label, multicentre phase 2 trial. *Lancet Oncol.* 17, 984–993.
- Planchard, D., Smit, E.F., Groen, H.J.M., Mazieres, J., Besse, B., Helland, Å., Giannoni, V., D'Amelio Jr, A.M., Zhang, P., Mookerjee, B., Johnson, B.E., 2017. Dabrafenib plus trametinib in patients with previously untreated BRAFV600E-mutant metastatic non-small-cell lung cancer: an open-label, phase 2 trial. *Lancet Oncol.* 18, 1307–1316.
- Proietti, I., Micheli, S., Di Fraia, M., Mambrin, A., Petrozza, V., Porta, N., Pacini, L., Calogero, A., Skroza, N., Potenza, C., 2020. A rare BRAF V600E mutation detected by next-generation sequencing in a superficial spreading melanoma: case report and potential diagnostic implications. *J. Eur. Acad. Dermatol. Venereol.* Epub ahead of print.
- Qu, K., Pan, Q., Zhang, X., Rodriguez, L., Zhang, K., Li, H., Ho, A., Sanders, H., Sfruzza, A., Cheng, S.M., Nguyen, D., Jones, D., Waldman, F., 2013. Detection of BRAF V600 mutations in metastatic melanoma: comparison of the Cobas 4800 and Sanger sequencing assays. *J. Mol. Diagn.* 15, 790–795.
- Rapp, U.R., Goldsborough, M.D., Mark, G.E., Bonner, T.I., Groffen, J., Reynolds Jr, F.H., Stephenson, J.R., 1983. Structure and biological activity of v-raf, a unique oncogene transduced by a retrovirus. *Proc. Natl. Acad. Sci. U. S. A.* 80, 4218–4222.
- Reyes, R., Mayo-de-Las-Casas, C., Teixidó, C., Cabrera, C., Marín, E., Vollmer, I., Jares, P., Garzón, M., Molina-Vila, M.A., Reguart, N., 2019. Clinical benefit from BRAF/MEK inhibition in a double non-V600E BRAF mutant lung adenocarcinoma: a case report. *Clin. Lung Cancer* 20, e219–e223.
- Ribas, A., Hodi, F.S., Callahan, M., Konto, C., Wolchok, J., 2013. Hepatotoxicity with combination of vemurafenib and ipilimumab. *N. Engl. J. Med.* 368, 1365–1366.
- Rijavec, E., Coco, S., Genova, C., Rossi, G., Longo, L., Grossi, F., 2019. Liquid biopsy in non-small cell lung cancer: highlights and challenges. *Cancers (Basel)* 12, 17.
- Ronaghi, M., Uhlén, M., Nyrén, P., 1998. A sequencing method based on real-time pyrophosphate. *Science* 281 (363), 365.
- Rothberg, J.M., Hinz, W., Rearick, T.M., Schultz, J., Mileski, W., Davey, M., Leamon, J.H., Johnson, K., Milgrew, M.J., Edwards, M., Hoon, J., Simons, J.F., Marran, D., Myers, J.W., Davidson, J.F., Branting, A., Nobile, J.R., Puc, B.P., Light, D., Clark, T.A., Huber, M., Branciforte, J.T., Stoner, I.B., Cawley, S.E., Lyons, M., Fu, Y., Homer, N., Sedova, M., Miao, X., Reed, B., Sabina, J., Feierstein, E., Schorn, M., Alanjary, M., Dimalanta, E., Dressman, B., Kaskasins, R., Sokolsky, T., Fidanza, J.A., Namsaraev, E., McKernan, K.J., Williams, A., Roth, G.T., Bustillo, J., 2011. An integrated semiconductor device enabling non-optical genome sequencing. *Nature* 475, 348–352.
- Russo, A., Lopes, A.R., McCusker, M.G., Garrigues, S.G., Ricciardi, G.R., Arensmeyer, K.E., Scilla, K.A., Mehra, R., Rolfo, C., 2020. New targets in lung cancer (Excluding EGFR, ALK, ROS1). *Curr. Oncol. Rep.* 22, 48.
- Salimian, K.J., Fazeli, R., Zheng, G., Ettinger, D., Maleki, Z., 2018. V600E BRAF versus non-V600E BRAF mutated lung adenocarcinomas: cytomorphology, histology, coexistence of other driver mutations and patient characteristics. *Acta Cytol.* 62, 79–84.
