

## Research Paper

# The biomarkers ATLAS: An audit on 1100 non-small cell lung cancer from an Italian knowledge-based database



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## ABSTRACT

**Aims:** To date, precision medicine has revolutionized the clinical management of Non-Small Cell Lung Cancer (NSCLC). International societies approved a rapidly improved mandatory testing biomarkers panel for the clinical stratification of NSCLC patients, but harmonized procedures are required to optimize the diagnostic workflow. In this context a knowledge-based database (Biomarkers ATLAS, <https://biomarkersatlas.com/>) was developed by a supervising group of expert pathologists and thoracic oncologists collecting updated clinical and molecular records from about 80 referral Italian institutions. Here, we audit molecular and clinical data from n = 1100 NSCLC patients collected from January 2019 to December 2020.

**Methods:** Clinical and molecular records from NSCLC patients were retrospectively collected from the two coordinating institutions (University of Turin and University of Naples). Molecular biomarkers (*KRAS*, *EGFR*, *BRAF*, *ROS1*, *ALK*, *RET*, *NTRK*, *MET*) and clinical data (sex, age, histological type, smoker status, PD-L1 expression, therapy) were collected and harmonized.

**Results:** Clinical and molecular data from 1100 (n = 552 mutated and n = 548 wild-type) NSCLC patients were systematized and annotated in the ATLAS knowledge-database. Molecular records from biomarkers testing were matched with main patients' clinical variables.

**Conclusions:** Biomarkers ATLAS (<https://biomarkersatlas.com/>) represents a unique, easily managing, and reliable diagnostic tool aiming to integrate clinical records with molecular alterations of NSCLC patients in the real-world Italian scenario.

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## 1. Introduction

In the era of precision medicine an increasing number of predictive biomarkers was clinically approved by international scientific societies to select advanced Non-Small Cell Lung Cancer (NSCLC) patients who are eligible for targeted therapies [1–3]. In this rapidly evolving scenario, a plethora of diagnostic specimens must be available for both morphological and molecular evaluation in order to optimize the therapeutic management of NSCLC patients [4,5]. Of note, adequacy of tissue samples represents one of the main gaps limiting biomarker testing in metastatic NSCLC, because scant biological specimens occur in a not negligible percentage of cases (20–25 %) [6–8]. However high-sensitive technologies, like new generation RT-PCR and Next Generation Sequencing (NGS) platforms have optimized the biomarker detection workflow within the routine diagnosis of NSCLC [9]. These platforms may assess diagnostically functional biomarkers from a series of different clinically routine tumor samples [9,10]. Remarkably, RT-PCR based platforms are affected by limited reference range and high-material consuming to successfully analyze approved biomarkers [11]. Conversely, NGS platforms enable to simultaneously detect several clinically relevant hot spot alterations across different key genes for the personalized treatment of solid tumors [12,13] but high-skilled personnel, technical cost and challenging data interpretation limit the spreading of this technology [10,14]. It has been demonstrated that harmonized procedures are still necessary to overcome undetected clinically relevant molecular alterations due to managing issue in pre-analytical and analytical phases as well as data reporting [15]. At the sight of these critical aspects, consulting comprehensive public available databases able to integrate different type of records (technical platform, molecular records, therapeutic regimen) for the management of NSCLC patients, could help to bridge this gap. Therefore a knowledge-based database (Biomarkers ATLAS, <https://biomarkersatlas.com/>) was developed by a supervising group of expert pathologists and thoracic oncologists aiming to collect updated clinical and molecular records from 80 referral Italian institutions including molecular biomarkers testing and clinical management of lung cancer patients [16]. These data provide a national real-world dataset of biomarkers testing results from NSCLC patients, aiming to support healthcare personnel in the routine diagnostic and therapeutic management of lung cancer patients.

## 2. Methods

Clinical and molecular data from advanced stage NSCLC patients were retrospectively collected from the two coordinating institutions (University of Turin and University of Naples) of ATLAS research network (<https://biomarkersatlas.com/>). Briefly, each record was included in the ATLAS database following the supervision from high-experienced pathologists, thoracic oncologists, and molecular biologists. In detail both molecular biomarkers (*KRAS*, *EGFR*, *BRAF*, *ROS1*, *ALK*, *RET*, *NTRK*, *MET*) and the main clinical features (sex, age, histological type, smoker status, PD-L1 expression, therapy) from enrolled patients were annotated in the ATLAS knowledge-database. Written informed consent was obtained from all the patients, in accordance with the general authorization to process personal data for scientific research purposes from “The Italian Data Protection Authority” (<http://www.garanteprivacy.it/web/guest/home/docweb/-/docwebdisplay/export/2485392>). All data was anonymously managed using numerical codes, and all samples were handled in compliance with the Helsinki Declaration (<https://www.wma.net/fr/news-post/en-matierede-transfert-des-taches-la-securite-des-patients-et-la-qualitedes-soins-devraient-etre-pr-imordiales/>).

Patients’ clinical, pathological, and molecular characteristics have been summarized either by descriptive statistics or categorical tables. Descriptive analyses have been performed, including means, standard deviations, medians, quartiles, and absolute/relative frequencies (with their respective two-sided 95 % confidence interval limits, where

relevant), according to the specific variables of interest.

## 3. Results

### 3.1. Patients’ characteristics

From January 2019 to December 2020, a total of 1100 advanced NSCLC patients collected from  $n = 2$  coordinator institutions were retrospectively enrolled, whose clinical, pathological and molecular features have been annotated within the ATLAS knowledge-database. With reference to the routine molecular testing results, the study population included  $n = 552$  (50.2 %) patients harboring an oncogenic driver alteration and  $n = 548$  (49.8 %) affected by wild-type disease. The targetable molecular biomarkers were distributed as follow:  $n = 152$  *EGFR* (13.8 %),  $n = 291$  *KRAS* (26.5%),  $n = 29$  *BRAF* (2.6 %) hot spot mutations,  $n = 46$  *ALK* (4.2 %),  $n = 15$  *RET* (1.4 %),  $n = 11$  *ROS1* (1.0 %), and  $n = 1$  *NTRK* (0.1 %) aberrant transcripts. Moreover  $n = 3$  *MET* exon 14 skipping (0.3 %) positive cases were annotated. (Fig. 1), five different clinical variables (age, smoking status, histology, PD-L1 expression level, and antitumor treatment) were orthogonally evaluated and matched with the molecular data. (Fig. 1A, 1B).

