RAS as a positive predictive biomarker: focus on lung and colorectal cancer patients

"RASAtlas": a real-world database to track RAS oncogenic mutations in lung and colorectal cancer

Umberto Malapelle[1#], Francesco Passiglia[2#], Chiara Cremolini[3,4#], Maria Lucia Reale[2], Francesco Pepe[1], Pasquale Pisapia[1], Diego Cortinovis[5], Alfonso De Stefano[6], Domenico Galetta[7], Fotios Loupakis[8], Fabio Pagni[9], Filippo Pietrantonio[10,11], Sara Pilotto[12], Andrea Sartore Bianchi[11,13], Calogero Lauricella[13], Hector Soto Parra[14], Marcello Tiseo[15], Giancarlo Troncone[1*], Silvia Novello[2]

[1] Department of Public Health, University of Naples Federico II, Naples, Italy

[2] Department of Oncology, San Luigi Gonzaga Hospital, University of Turin, Orbassano, Italy

[3]Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy.

[4]Unit of Medical Oncology 2, Azienda Ospedaliero-Universitaria Pisana, Pisa, Italy.

[5] SC Oncologia Medica, SS Lung Unit Asst Ospedale San Gerardo, Monza, Italy.

[6] Abdominal Oncology Unit, Istituto Nazionale Tumori IRCCS Fondazione Pascale, Naples, Italy.

[7] Medical Thoracic Oncology Unit, IRCCS Istituto Tumori "Giovanni Paolo II," Bari, Italy.

[8] Unit of Oncology 1, Department of Clinical and Experimental Oncology, Istituto Oncologico Veneto, IRCCS, Padua, Italy.

[9] Department of Medicine and Surgery, Pathology, San Gerardo Hospital, University of Milano-Bicocca, Monza, Italy.

[10] Department of Medical Oncology, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy.

[11] Oncology and Hemato-oncology Department, University of Milan, Milan, Italy.

[12] U.O.C. of Oncology, Azienda Ospedaliera Universitaria Integrata, University of Verona, Verona, Italy.

[13] Niguarda Cancer Center, Grande Ospedale Metropolitano Niguarda, Milano, Italy.

[14] Department of Oncology, Medical Oncology, University Hospital Policlinico-San Marco, Catania, Italy.

[15] Department of Medicine and Surgery, University of Parma, and Medical Oncology Unit, University Hospital of Parma, Parma, Italy.

All authors, except for Umberto Malapelle, Maria Lucia Reale, Chiara Cremolini, Francesco Passiglia, Francesco Pepe, Pasquale Pisapia, Giancarlo Troncone and Silvia Novello, are listed alphabetically.

[#] equally contribution.

***Correspondance:**

Giancarlo Troncone

E-mail: giancarlo.troncone@unina.it

Department of Public Health, University of Naples Federico II, 80131, Naples, Italy

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Abstract

Rat sarcoma (*RAS*) oncogenes are among the most studied oncogenes during the last decades. Taking into account all human tumors, Kirsten Rat Sarcoma Viral Oncogene Homolog (*KRAS*) gene is the most frequently mutated (about 22%) among the three isoforms, followed by Neuroblastoma RAS Viral Oncogene Homolog (*NRAS*) (8%) and Harvey Rat Sarcoma Viral Oncogene Homolog (*HRAS*) (3%). During the last years, careful attention has been paid on *KRAS* and *NRAS* gene mutations in non-small cell lung cancer (NSCLC) and colorectal cancer (CRC) patients due to their prognostic and predictive roles. In particular, several literature data have been currently available on the possibility to treat with targeted therapies NSCLC and CRC *KRAS*- and *NRAS*-mutated patients. Here, we review the current literature on *KRAS* and *NRAS* in NSCLC and CRC patients and provide an overview of the real-world practice in different Italian laboratories. Based on this, we propose a knowledge base database (www.rasatlas.com) to help the healthcare personnel in the management of *RAS* gene mutations in the landscape of precision oncology.

Commentato [ASB1]: The rasatlas website seems to me to be a notable "plus" of the paper and should be cited also in the abstract

Keywords: NSCLC, CRC, NGS, KRAS, NRAS.