- Sauer, S., Lehrach, H., Reinhardt, R., 2003. MALDI mass spectrometry analysis of single nucleotide polymorphisms by photocleavage and charge-tagging. *Nucleic Acids Res.* 31, e63.
- Schirosi, L., Strippoli, S., Gaudio, F., Graziano, G., Popescu, O., Guida, M., Simone, G., Mangia, A., 2016. Is immunohistochemistry of BRAF V600E useful as a screening tool and during progression disease of melanoma patients? *BMC Cancer* 16, 905.
- Sereno, M., Moreno, V., Moreno Rubio, J., Gómez-Raposo, C., García Sánchez, S., Hernández Jurdado, R., Falagan, S., Zambrana Tébar, F., Casado Sáenz, E., 2015. A significant response to sorafenib in a woman with advanced lung adenocarcinoma and a BRAF non-V600E mutation. *Anticancer Drugs* 26, 1004–1007.
- Siravegna, G., Mussolin, B., Venesio, T., Marsoni, S., Seoane, J., Dive, C., Papadopoulos, N., Kopetz, S., Corcoran, R.B., Siu, L.L., Bardelli, A., 2019. How liquid biopsies can change clinical practice in oncology. *Ann. Oncol.* 30, 1580–1590.
- Smit, E., 2014. BRAF mutations in non-small-cell lung cancer. *J. Thorac. Oncol.* 9, 1594–1595.
- Sousa, A.C., Silveira, C., Janeiro, A., Malveiro, S., Oliveira, A.R., Felizardo, M., Nogueira, F., Teixeira, E., Martins, J., Carmo-Fonseca, M., 2020. Detection of rare and novel EGFR mutations in NSCLC patients: implications for treatment-decision. *Lung Cancer* 139, 35–40.
- Sullivan, R.J., Infante, J.R., Janku, F., Wong, D.J.L., Sosman, J.A., Keedy, V., Patel, M.R., Shapiro, G.I., Mier, J.W., Tolcher, A.W., Wang-Gillam, A., Sznol, M., Flaherty, K., Buchbinder, E., Carvajal, R.D., Varghese, A.M., Lacouture, M.E., Ribas, A., Patel, S.P., DeCrescenzo, G.A., Emery, C.M., Groover, A.L., Saha, S., Varterasian, M., Welsch, D.J., Hyman, D.M., Li, B.T., 2018. First-in-class ERK1/2 inhibitor ulixertinib (BVD-523) in patients with MAPK mutant advanced solid tumors: results of a phase I dose-escalation and expansion study. *Cancer Discov.* 8, 184–195.
- Sullivan, R.J., Hamid, O., Gonzalez, R., Infante, J.R., Patel, M.R., Hodi, F.S., Lewis, K.D., Tawbi, H.A., Hernandez, G., Wongchenko, M.J., Chang, Y., Roberts, L., Ballinger, M., Yan, Y., Cha, E., Hwu, P., 2019. Atezolizumab plus cobimetinib and vemurafenib in BRAF-mutated melanoma patients. *Nat. Med.* 25, 929–935.
- Sullivan, R.J., Hollebecque, A., Flaherty, K.T., Shapiro, G.I., Rodon Ahnert, J., Millward, M.J., Zhang, W., Gao, L., Sykes, A., Willard, M.D., Yu, D., Schade, A.E., Crowe, K., Flynn, D.L., Kaufman, M.D., Henry, J.R., Peng, S.B., Benhadji, K.A., Conti, L., Gordon, M.S., Tiu, R.V., Hong, D.S., 2020. A phase I study of LY3009120, a Pan-RAF inhibitor, in patients with advanced or metastatic cancer. *Mol. Cancer Ther.* 19, 460–467.
- Tan, I., Stinchcombe, T.E., Ready, N.E., Crawford, J., Datto, M.B., Nagy, R.J., Lanman, R. B., Gu, L., Clarke, J.M., 2019. Therapeutic outcomes in non-small cell lung cancer with BRAF mutations: a single institution, retrospective cohort study. *Transl. Lung Cancer Res.* 8, 258–267.