In details,  $n = 369$  (33.5 %) were females and 668 (60.8 %) males, respectively. Moreover, the vast majority of NSCLC patients were > 60–80 years/old (687; 65.2 %). Among them, 648 (58.9 %) NSCLC patients were diagnosed as Adenocarcinoma (ADC). Moreover,  $n = 409$  (37.1 %), 189 (17.2 %) and 178 (16.2 %) PD-L1 < 1, 1–49 and > 50 PD-L1 expression level was observed. Then, 94 (8.5 %) 92 (8.4 %) 33 (3.0 %) 175 (15.9 %) smokers, never smoker, former (<10p/y) and (>10p/y) smokers were retrieved, respectively. With reference to the therapeutic management, 114 (10.4 %), 128 (11.6 %), 34 (3.1 %) and 33 (3.0 %) of received target therapy, chemotherapy, immunotherapy and chemo-immunotherapy, respectively as first-line treatment (Table 1).

Moreover, each mutation in the ATLAS knowledge-database has been matched to the pubmed indexed references as well as to [clinicaltrials.gov](https://clinicaltrials.gov) website (Biomarkers ATLAS, <https://biomarkersatlas.com/>).

### 3.2. EGFR

*EGFR* mutated patients showed a low number of male (54 out of 152, 35.5 %) in comparison with female cases (98 out of 152, 64.5 %). As expected, the majority of cases were ADC (91.6 %) while remaining cases highlighted NOS (4.9 %) and SCC (0.7 %). From an epidemiological point of view, *EGFR* mutated patients were categorized as follows: age > 60–80 (66.2 %), age 40–60 (17.9 %), age > 80 (14.6 %) and age < 40 (1.3 %). Most of *EGFR*-muted patients were never-smokers (52.1 %), in line with literature data. Conversely a small percentage (7.3 %) of current smokers harbor an *EGFR* mutation. As far as PD-L1 status has been concerned, *EGFR* mutated tumors showed PD-L1 expression level < 1 % in  $n = 67$  (59.3 %) cases,.. Almost all these patients (95.6 %) received upfront target therapy while the remaining subgroup (4.4 %) were treated with chemotherapy and the first-line treatment was not registered for  $n = 61$  cases. Overall, molecular alterations were detected in  $n = 149$  (98.0 %) cases by adopting an NGS-based diagnostic approach while only 2.0 % used RT-PCR (Table 2).

Remarkably, most common *EGFR* hotspot mutations were detected in exons 19 and 21. A detailed list of all the annotated mutations has been reported in Table 3.

### 3.3. BRAF

*BRAF* mutated NSCLC patients showed a comparable number of male (16 out of 29, 55.0 %) and female cases (13 out of 29, 45.0 %). As expected, the vast majority of cases were ADC (78.6 %). From an epidemiological point of view, *BRAF* mutated patients were categorized as follows: age > 60–80 (75.0 %), age 40–60 (21.4 %), and age < 40 (3.6 %). Regarding smoking habits most frequently Former > 10p/y (66.7 %)