1. RAS genes: an overview

Rat sarcoma (*RAS*) oncogenes are among the most studied oncogenes during the last decades.[1] As early as 1960s, it has been demonstrated the role of *RAS* viral oncogenes (Harvey Rat Sarcoma Viral Oncogene Homolog [*HRAS*] and Kirsten Rat Sarcoma Viral Oncogene Homolog [*KRAS*]), carried by retroviruses, to induce the generation of sarcomas in rats.[2, 3] Subsequently, normal counterparts of these genes were identified within human genome.[4, 5] In particular, *HRAS* gene is located on chromosome 11p15.5, whereas *KRAS* gene is placed on chromosome 12p12.1.[6] The turning point in the identification of the oncogenic role of RAS oncogenes in human cells occurred in 1982.[7, 8] Besides *HRAS* and *KRAS*, a third member of *RAS* oncogene family, named Neuroblastoma RAS Viral Oncogene Homolog (*NRAS*), was later identified in human neuroblastoma cell lines.[9] *NRAS* gene is located on chromosome 1p13.2.[6]

Overall, RAS proteins are G-proteins with Guanosine Triphosphate (GTP)ase function that act as molecular switches in the regulation of different pathways involved in promoting cell proliferation, differentiation and survival.[10] Briefly, in normal conditions, after the activation of receptor tyrosine kinases (RTKs), G-protein coupled receptors (GPCRs) or integrin family members, guanine nucleotide exchange factors (GEF) proteins are recruited to favor guanosine diphosphate (GDP) dissociation and GTP binding. In fact, the association between RAS-GEF proteins determines a conformational change resulting in a RAS protein limited affinity for GDP, enabling the substitution with GTP, and its consequent activation.[11, 12] Conversely, the GTP hydrolysis and the consequent inactivation of RAS protein is highly increased by GTPase Activating proteins (GAPs).[12, 13] Finally, RAS proteins activation is associated with the downstream recruitment and activation of the mitogen-activated protein kinase (MAPK) pathway.[14]

Considering all human tumors, *KRAS* gene is the most frequently mutated (about 22%) among the three isoforms, followed by *NRAS* (8%) and *HRAS* (3%).[15] Regarding mutation types, the most common genomic alterations are single nucleotide variations determining amino acids substitutions

within codons 12, 13 and 61, resulting in the increasing affinity for GTP and constitutive activation of RAS proteins.[16, 17] However, 80% of *KRAS* mutations are discovered within codon 12, whereas mutations in *NRAS* are more common identified (60%) in codon 61.[10, 17]

Regarding the oncogenic role of *RAS* genes, The Cancer Genome Atlas (TCGA) project highlighted that *RAS* gene mutations occur in about 20–30% of all human cancers.[18] The increasing attention for *RAS* genes, in particular *KRAS* and *NRAS*, in colorectal cancer (CRC) and non-small cell lung cancer (NSCLC) patients is associated to the role of these genes mutations as predictive biomarkers of response/resistance to targeted treatments. In particular, *KRAS* and *NRAS* mutations play a negative predictive role of response to the anti-epidermal growth factor receptor (EGFR) monoclonal antibodies in metastatic CRC patients.[19] On the other hand, even though being highly attractive as a direct target for cancer therapy, attempts to target RAS oncogenic products have been largely unsuccessful for several reasons and RAS proteins were long considered "undruggable" until the discovery of the so called switch-II pocket in 2013 by the Shokat Laboratory. The resulting recent development of inhibitors for the oncogenic exon 2 p.G12C mutant of KRAS is opening new therapeutic avenues and make this mutation a positive predictive marker in advanced stages NSCLC patients when considering the administration of a new generation of tyrosine kinase inhibitors (TKIs), such as AMG510 and MRTX849.[20]

Here, we focus our attention on *KRAS* and *NRAS* mutations in CRC and NSCLC.

Commentato [ASB2]: J. M. Ostrem, U. Peters, M. L. Sos, J. A. Wells and K. M. Shokat, Nature, 2013, 503, 548–551.