- Tan, A.C., Lai, G.G.Y., Tan, G.S., Poon, S.Y., Doble, B., Lim, T.H., Aung, Z.W., Takano, A., Tan, W.L., Ang, M.K., Tan, B.S., Devanand, A., Too, C.W., Gogna, A., Ong, B.H., Koh, T.P.T., Kanesvaran, R., Ng, Q.S., Jain, A., Rajasekaran, T., Lim, A.S.T., Lim, W. T., Toh, C.K., Tan, E.H., Lim, T.K.H., Tan, D.S.W., 2020. Utility of incorporating next-generation sequencing (NGS) in an Asian non-small cell lung cancer (NSCLC) population: Incremental yield of actionable alterations and cost-effectiveness analysis. *Lung Cancer* 139, 207–215.
- Tang, K., Fu, D., Kötter, S., Cotter, R.J., Cantor, C.R., Köster, H., 1995. Matrix-assisted laser desorption/ionization mass spectrometry of immobilized duplex DNA probes. *Nucleic Acids Res.* 23, 3126–3131.
- Tran, N.H., Wu, X., Frost, J.A., 2005. B-Raf and Raf-1 are regulated by distinct autoregulatory mechanisms. *J. Biol. Chem.* 280, 16244–16253.
- Trinh, V.A., Davis, J.E., Anderson, J.E., Kim, K.B., 2014. Dabrafenib therapy for advanced melanoma. *Ann. Pharmacother.* 48, 519–529.
- Trovisco, V., Soares, P., Sobrinho-Simões, M., 2006. B-RAF mutations in the etiopathogenesis, diagnosis, and prognosis of thyroid carcinomas. *Hum. Pathol.* 37, 781–786.
- Van Alst, L., Barr, M., Marcus, S., Polverino, A., Wigler, M., 1993. Complex formation between RAS and RAF and other protein kinases. *Proc Natl Acad Sci U S A.* 90, 6213–6217.
- Vigiari, E., Malapelle, U., de Luca, C., Bellevicine, C., Troncone, G., 2015. Challenges and opportunities of next-generation sequencing: a cytopathologist's perspective. *Cytopathology* 26, 271–283.
- Vigiari, E., Malapelle, U., Iaccarino, A., Acanfora, G., Pisapia, P., Clery, E., De Luca, C., Bellevicine, C., Troncone, G., 2019. PD-L1 expression on routine samples of non-small cell lung cancer: results and critical issues from a 1-year experience of a centralised laboratory. *J. Clin. Pathol.* 72, 412–417.
- Vojtek, A.B., Hollenberg, S.M., Cooper, J.A., 1993. Mammalian Ras interacts directly with the serine/threonine kinase Raf. *Cell* 74, 205–214.
- Wang, S., Yu, B., Ng, C.C., Mercorella, B., Selinger, C.L., O'Toole, S.A., Cooper, W.A., 2015. The suitability of small biopsy and cytology specimens for EGFR and other mutation testing in non-small cell lung cancer. *Transl. Lung Cancer Res.* 4, 119–125.
- Warne, P.H., Viciana, P.R., Downward, J., 1993. Direct interaction of Ras and the amino-terminal region of Raf-1 in vitro. *Nature* 364, 352–355.
- Wasylyk, C., Wasylyk, B., Heidecker, G., Huleihel, M., Rapp, U.R., 1989. Expression of raf oncogenes activates the PEAI transcription factor motif. *Mol. Cell. Biol.* 9, 2247–2250.
- Wu, Z., Yang, Z., Dai, Y., Zhu, Q., Chen, L.A., 2019. Update on liquid biopsy in clinical management of non-small cell lung cancer. *Oncol. Ther.* 12, 5097–5109.
- Yaeger, R., Corcoran, R.B., 2019. Targeting alterations in the RAF-MEK pathway. *Cancer Discov.* 9, 329–341.
- Zhang, X.F., Settleman, J., Kyriakis, J.M., Takeuchi-Suzuki, E., Elledge, S.J., Marshall, M. S., Bruder, J.T., Rapp, U.R., Avruch, J., 1993. Normal and oncogenic p21ras proteins bind to the amino-terminal regulatory domain of c-Raf-1. *Nature* 364, 308–313.
- Zheng, H.Y., Wang, H.B., Shen, F.J., Tong, Y.Q., Yao, Q., Qiao, B., Sun, S., Li, Y., 2020. EGFR gene mutation and methodological evaluation in 399 patients with non-small cell lung cancer. *Curr. Issues Pharm. Med. Sci. Pract.* 40, 78–84.
- Zhu, M.L., Zhou, L., Sadri, N., 2018. Comparison of targeted next generation sequencing (NGS) versus isolated BRAF V600E analysis in patients with metastatic melanoma. *Virchows Arch.* 473, 371–377.

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