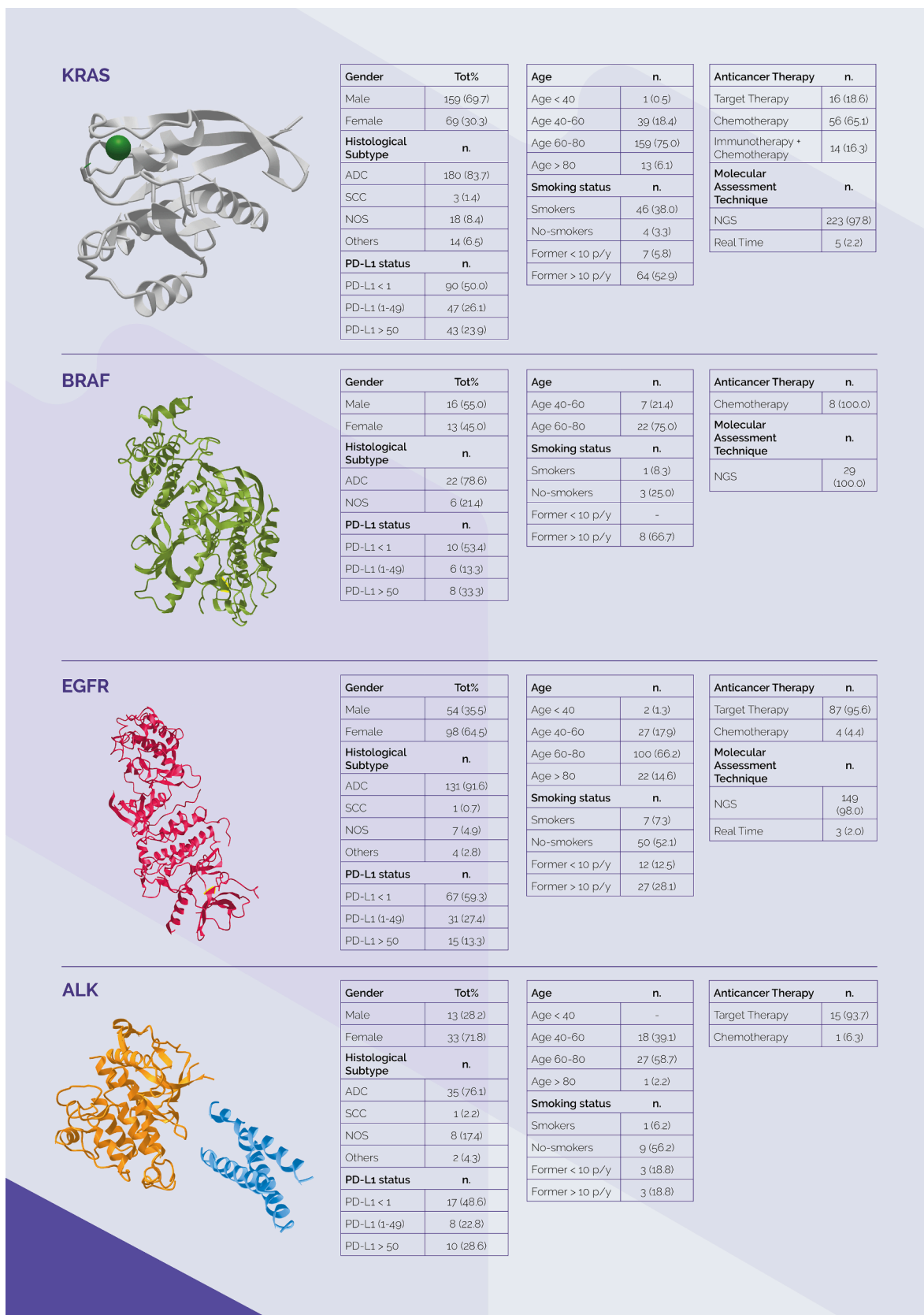


Fig. 1. Panel of molecular biomarkers matched with clinical variables in in ATLAS knowledge-database.

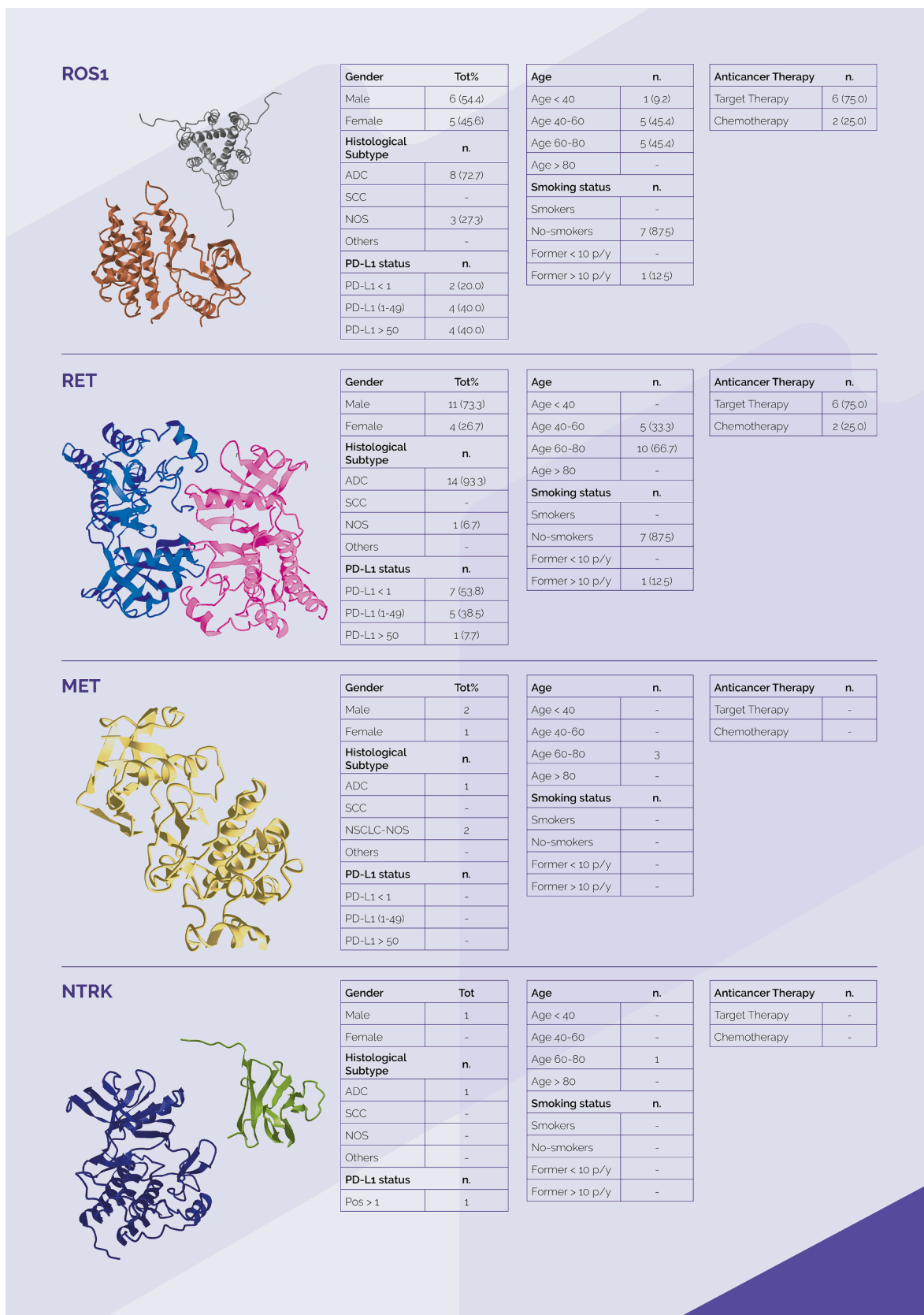


Fig. 1. (continued).

patients. As far as PD-L1 status was concerned, *BRAF* mutated patients showed PD-L1 negative disease in n = 8 (53.4 %) cases, whereas an expression rate > 50 % and ranging from 1 to 50 % were observed in n =

33.3 % and n = 13.3 % of cases, respectively. All these patients were treated with first-line chemotherapy since targeted therapy was not approved yet in Italy at that time, while treatment administration was