2. RAS mutations in non-small cell lung cancer: therapeutic strategies and clinical evidence

Commentato [MT3]: Andrebbe uniformata la parte lung e colon per quanto riguarda AMG; indicato sempre come AMG510 nel lung e Sotorasib nel colon

Since their discovery, *RAS* mutations have represented an appealing target for the clinical treatment of NSCLC patients, considering their high prevalence, reported to be around 30% in lung adenocarcinomas.[21] Differently from other targetable oncogenes, such as *EGFR*, V-Raf Murine Sarcoma Viral Oncogene Homolog B (*BRAF*) mutations as well as Anaplastic Lymphoma Kinase (*ALK*)/ ROS Proto-Oncogene 1, Receptor Tyrosine Kinase (*ROS1*) rearrangements, the prevalence of *KRAS* mutations was reported to be higher in smoker patients and Western population.[22] From the

clinical point of view, *KRAS* has been historically considered as a negative prognostic factor in surgically resected NSCLC patients [23, 24] as well as a negative predictor of EGFR TKIs responsiveness when co-occurring with *EGFR* sensitizing mutations in the advanced stage setting.[25] No significant association between *KRAS* mutation status and survival outcomes have emerged from clinical studies evaluating different programmed death-1/ligand-1(PD-1/PD-L1) inhibitors either as single agent or in combination with chemotherapy, thus suggesting that the immune-checkpoint blockade may be considered as an effective and valid treatment option in metastatic NSCLC patients, regardless of *KRAS* mutations.[26-29]

Initial research efforts to *RAS* mutations therapeutic targeting have largely focused on the indirect inhibition of downstream regulators, including the Rapidly accelerated fibrosarcoma (RAF)/extracellular signal-regulated kinase (MEK)/extracellular signal-regulated kinase (ERK) as well as phosphatidylinositol 3-kinase (PI3K)/Protein kinase B (AKT)/mammalian target of rapamycin (mTOR) signaling pathways. Particularly two MEK small molecule inhibitors, Trametinib and Selumetinib, either as single agent or in combination with chemotherapy, have been investigated within different randomized phase II/III clinical trials, [30-32] overall showing disappointing results in previously treated population. The evidence of Focal adhesion kinase (*FAK*) upregulation under MEK-TKI therapy provided the rational to test the dual FAK/MEK inhibitor, VS-6766, in combinations with the FAK inhibitor, Defactinib, across different solid tumors harboring *KRAS* mutations. The preliminary data of this phase I basket trial have recently shown a promising activity and tolerability profile in the small cohort of NSCLC patients, with a disease control rate of 90% and an objective response rate of 10%, especially in the subgroup of exon 2 p.G12V mutant tumors, thus gathering further investigation in this molecular subset.[33] The association of MEK and PI3K/mTOR inhibitors were also tested in early phase I clinical studies,[34, 35] showing interesting signals in terms of activity but high incidence of severe adverse events, which precluded any further clinical development. Another investigated approach was the inhibition of farnesyltransferase (FTase), which acts as a key regulator of RAS localization within tumor cells. However different

molecules, such as tipifarnib and lopafarnib, failed to show any relevant activity in NSCLC clinical studies. [36-38]

These findings overall suggested that RAS indirect inhibition through downstream signaling and post-translational modification targeting is not an effective strategy for *KRAS*-positive NSCLC treatment. The biological heterogeneity of *RAS*-mutant diseases, the incomplete inhibition of *RAS* molecular pathway, the simultaneous activation of alternative downstream feedback signaling were the main reasons limiting clinical efficacy of these treatment approaches in the clinical setting.