**Table 1**  
Clinical and molecular records of 1100 NSCLC patients from ATLAS database.

| Variable                    | n.1100    | %        |
|-----------------------------|-----------|----------|
| <b>Gender</b>               |           |          |
| Male                        | 668       | 60.8     |
| Female                      | 369       | 33.5     |
| Unknown                     | 63        | 5.7      |
| <b>Histological Subtype</b> |           |          |
| ADC                         | 648       | 58.9     |
| SCC                         | 34        | 3.1      |
| NOS                         | 144       | 13.1     |
| Others                      | 58        | 5.3      |
| Unknown                     | 216       | 19.6     |
| <b>Age</b>                  |           |          |
| Age < 40                    | 5         | 0.5      |
| Age 40–60                   | 172       | 15.5     |
| Age > 60–80                 | 687       | 62.5     |
| Age > 80                    | 66        | 6.0      |
| Unknown                     | 170       | 15.5     |
| <b>PD-L1</b>                |           |          |
| <1                          | 408       | 37.1     |
| 1–49                        | 189       | 17.2     |
| >50                         | 178       | 16.2     |
| Unknown                     | 325       | 29.5     |
| <b>Smoking status</b>       |           |          |
| Smokers                     | 94        | 8.5      |
| No-smokers                  | 92        | 8.4      |
| Former < 10p/y              | 33        | 3.0      |
| Former > 10p/y              | 175       | 15.9     |
| Unknown                     | 706       | 64.2     |
| <b>Anticancer Therapy</b>   | <b>n.</b> | <b>%</b> |
| Target Therapy              | 114       | 10.4     |
| Chemotherapy                | 128       | 11.6     |
| Immunotherapy               | 34        | 3.1      |
| Chemo + Immunotherapy       | 33        | 3.0      |
| Unknown                     | 791       | 71.9     |

not registered in n = 21 cases. (Table 4).

Similarly, all molecular data were retrieved by adopting NGS based approaches. A total of n = 29 hot BRAF spot mutations were observed. Remarkably, most common BRAF hotspot mutations were equally distributed among exons 15 and 11, with a detailed list of annotated mutations reported in Table 5.

### 3.4. KRAS

Clinical data were available for 228 KRAS mutated patients registered in the ATLAS dataset. They showed a remarkable difference in terms of sex distribution (male 159 out of 228, 69.7 % and female 69 out of 228, 30.3 %). As expected, the vast majority of cases were ADC (83.7 %). From an epidemiological point of view, KRAS positive patients were categorized as follows: age > 60–80 (75.0 %), age 40–60 (39.0 %), age > 80 (6.1 %) and age < 40 (0.5 %). Regarding smoking habits more frequently former > 10p/y KRAS mutated patients (52.9 %) were observed, in line with literature data. As far as PD-L1 status has been concerned, KRAS mutated patients showed PD-L1 expression < 1 % in 50.0 % of cases, whereas an expression ranging from 1 to 50 and ≥ 50 % was observed in 26.1 % and 23.9 % of cases, respectively. These patients were predominantly treated with chemotherapy in 65.1 % of cases. (Table 6) Overall, molecular alterations were detected in n = 223 (97.8 %) cases by adopting NGS-based approach. A total of n = 291 hot spot mutations were identified. Remarkably, most common KRAS hotspot mutations were observed in exon 2, with a detailed list of annotated mutations available in Table 7.

### 3.5. Aberrant transcripts

Clinically approved gene rearrangements among ALK, ROS1, RET, NTRK genes and molecular alterations promoting MET Δ14 skipping were taken into account. Aberrant transcripts positive patients showed a

**Table 2**  
Clinical records of EGFR positive NSCLC patients from ATLAS database.

| Variables                   | Tot (%)    | Common (%) | Uncommon (%) | Ex 20 insertions (%) |
|-----------------------------|------------|------------|--------------|----------------------|
| <b>Gender</b>               |            |            |              |                      |
| Male                        | 54 (35.5)  | 28 (30.8)  | 23 (41.8)    | 3 (50.0)             |
| Female                      | 98 (64.5)  | 63 (69.2)  | 32 (58.2)    | 3 (50.0)             |
| <b>Histological Subtype</b> |            |            |              |                      |
| ADC                         | 131 (91.6) | 79 (86.8)  | 47 (85.5)    | 5 (100.0)            |
| SCC                         | 1 (0.7)    | –          | 1 (1.8)      | –                    |
| NOS                         | 7 (4.9)    | 2 (2.2)    | 5 (9.1)      | –                    |
| Others                      | 4 (2.8)    | 3 (3.3)    | 1 (1.8)      | –                    |
| <b>PD-L1 status</b>         |            |            |              |                      |
| PD-L1 < 1                   | 67 (59.3)  | 41 (45.1)  | 24 (43.6)    | 2 (33.3)             |
| PD-L1 (1–49)                | 31 (27.4)  | 17 (18.7)  | 13 (23.6)    | 1 (16.7)             |
| PD-L1 > 50                  | 15 (13.3)  | 10 (11.0)  | 4 (7.3)      | 1 (16.7)             |
| <b>Age</b>                  |            |            |              |                      |
| Age < 40                    | 2 (1.3)    | 2 (2.2)    | –            | –                    |
| Age 40–60                   | 27 (17.9)  | 15 (16.5)  | 12 (21.8)    | –                    |
| Age 60–80                   | 100 (66.2) | 66 (72.5)  | 30 (54.4)    | 4 (66.7)             |
| Age > 80                    | 22 (14.6)  | 7 (7.7)    | 13 (23.6)    | 2 (33.3)             |
| <b>Smoking status</b>       |            |            |              |                      |
| Smokers                     | 7 (7.3)    | 4 (4.4)    | 3 (5.5)      | –                    |
| No-smokers                  | 50 (52.1)  | 26 (28.6)  | 23 (41.8)    | 1 (16.7)             |
| Former < 10p/y              | 12 (12.5)  | 9 (9.9)    | 3 (5.5)      | –                    |
| Former > 10p/y              | 27 (28.1)  | 12 (13.2)  | 13 (23.6)    | 2 (33.3)             |
| <b>Anticancer Therapy</b>   |            |            |              |                      |
| Target Therapy              | 87 (95.6)  | 47 (51.6)  | 40 (72.7)    | –                    |
| Chemotherapy                | 4 (4.4)    | 3 (3.3)    | –            | 1 (16.7)             |
| <b>Molecular Assessment</b> |            |            |              |                      |
| <b>Technique</b>            |            |            |              |                      |
| NGS                         | 149 (98.0) | 88 (96.7)  | 55 (100.0)   | 6 (100.0)            |
| Real Time                   | 3 (2.0)    | 3 (3.3)    | –            | –                    |

comparable number of male (34 out of 76, 44.7 %) and female cases (42 out of 76, 55.3 %). As expected, the vast majority of cases were ADC (77.7 %) From an epidemiological point of view, aberrant transcripts positive patients were categorized as follows: age > 60–80 (59.3 %), age 40–60 (38.1 %), age > 80 (1.3 %) and age < 40 (1.3 %). Regarding smoking habits most frequently never smokers aberrant rearranged patients (55.9 %) were observed, in line with literature data. As far as PD-L1 status is concerned, these patients showed PD-L1 expression < 1 % in 44.1 % of cases, whereas an expression ranging from 1 to 50 and > 50 % were observed in 30.5 % and 25.4 %, respectively.. These patients were treated with targeted agents (76.5 %) or chemotherapy (23.5 %). (Table 8) Overall, molecular alterations were detected in n = 63 (82.9 %) cases by adopting NGS-based technology.. Remarkably, most common aberrant transcripts were detected in ALK. (Complete list of fusion genes is available in Table 9).

## 4. Discussion

In the era of precision medicine, the rapidly increasing number of predictive biomarkers has revolutionized the clinical management of lung cancer patients. This heterogeneous scenario requires novel supporting tools for healthcare personnel helping to adequately decipher both clinical and molecular data from NSCLC patients. Technical strategies approached by each European country should be optimized at the sight of technical (number of molecular analysis/years, number of approved biomarkers) and administrative critical issues (reimbursement

**Table 3**  
List of *EGFR* mutations in NSCLC patients from ATLAS database.

| Nucleotide substitution | Amino acid change    | N = 172 | %      |
|-------------------------|----------------------|---------|--------|
| <b>Exon 18</b>          |                      |         |        |
| c.2117 T > C            | p.I706T              | 1       | 0.6 %  |
| c.2155G > T             | p.G719C              | 3       | 1.6 %  |
| c.2155G > A             | p.G719S              | 2       | 1.2 %  |
| c.2170G > A             | p.G724S              | 1       | 0.6 %  |
| c.2125G > A             | p.E709K              | 1       | 0.6 %  |
| c.2152C > G             | p.L718V              | 2       | 1.2 %  |
| c.2127_2129del          | p.E709_T710delinsD   | 2       | 1.2 %  |
| <b>Exon 19</b>          |                      |         |        |
| c.2236_2250del          | p.E746_A750del       | 56      | 32.6 % |
| c.2237_2251del          | p.E746_T751delinsA   | 2       | 1.2 %  |
| c.2240_2257del          | p.L747_P753delinsS   | 6       | 3.4 %  |
| c.2239_2256del          | p.L747_S752del       | 2       | 1.2 %  |
| c.2264C > A             | p.A755D              | 1       | 0.6 %  |
| c.2240_2254del          | p.L747_T751del       | 5       | 2.8 %  |
| c.2237_2255delins       | p.E746_S752delinsV   | 7       | 4.0 %  |
| c.2238_2255del          | p.E746_S752delinsD   | 1       | 0.6 %  |
| c.?                     | p.I744_K745insKIPVAI | 2       | 1.2 %  |
| p.S752_I759del          | p.S752_I759del       | 2       | 1.2 %  |
| <b>Exon 20</b>          |                      |         |        |
| c.2300_2308dup          | p.A767_V769dup       | 2       | 1.2 %  |
| c.2369C > T             | p.T790M              | 10      | 5.8 %  |
| c.2375 T > C            | p.L792P              | 1       | 0.6 %  |
| c.2308_2309insCCAGCGTGG | p.M766_A767ins       | 1       | 0.6 %  |
| c.2311delinsGGTT        | p.N771delinsGY       | 2       | 1.2 %  |
| c.?                     | p.S768_D760dup       | 1       | 0.6 %  |
| c.2281G > T             | p.D761Y              | 1       | 0.6 %  |
| c.2303G > T             | p.S768I              | 4       | 2.2 %  |
| c.2389 T > A            | p.C797S              | 1       | 0.6 %  |
| <b>Exon 21</b>          |                      |         |        |
| c.2572C > A             | p.L858M              | 2       | 1.2 %  |
| c.2612C > A             | p.A871E              | 1       | 0.6 %  |
| c.2573 T > G            | p.L858R              | 44      | 25.6 % |
| c.2582 T > A            | p.L861Q              | 6       | 3.4 %  |

**Table 4**  
Clinical records of *BRAF* positive NSCLC patients from ATLAS database.

| Variables                             | Tot        | p.V600E (%) | Non-p.V600E (%) |
|---------------------------------------|------------|-------------|-----------------|
| <b>Gender</b>                         |            |             |                 |
| Male                                  | 16 (55.0)  | 2 (25.0)    | 14 (66.7)       |
| Female                                | 13 (45.0)  | 6 (75.0)    | 7 (33.3)        |
| <b>Histological Subtype</b>           |            |             |                 |
| ADC                                   | 22 (78.6)  | 7 (87.5)    | 15 (75.0)       |
| NOS                                   | 6 (21.4)   | 1 (12.5)    | 5 (25.0)        |
| <b>PD-L1 status</b>                   |            |             |                 |
| PD-L1 < 1                             | 10 (53.4)  | 3 (37.8)    | 7 (43.8)        |
| PD-L1 (1–49)                          | 6 (13.3)   | –           | 6 (37.4)        |
| PD-L1 > 50                            | 8 (33.3)   | 5 (62.2)    | 3 (18.8)        |
| <b>Age</b>                            |            |             |                 |
| Age 40–60                             | 7 (21.4)   | 3 (37.5)    | 4 (19.0)        |
| Age 60–80                             | 22 (75.0)  | 5 (62.5)    | 17 (81.0)       |
| <b>Smoking status</b>                 |            |             |                 |
| Smokers                               | 1 (8.3)    | –           | 1 (11.1)        |
| No-smokers                            | 3 (25.0)   | 2 (66.7)    | 1 (11.1)        |
| Former > 10p/y                        | 8 (66.7)   | 1 (33.3)    | 7 (77.8)        |
| <b>Anticancer Therapy</b>             |            |             |                 |
| Chemotherapy                          | 8 (100.0)  | 2 (100.0)   | 6 (100.0)       |
| <b>Molecular Assessment Technique</b> |            |             |                 |
| NGS                                   | 29 (100.0) | 8 (100.0)   | 21 (100.0)      |