A deeper understanding of RAS biology along with a better characterization of the protein structure have recently allowed to identify novel druggable pockets within specific protein domains, thus leading to the development of a new class of highly selective and potent compounds directly targeting specific *KRAS* mutations. Among the different drugs currently under investigation in clinical studies, both AMG510 (Sotorasib) and MRTX849 (Adagrasib) are irreversible covalent small molecule inhibitors able to keep KRAS locked in its inactive state, showing promising activity in NSCLC patients harboring *KRAS* exon 2 p.G12C mutation.[39-41] Early results coming from phase I studies have recently shown: objective response rate (ORR) of 32.2%, disease control rate (DCR) of 88.1%, median progression free survival (PFS) of 6.3 months, and median duration of response of 10.9 months, in the small cohort of 59 *KRAS* exon 2 p.G12C-positive NSCLC patients receiving AMG510 in advanced lines of treatment for their metastatic disease within the CodeBreak 101 trial.[42] Although the median follow-up is shorter as yet, the administration of MRTX849 within the KRYSTAL-1 study [43] has been associated to an ORR of 45% and a DCR of 96% in a similar cohort of 51 molecularly selected advanced NSCLC patients harboring *KRAS* exon 2 p.G12C mutations, with 64% ORR in the small subgroup of patients harboring co-occurring Serine/Threonine Kinase 11 (*STK11*) mutations. Overall, these preliminary data revealed that direct KRAS targeting, by using covalent small molecules inhibitors, not only is feasible, but produced a relevant and durable clinical benefit along with a tolerable safety profile in heavily pre-treated NSCLC patients harboring *KRAS* exon 2 p.G12C mutations.

Commentato [MT4]: Aggiungerei qui una frase sulla frequenza della mutazione G12C, come fatto anche nel paragrafo del colon, tipo “the prevalence of the KRAS G12C mutation in NSCLC is approximately 12%-14% (40%-50% of all KRAS mutations)”.

Confirmatory results emerging from the ongoing phase II/III randomized trials will tell us about the real efficacy of these agents in earlier lines of treatment as well as about any potential differences regarding their therapeutic activity/tolerability. Of course, the recent advent of effective drugs targeting *KRAS* oncogene represented one of the major breakthroughs of lung cancer research over the last few years, showing unprecedent response in a heavily pre-treated, molecularly selected population harboring *KRAS* exn 2 p.G12C mutations. However the ORR emerging from these studies clearly suggested that both the tumor heterogeneity of *KRAS*-mutant disease, which we know is characterized by high incidence of co-occurring mutations involving other genes, as well as the early adaptation to KRAS targeted inhibitors may significantly affect the clinical activity of these agents.[44, 45] The issue of innate/acquired resistance to the novel KRAS inhibitors has been recently assessed by several works and require further investigation in dedicate studies, while a deeper understanding of the multiple molecular networks involving the mutant *RAS* tumor cell oncogenic signaling provided the biological rational to the design of clinical trials exploring the role of treatment combinations.[46, 47] Particularly the Src homology region 2 (SH2)-containing protein tyrosine phosphatase 2 (SHP2),[48] the PD-1/PD-L1 axis [49] as well as the cyclin dependent kinase (CDK)4/6 pathway,[50] emerged as the most promising targets for complementary and synergistic therapeutic strategies including either KRAS or MEK inhibitors, and are currently being tested within early phase clinical trials whose results are eagerly awaited. Finally pan-RAS inhibition represents an alternative promising treatment approach aiming to block *RAS* oncogene regardless of the specific kind of mutation.[51] Among the different drugs, the Son of Sevenless (SOS1) selective BI 1701963 inhibitor has shown the most promising profile in pre-clinical models and has been further advanced to the clinical investigation, with ongoing studies exploring the activity and tolerability of this compound either alone and in combination with MEK inhibitors.

3. RAS mutations in colorectal cancer: from negative selection to positive prediction

Mutations affecting *RAS* genes have been known for long as early genomic events driving CRC carcinogenesis and progression in the adenoma-carcinoma sequence. The treatment of *RAS* mutated CRC is a tough challenge for medical oncologists for several reasons.

First of all, *RAS* mutations have a negative prognostic impact in metastatic CRC (mCRC), being associated with shorter survival in multiple series from both clinical trials and real-life practice.[52] A mild negative effect on patients' survival was reported also in early stages of disease, and especially among patients with microsatellite stable tumors. [53-55]

Secondly, the assessment of *KRAS* exon 2 (codon 12 and 13) molecular status entered the therapeutic management of mCRC patients as the first biomarker useful to drive treatment choices in the clinical practice. In fact, after seminal translational works indicating that RAS mutations by-pass the blockade exerted by EGFR-targeted monoclonal antibodies, the *post-hoc* analysis of a phase III randomized trial of panitumumab versus best supportive care (BSC) in pre-treated mCRC clearly demonstrated lack of benefit from the anti-EGFR among patients bearing *KRAS* mutated tumors.[56] Based on these results, the use of both cetuximab and panitumumab was restricted to the subgroup of patients with *KRAS* wild-type tumours.