cost, high-skilled personnel) [17] encountered by referral Italian institutions. In addition, rapidly increasing number of mandatory testing biomarkers approved by international societies requires advanced diagnostic tools taking into account the upcoming biomarkers routinely tested in clinical practice [18] At the sight of the technical issues impacting on the diagnostic availability of tissue specimens for molecular profiling of NSCLC patients, liquid biopsy (peripheral blood) is

**Table 5**  
List of *BRAF* mutations in NSCLC patients from ATLAS database.

| Nucleotide substitution | Amino acid change | N = 29 | %      |
|-------------------------|-------------------|--------|--------|
| <b>Exon 11</b>          |                   |        |        |
| c.1405G > C             | p.G469Q           | 1      | 3.4 %  |
| c.1406G > T             | p.G469V           | 4      | 13.8 % |
| c.1406G > C             | p.G469A           | 6      | 20.8 % |
| c.1405G > A             | p.G469R           | 2      | 6.9 %  |
| c.1396G > A             | p.G466R           | 1      | 3.4 %  |
| <b>Exon 15</b>          |                   |        |        |
| c.1405G > C             | p.N581S           | 1      | 3.4 %  |
| c.1799 T > A            | p.V600E           | 8      | 27.7 % |
| c.1786G > C             | p.G596R           | 2      | 6.9 %  |
| c.1801A > G             | p.K601E           | 2      | 6.9 %  |
| c.1756G > A             | p.E586K           | 1      | 3.4 %  |
| c.1794_1796dup          | p.T599dup         | 1      | 3.4 %  |

**Table 6**  
Clinical records of *KRAS* positive NSCLC patients from ATLAS database.

| Variables                             | Tot        | p.G12C (%)  | Non-p.G12C (%) |
|---------------------------------------|------------|-------------|----------------|
| <b>Gender</b>                         |            |             |                |
| Male                                  | 159 (69.7) | 75 (75.0)   | 84 (65.5)      |
| Female                                | 69 (30.3)  | 25 (25.0)   | 44 (34.4)      |
| <b>Histological Subtype</b>           |            |             |                |
| ADC                                   | 180 (83.7) | 79 (85.8)   | 101 (82.1)     |
| SCC                                   | 3 (1.4)    | 1 (1.1)     | 2 (1.6)        |
| NOS                                   | 18 (8.4)   | 10 (10.9)   | 8 (6.5)        |
| Others                                | 14 (6.5)   | 2 (2.2)     | 12 (9.8)       |
| <b>PD-L1 status</b>                   |            |             |                |
| PD-L1 < 1                             | 90 (50.0)  | 34 (43.6)   | 56 (54.9)      |
| PD-L1 (1–49)                          | 47 (26.1)  | 21 (26.9)   | 26 (25.5)      |
| PD-L1 > 50                            | 43 (23.9)  | 23 (29.5)   | 20 (19.6)      |
| <b>Age</b>                            |            |             |                |
| Age < 40                              | 1 (0.5)    | –           | 1 (0.8)        |
| Age 40–60                             | 39 (18.4)  | 11 (11.1)   | 28 (24.8)      |
| Age 60–80                             | 159 (75.0) | 77 (77.8)   | 82 (72.6)      |
| <b>Age &gt; 80</b>                    |            |             |                |
| 13 (6.1)                              | 11 (11.1)  | 2 (1.8)     |                |
| <b>Smoking status</b>                 |            |             |                |
| Smokers                               | 46 (38.0)  | 17 (36.2)   | 29 (39.2)      |
| No-smokers                            | 4 (3.3)    | –           | 4 (5.4)        |
| Former < 10p/y                        | 7 (5.8)    | 1 (2.1)     | 6 (8.1)        |
| Former > 10p/y                        | 64 (52.9)  | 29 (61.7)   | 35 (47.3)      |
| <b>Anticancer Therapy</b>             |            |             |                |
| Immunotherapy                         | 16 (18.6)  | 4           | 12             |
| Chemotherapy                          | 56 (65.1)  | 27          | 29             |
| Immunotherapy + Chemotherapy          | 14 (16.3)  | 4           | 10             |
| <b>Molecular Assessment Technique</b> |            |             |                |
| NGS                                   | 223 (97.8) | 100 (100.0) | 123 (96.1)     |
| Real Time                             | 5 (2.2)    | –           | 5 (3.9)        |

rapidly emerging as an integrative source of nucleic acids to successfully evaluate actionable alterations in NSCLC patients [19]. Moreover, data analysis needs harmonized, comprehensive and continuously updated knowledge-based databases containing technical, molecular and clinical records to optimize clinical administration of solid tumor patients. In this context, the Italian knowledge database Biomarkers ATLAS, <https://biomarkersatlas.com/>) has been developed aiming to collect clinical-pathological-molecular variables as well as diagnostic and therapeutic practices from NSCLC patients in the real-world setting. Data recorded by participating institutions are freely available either by web or mobile APP access for healthcare personnel, patient’s advocacies (Women Against Lung Cancer Europe, WALCE) as well as national institutions, like regulatory agencies, providing an updated and complete overview of the epidemiological picture of predictive biomarkers in lung cancer patients. In this audit, we have annotated records from 1100

**Table 7**  
List of KRAS mutations in NSCLC patients from ATLAS database.

| Nucleotide substitution | Amino acid change | N = 291 | %      |
|-------------------------|-------------------|---------|--------|
| <b>Exon 2</b>           |                   |         |        |
| c.35G > C               | p.G12A            | 17      | 5.9 %  |
| c.34G > T               | p.G12C            | 129     | 44.5 % |
| c.35G > A               | p.G12D            | 38      | 13.0 % |
| c.35G > C               | p.G12R            | 3       | 1.0 %  |
| c.35G > T               | p.G12V            | 44      | 15.2 % |
| c.35G > C               | p.G12S            | 2       | 0.7 %  |
| c.34_35delinsTT         | p.G12F            | 5       | 1.7 %  |
| c.57G > C               | p.L19F            | 1       | 0.3 %  |
| c.37G > T               | p.G13C            | 14      | 4.8 %  |
| c.38G > A               | p.G13D            | 5       | 1.7 %  |
| c.37G > A               | p.G13S            | 2       | 0.7 %  |
| c.36 T > C              | p.G12=            | 1       | 0.3 %  |
| c.34_35delinsAT         | p.G12I            | 1       | 0.3 %  |
| <b>Exon 3</b>           |                   |         |        |
| c.183A > T              | p.Q61H            | 17      | 5.9 %  |
| c.182A > T              | p.Q61L            | 6       | 2.0 %  |
| c.181C > A              | p.Q61K            | 1       | 0.3 %  |
| c.175G > A              | p.A59T            | 1       | 0.3 %  |
| <b>Exon 4</b>           |                   |         |        |
| c.436G > A              | p.A146T           | 2       | 0.7 %  |
| c.437C > T              | p.A146V           | 2       | 0.7 %  |

NSCLC patients, including n = 552 (50.2 %) with and 548 (49.8 %) without targetable molecular alterations. The inclusion of WT cases within the ATLAS database allowed a reliable epidemiological estimation of cited molecular alteration within real-world representative series of NSCLC patients, which is in line with literature data [3,20,21]. This experience highlighted the central role of NGS-based analysis in the molecular testing of NSCLC patients. Indeed, this approach optimizes the technical management of diagnostic tissue samples, including scant specimen, elected for molecular analysis. Additionally, NGS-based testing strategy is cheaper and saves analytical time, as previously reported [18,22]. However, RT-PCR based testing strategy may represent a useful approach enabled to confirm borderline molecular results previously tested with orthogonal technology or to generate a molecular report in a clinically relevant time [23,24]. In the era of immunotherapy administration, another advantage of this database is represented by the integrative data of PD-L1 expression with conventional predictive molecular biomarkers. It has been observed conflicting data regarding clinical benefit derived from the administration of target drugs in the case of concomitant PD-L1 high expression levels and oncogenic driver alterations [25,26]. As previously shown [27], a rapidly increasing number of “uncommon” molecular alterations needs further investigations. Taking into account the pivotal role of liquid biopsy in the clinical administration of NSCLC patients, the integration of technical, clinical and molecular cfDNA derived data in ATLAS knowledge-database will improve the amount of records in a public repository from real world series of NSCLC patients [28]. This study highlights some limitations. Firstly, enrolled patients may represent a selected patient population because only patients elected to molecular analysis were taken into account; secondly, clinical and demographic features are available for a restricted series of patients. Although this preliminary data collection derives only from two Italian institutions, we have developed a fully integrated real-world registry offering a unique opportunity to provide a reliable picture about the epidemiological distribution of molecular alterations as well as the diagnostic and therapeutic management of NSCLC patients coming from the real-world Italian scenario.

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**Table 8**  
Clinical records of aberrant transcripts positive NSCLC patients from ATLAS database.

| Variables                             | n. | %      | ALK (%)   | ROS1 (%) | RET (%)   |
|---------------------------------------|----|--------|-----------|----------|-----------|
| <b>Gender</b>                         |    |        |           |          |           |
| Male                                  | 34 | 44.7 % | 13 (28.2) | 6 (54.4) | 11 (73.3) |
| Female                                | 42 | 55.3 % | 33 (71.8) | 5 (45.6) | 4 (26.7)  |
| <b>Histological Subtype</b>           |    |        |           |          |           |
| ADC                                   | 57 | 77.7 % | 35 (76.1) | 8 (72.7) | 14 (93.3) |
| SCC                                   | 1  | 1.3 %  | 1 (2.2)   | –        | –         |
| NOS                                   | 12 | 9.2 %  | 8 (17.4)  | 3 (27.3) | 1 (6.7)   |
| Others                                | 2  | 11.8 % | 2 (4.3)   | –        | –         |
| <b>PD-L1 status</b>                   |    |        |           |          |           |
| PD-L1 < 1                             | 26 | 44.1 % | 17 (48.6) | 2 (20.0) | 7 (53.8)  |
| PD-L1 (1–49)                          | 18 | 30.5 % | 8 (22.8)  | 4 (40.0) | 5 (38.5)  |
| PD-L1 > 50                            | 15 | 25.4 % | 10 (28.6) | 4 (40.0) | 1 (7.7)   |
| <b>Age</b>                            |    |        |           |          |           |
| Age < 40                              | 1  | 1.3 %  | –         | 1 (9.2)  | –         |
| Age 40–60                             | 29 | 38.1 % | 18 (39.1) | 5 (45.4) | 5 (33.3)  |
| Age 60–80                             | 45 | 59.3 % | 27 (58.7) | 5 (45.4) | 10 (66.7) |
| Age > 80                              | 1  | 1.3 %  | 1 (2.2)   | –        | –         |
| <b>Smoking status</b>                 |    |        |           |          |           |
| Smokers                               | 3  | 8.8 %  | 1 (6.2)   | –        | 2 (20.0)  |
| No-smokers                            | 19 | 55.9 % | 9 (56.2)  | 7 (87.5) | 3 (30.0)  |
| Former < 10p/y                        | 4  | 11.8 % | 3 (18.8)  | –        | 1 (10.0)  |
| Former > 10p/y                        | 8  | 23.5 % | 3 (18.8)  | 1 (12.5) | 4 (40.0)  |
| <b>Anticancer Therapy</b>             |    |        |           |          |           |
| Target Therapy                        | 26 | 76.5 % | 15 (93.7) | 6 (75.0) | 5 (50.0)  |
| Chemotherapy                          | 8  | 23.5 % | 1 (6.3)   | 2 (25.0) | 5 (50.0)  |
| <b>Molecular Assessment Technique</b> |    |        |           |          |           |
| NGS                                   | 63 | 82.9 % | –         | –        | –         |
| Real Time                             | 13 | 27.1 % | –         | –        | –         |