Commentato [ASB5]: PMID: 17363584, PMID: 16618717, PMID: 28148677

A refinement of the selection of candidates to anti-EGFRs was subsequently achieved thanks to the retrospective analyses of several randomized trials investigating the addition of an anti-EGFR agent to standard chemotherapy regimens. Again, lack of benefit (and possibly a detrimental effect) from anti-EGFRs was shown in patients with tumors bearing not only *KRAS* exon 2 but also *NRAS* exon 2 and *KRAS* and *NRAS* exon 3 (codon 59 and 61) and 4 (codon 117 and 146) mutations.[57-59] As a consequence, the use of anti-EGFR monoclonal antibodies was restricted to patients with *RAS* wild-type tumors.

More recently, the emergence of *RAS* mutations has been identified as a mechanism of acquired resistance to anti-EGFRs, since *RAS* mutated clones have been found at the time of disease progression in tumors initially sensitive and then become resistant to anti-EGFR-containing regimens.[60] The same mutations were evident in circulating tumor DNA extracted from liquid

biopsies collected throughout the therapeutic route of mCRC patients. This led to hypothesize the opportunity to obtain a dynamic track of anti-EGFR sensitivity in the different phases of the treatment history, and therefore to build a "molecularly-informed" *continuum of care* for mCRC patients.[61]

Thirdly, the presence of *RAS* mutations has been recently associated with resistance to other targeted strategies in mCRC, including human epidermal growth factor receptor 2 (HER2) inhibition in HER2-positive tumors.[62] Though in the absence of a formal demonstration due to the lack of randomized trials and the small numbers of treated patients, the explanation supporting this clinical finding is biologically sound, thus making the use of anti-HER2 agents more reasonable in HER2-positive *RAS* wild-type tumors.

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More recently, the subgroup analysis of the Keynote-177 trial investigating the upfront use of the checkpoint inhibitor pembrolizumab *versus* a standard treatment of cytotoxic doublets plus a biologic agent as first-line therapy in patients with microsatellite instable mCRC, showed a differential effect of the anti-PD1 according to *RAS* mutational status, suggesting lack of benefit among patients with *RAS* mutated tumors.[63] Due to the exploratory nature of this unplanned subgroup analysis, these data should be merely regarded as hypothesis generating, and potentially useful to investigate whether the immune suppressive effect on tumor microenvironment of *RAS* mutations suggested in preclinical experiences is evident also in microsatellite instable tumors.

Commentato [ASB7]: Update with published NEJM

The negative prognostic impact of *RAS* mutations, its negative predictive role with regard to available targeted agents, the lack of specific targeted approaches able to turn a relative advantage for cancer cells into an *Achille's heel*, make therapeutic options for these tumors, and therefore for around a half of affected patients, very limited.

More recently, the development of anti-KRAS exon 2 p.G12C agents opened a new perspective at least for a subgroup of patients with *RAS* mutated tumors. The percentage of this mutation among all *KRAS* mutations according to literature data spans from 6% to 17%.[64, 65] In a series of consecutive patients treated in the daily clinical practice, as compared with other *KRAS* mutations, *KRAS* exon 2 p.G12C was more frequent among men, it was associated with a higher occurrence of liver and lung

metastases and a lower frequency of peritoneal spread. Interestingly, patients bearing *KRAS* exon 2 p.G12C mutated tumors had shorter OS than patients with other *KRAS* mutations.

In the mCRC cohort of the phase I CodeBreak 100 trial including 42 highly pretreated patients the response rate with sotorasib was 7.1% and the disease control rate was 73.8%. Objective responses were observed with a daily dose of 960 mg. Among the 25 patients receiving the 960 mg daily dose the response rate and the disease control rate raised at 12% and 80%, respectively.[66] Encouraging preclinical and early clinical results were reported also with the *KRAS* exon 2 p.G12C inhibitor MRTX894.[67]

Though acknowledging signals of activity of this targeted approach, results reported in mCRC are definitely less encouraging than those evidenced in NSCLC. In an effort to disclose the reasons of such inconsistent results, Amodio *et al* identified a higher ERK rebound following G12C inhibition in *KRAS* exon 2 p.G12C mutated CRC cells than in NSCLC cells.[68] EGFR signaling was identified as a predominant mechanism of resistance, thus opening the way to the combination of a *KRAS* exon 2 p.G12C inhibitor with an anti-EGFR agent as a potentially efficacious strategy in mCRC, able to overcome resistance to the *KRAS* exon 2 p.G12C inhibitor as single agent.

These findings show evident similarities with the steps that led to the demonstration of the efficacy of the BRAF inhibitor encorafenib in combination with the anti-EGFR cetuximab in the treatment of *BRAF* exon 15 p.V600E mutated mCRC. While BRAF inhibitors as single agents showed very limited activity in mCRC differently from advanced melanoma,[69] the identification of the hyperactivation of EGFR as a mechanism of resistance to BRAF inhibition led to the investigation of the double targeted strategy that showed a significant OS improvement in pre-treated *BRAF* mutated mCRC patients. [70, 71]

Moreover, since in immune-competent mice, treatment with AMG510 resulted in a pro-inflammatory tumour microenvironment its combination with checkpoint inhibitors appears worth of investigation.[72]

Again, the combination of BRAF inhibitors and immunotherapy seems promising in early phase trials in *BRAF* mutant mCRC.[73]

Commentato [MT8]: Toglierei

4. Real world dataset: www.rasatlas.com

Real world data were retrospectively collected from the last two years of activities of 12 referral institutions specialized in lung (n = 7) and colorectal cancer (n = 5) molecular testing. 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/).

Regarding the whole cohort of NSCLC analyzed samples (n = 1053), 583 (55.4%) and 470 (44.6%) were histological and cytological specimens, respectively. Considering the histological subtypes, 490 (84.0%) small biopsies and 93 (16.0%) surgical resections were analyzed. Among the cytological cohort, 284 (59.2%) cell blocks and 186 (40.8%) direct smears were considering for the analysis. Moreover, 840 (79.8%), 134 (12.7%), 73 (6.9%) and 6 (0.6%) out of 1053 patients were diagnosed as adenocarcinomas (ADCs), neuroendocrine carcinomas (NECs), squamous cell-carcinomas (SCCs) and a mixed adenocarcinomas and squamous cell carcinomas (ADCs plus SCCs), respectively. Considering the detection technology used to perform lung cancer molecular analysis among the 7 different Italian Institutions, the most adopted approach was the combination of next generation sequencing (NGS) platform and real time polymerase chain reaction (RT-PCR) system (n=4, 57.2%), in two (28.5%) institutions only NGS system was adopted in the routine practice, while one (14.3%) participating institution performed molecular analysis by using massARRAY system (Agena Bioscience, San Diego CA, USA). Of note, in all the participating centers adopting NGS technology, the Ion S5™ System (ThermoFisher Scientifics, Waltham, MA) was employed.

Overall, 23.8% (251/1053) patients harbored a *RAS* genes mutation. Almost all of them (99.2%, 249/251) were reported in the *KRAS* gene, whereas only two (0.8%) patients harbored a *NRAS* mutation. In detail, exon 2 p.G12C was the most represented *KRAS* alteration (110/249; 44.3%),

followed by exon 2 p.G12V (43/249; 17.5%) and exon 2 p.G12D (27/249; 10.8%) point mutations. Briefly, a wide range of different *KRAS* mutations (n=15 with <10 % of frequency detection) were also identified within the tested population (Table 1). The only two identified *NRAS* point mutations were the exon 2 p.G12A and the exon 3 p.Q61L.