**Table 9**  
List of aberrant transcripts in NSCLC patients from ATLAS database.

| Aberrant Transcript       | N = 76 | %      |
|---------------------------|--------|--------|
| <b>ALK</b>                |        |        |
| ALK (ex 20) - EMLA4(ex13) | 15     | 19.7 % |
| ALK (ex20) - EML4 (ex20)  | 3      | 3.9 %  |
| ALK (ex20) - EML4 (ex6)   | 12     | 15.9 % |
| ALK (ex20) - EML4 (ex18)  | 3      | 3.9 %  |
| HIP1(28) - ALK(20)        | 1      | 1.3 %  |
| ALK-UNKNOWN               | 12     | 15.9 % |
| <b>ROS1</b>               |        |        |
| ROS1 (ex34) - CD74 (ex6)  | 7      | 9.2 %  |
| ROS1(32) - SCL34A2(13)    | 1      | 1.3 %  |
| ROS1(34) - SCL34A2(13)    | 1      | 1.3 %  |
| ROS1(32) - SCL34A2(4)     | 1      | 1.3 %  |
| ROS1 - UNKNOWN            | 1      | 1.3 %  |
| <b>RET</b>                |        |        |
| RET (ex12) - KIF5B (ex15) | 7      | 9.2 %  |
| RET (12) - CCDC6(1)       | 4      | 5.3 %  |
| RET - UNKNOWN             | 4      | 5.3 %  |
| <b>NTRK</b>               |        |        |
| NTRK3 – KANK1             | 1      | 1.3 %  |
| <b>MET</b>                |        |        |
| MET Δ 14                  | 3      | 3.9 %  |

## CRedit authorship contribution statement

**Umberto Malapelle:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Francesco Passiglia:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Francesco Pepe:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Pasquale Pisapia:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Formal analysis, Data curation, Conceptualization. **Maria Lucia Reale:** Conceptualization, Data curation, Formal analysis, Methodology, Software, Supervision, Validation, Writing – original draft, Writing – review & editing. **Diego Cortinovis:** Data curation, Investigation, Methodology, Resources, Validation, Writing – review & editing. **Filippo Frassetto:** Writing – review & editing, Validation, Methodology, Investigation, Data curation. **Domenico Galetta:** Writing – review & editing, Validation, Methodology, Investigation, Data curation. **Edoardo Garbo:** Writing – review & editing, Validation, Methodology, Investigation, Data curation. **Paolo Graziano:** Writing – review & editing, Validation, Methodology, Investigation, Data curation. **Fabio Pagni:** Writing – review & editing, Validation, Methodology, Investigation, Data curation. **Giulia Pasello:** Writing – review & editing, Validation, Resources, Methodology, Investigation, Data curation. **Pierluigi Piovano:** Validation, Resources, Methodology, Investigation, Data curation, Writing – review & editing. **Sara Pilotto:** Data curation, Investigation, Methodology, Resources, Validation, Writing – review & editing. **Marcello Tiseo:** Data curation, Investigation, Methodology, Resources, Validation, Writing – review & editing. **Carlo Genova:** Writing – review & editing, Data curation, Investigation, Methodology, Resources, Validation. **Luisella Righi:** Data curation, Investigation, Methodology, Resources, Validation, Writing – review & editing. **Giancarlo Troncone:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Conceptualization, Data curation, Formal analysis, Methodology. **Silvia Novello:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Methodology, Formal analysis, Data curation, Conceptualization.

## Declaration of competing interest

Umberto Malapelle has received personal fees (as consultant and/or speaker bureau) from Boehringer Ingelheim, Roche, MSD, Amgen, Thermo Fisher Scientific, Eli Lilly, Diaceutics, GSK, Merck and AstraZeneca, Janssen, Diatech, Novartis and Hedera for work performed. Francesco Passiglia Francesco declared consultant/advisory fee from AstraZeneca, Janssen, Amgen, Sanofi, Bristol Myer Squibb, Merck Sharp and Dohne, Roche, Beigene, Novartis, Thermofisher Scientific. Pasquale Pisapia reports speaking fees from Novartis outside the submitted work. Diego Cortinovis has received personal fees (as consultant and/or speaker bureau) from Advisory Amgen, AstraZeneca, BMS, MSD, Novartis, Roche, Janssen, Sanofi Genzyme. Domenico Galetta has received personal fees (as consultant and/or speaker bureau) from Astra-Zeneca, Pfizer, Eli-Lilly, BMS, MSD, Novartis, Amgen, Roche for work performed outside of the current study. Paolo Graziano has received personal fees (as consultant and/or speaker bureau) from: Amgen, AstraZeneca, BMS, Eli Lilly, MSD, Novartis, Roche, Pfizer, Boehringer Ingelheim unrelated to the current work. Fabio Pagni has received personal fees (as consultant and/or speaker bureau) from Novartis, Roche, MSD, Amgen, GSK and AstraZeneca, for work performed outside of the current study. Giulia Pasello has received personal fees (as consultant and/or speaker bureau) from: Amgen, AstraZeneca, BMS, Eli Lilly, MSD, Novartis, Roche, Janssen, AstraZeneca, Roche unrelated to the current work. Sara Pilotto reports personal fees (invited speaker, advisory board) from AstraZeneca, Eli-Lilly, Novartis, AMGEN, Takeda, Sanofi, Bristol Myers Squibb, MSD and Roche; and research

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