Overall, 1523 archived data from CRC patients were retrieved for our analysis. In particular, 728/1523 patients (47.8%) showed *K*- and/or *NRAS* mutations. Among them, 657/728 (90.2%), 59/728 (8.2%), 7/728 (1.0%); 4/728 (0.5%) and 1/728 (0.1%) cases harbored *KRAS*, *NRAS*, *KRAS* plus *KRAS*, *KRAS* plus *NRAS* and *NRAS* plus *NRAS* point mutations. All samples were diagnosed as ADC. Regarding histological samples, 1118/1523 (73.4%) surgical resections and 405/1523 (26.6%) small biopsies were considered for the analysis. As far as methodological approach is concerned, this scenario appears heterogeneous because two (40.0%) out of five institutions adopted an NGS-based diagnostic workflow to carry out molecular analysis, whereas the remaining centers equally adopted (1/5, 20%) NGS platform in combination with RT-PCR approach, massARRAY system and direct sanger Sequencing platform in association with high resolution melting analysis system (HRMA). Also, in this case, all participating centers adopting NGS used the Ion S5™ System (ThermoFisher Scientifics). Regarding regards *KRAS* mutational status, exon 2 p.G12V point mutation was identified in the large part of cases (178/676; 26.4%), moreover exon 2 p.G12D and p.G13D mutations were respectively detected in 129/676 (19.2%) and 111/676 (16.5%) cases; a not negligible number of *KRAS* mutations was also identified in 29 different hot spot regions. (Table 2). Interestingly, the highest number of *NRAS* mutations concerns with exon 3 p.Q61K, (17/62; 27.4%) p.Q61R (8/62; 12.9%), p.Q61L (7/62; 11.3%), on the other side a not negligible fraction of *NRAS* mutations was distributed among exon 2 and 4. (Table 3).

The data collected from 12 different Italian institutions (7 for lung cancer and 5 for colorectal cancer; Tables 1, 2, 3) supervised by a group of experienced pathologists and oncologists (UM, FL, GT, SN) and summarized in real word dataset section were compared with the data reported in the COSMIC database (www.cosmic.com; last access 26/11/2020) and exploited to build-up a periodically updated

Commentato [MT9]: Grafici a torta forse potrebbero essere più appealing; che ne dite?

user friendly knowledge base database (www.rasatlas.com) to help the healthcare personnel in the management of *RAS* gene mutations in the landscape of precision oncology (Figure 1).

Conclusion

KRAS and *NRAS* mutations play a pivotal role in the management of advanced stages NSCLC and CRC patients. To date, several clinical trials have demonstrated the efficacy of targeted therapies in these settings of patients. For this reason, *KRAS* and *NRAS* mutational status assessment is pivotal for the adequate management of advanced stages NSCLC and CRC patients. Besides the prognostic role of these alterations in either NSCLC or CRC patients, novel targeted therapies are being under investigation to selectively treat *KRAS*- and *NRAS*-mutated patients. To this end, and in order to avoid any patient behind, it is crucial to better define the different mutations that may arise in these two genes. In conclusion, in this complex scenario, we reviewed literature and real-world practice collected from 12 different Italian institutions and summarized in a knowledge base database (www.rasatlas.com) to help the healthcare personnel in the management of *RAS* gene mutations in the landscape of precision oncology.

Tables and Figure legend

Table 1. Distribution of *KRAS* mutations in NSCLC patients: Italian experience.

Table 2. Distribution of *KRAS* mutations in CRC patients: Italian experience.

Table 3. Distribution of *NRAS* mutations in CRC patients: Italian experience.

Figure 1. www.rasatlas.com home page.

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Authors' contribution

Conceptualization: Umberto Malapelle, Maria Lucia Reale, Chiara Cremolini, Francesco Passiglia, Giancarlo Troncone, Silvia Novello.

Methodology: all authors.

Software: all authors.

Validation: all authors.

Formal analysis: all authors.

Investigation: all authors.

Resources: all authors.

Data Curation: all authors.

Writing - Original Draft: Umberto Malapelle, Maria Lucia Reale, Chiara Cremolini, Francesco Passiglia, Francesco Pepe, Pasquale Pisapia, Giancarlo Troncone, Silvia Novello.

Writing - Review & Editing: all authors.

Visualization: all authors.

Supervision: Giancarlo Troncone, Silvia Novello.

Project administration: Umberto Malapelle, Maria Lucia Reale, Chiara Cremolini, Francesco Passiglia, Fotios Loupakis, Giancarlo Troncone, Silvia Novello.

Conflict of interest

Umberto Malapelle has received personal fees (as consultant and/or speaker bureau) from Boehringer Ingelheim, Roche, MSD, Amgen, ThermoFisher Scientifics, Diaceutics, GSK, Merck and AstraZeneca, 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. The other authors declare no potential conflicts of interest.

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