Original Article

Frequency of actionable alterations in epidermal growth factor receptor *(EGFR)* wild type non-small cell lung cancer: experience of the Wide Catchment Area of Romagna (AVR)

Elisa Chiadini[1], Matteo Canale[1], Angelo Delmonte[2], Claudio Dazzi[3], Claudia Casanova[3], Laura Capelli[1], Marita Mariotti[2], Maximilian Papi[4], Alessandro Gamboni[5], Maurizio Puccetti[6], Sara Bravaccini[1], Alessandra Dubini[7], Daniele Calistri[1], Lucio Crinò[2], Paola Ulivi[1]

[1]Biosciences Laboratory, [2]Department of Medical Oncology, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola, Italy; [3]Medical Oncology Unit, S. Maria delle Croci Hospital, Ravenna, Italy; [4]Oncology and Oncohematology Unit, Infermi Hospital, Rimini, Italy; [5]Medical Oncology Unit, Infermi Hospital, Faenza, Italy; [6]Pathology Unit, S. Maria delle Croci Hospital, Ravenna, Italy; [7]Pathology Unit, Morgagni-Pierantoni Hospital, Forlì, Italy

Contributions: (I) Conception and design: P Ulivi; (II) Administrative support: None; (III) Provision of study materials or patients: M Puccetti, A Dubini; (IV) Collection and assembly of data: A Delmonte, C Dazzi, C Casanova, M Mariotti, M Papi, A Gamboni; (V) Data analysis and interpretation: P Ulivi; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Paola Ulivi. Biosciences Laboratory, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Via P. Maroncelli 40, 47014 Meldola, Italy. Email: paola.ulivi@irst.emr.it.

Background: Molecular diagnostics for non-small cell lung cancer (NSCLC) has become the standard of care for personalized treatment. Epidermal growth factor receptor (*EGFR*) mutation and *EML4-ALK* translocation represent the two most important alterations in first-line treatment decision-making. However, other potentially targetable alterations are also present.

Methods: One thousand consecutive NSCLC patients with *EGFR* wild type (wt) tumors diagnosed by routine molecular analysis were considered. *KRAS*, *BRAF*, *ERBB2*, *PIK3CA*, *NRAS*, *ALK*, *MAP2K1*, *RET* and *DDR2* gene mutations were analyzed using the multiparametric Sequenom MassARRAY® platform. *EML4-ALK* and *ROS1* rearrangements were also assessed by fluorescent *in situ* hybridization. *HER4* status was determined by direct sequencing.

Results: Three hundred and forty-eight (34.8%), 31 (3.1%), 39 (4.4%), 14 (1.8%), 6 (0.7%), 16 (1.8%), 5 (0.6%) and 9 (0.9%) patients showed an alteration in *KRAS*, *BRAF*, *ALK*, *ROS1*, *NRAS*, *PIK3CA*, *MAPK1/2* and *HER2* genes, respectively. Of the 657 patients for whom all markers were determined, 318 (48%) patients had at least one alteration. Eight patients showed overlapping mutations, 4 *KRAS* mutation/*EML4-ALK* translocation, one *KRAS* mutation/*ROS1* rearrangement, 2 *KRAS/PIK3CA* mutations, and one *BRAF/PIK3CA* mutations.

Conclusions: About 50% of our patients had a potentially targetable alteration, confirming the usefulness of a multiparametric approach for routine molecular diagnostics aimed at identifying potential therapeutic targets.

Keywords: Non-small cell lung cancer (NSCLC); targeted therapy; epidermal growth factor receptor (EGFR); multitarget analysis; Formalin-fixed paraffin-embedded samples (FFPE samples)

Submitted Apr 11, 2018. Accepted for publication Jun 15, 2018.
doi: 10.21037/jtd.2018.07.22
View this article at: http://dx.doi.org/10.21037/jtd.2018.07.22

Introduction

Targeted therapy for non-small cell lung cancer (NSCLC) has transformed the outcome of patients carrying specific molecular alterations. In particular, epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs), such as gefitinib, erlotinib or afatinib, and anti-ALK agents, such as crizotinib, have changed the natural history of adenocarcinoma patients carrying specific *EGFR* mutations or *EML4-ALK* translocation/*ROS1* rearrangements, respectively (1-5). Other potentially targetable alterations have been identified in lung cancer. Of these, *BRAF* and *HER2* mutations are present in about 3% and 2% of patients with lung adenocarcinoma, respectively (6-8) and represent possible targets for therapy using anti BRAF (vemurafenib or dabrafenib) or anti-HER2 (trastuzumab, dacomitinib, etc.) agents (8-11). Moreover, *mesenchymal-epithelial transition factor* (*MET*) alterations (mutation or amplification) would seem to identify a subset of patients who are more likely to respond to crizotinib (12,13). In addition, other potentially targetable alterations have been found in several genes, including *NTRK1*, *PIK3CA*, *HER4*, *NRAS* (6,14-18), and the frequency of these alterations differs in different ethnicities. The number of clinical trials aimed at analyzing the effect of targeted drugs specific for these different alterations is thus expected to increase enormously in the near future.

In the present study we evaluated a large Italian cohort of NSCLC patients, all *EGFR* wild type (wt) according to diagnostic molecular analysis, to verify the frequency of potentially targetable alterations in relation to clinical pathological characteristics of patients.

Methods

Patients

We evaluated a cohort of 1,000 patients, all recruited from the Wide Catchment Area of Romagna (AVR), with histologically or cytologically confirmed advanced NSCLC classified as EGFR wt by routine diagnostic molecular analysis from January 2013 to December 2016. The clinical pathological characteristics of patients are reported in *Table 1*. The study was approved by our institutional Review Board and all patients gave written informed consent.

Biological samples

Formalin-fixed paraffin-embedded (FFPE) histological samples, cytological FFPEs (cell blocks) or cytological smears were available for molecular analysis. Biological samples were evaluated and selected by AVR pathologists. Tumor specimens comprising at least 50% tumor cells were selected and underwent DNA extraction.

EML4-ALK and ROS1 determinations

Selected FFPE histological or cytological sections and cytological samples were used to perform EML4-ALK and ROS1 determinations. FISH assay was performed using a break-apart ALK or ROS1 probe (Vysis LSI Dual Color, Break Apart Rearrangement Probe; Abbott/Vysis, Illinois, IL, USA. *ALK* and *ROS1* rearrangements were scored as positive when ≥15% of tumor cells displayed split signals or isolated signals containing a kinase domain (red for *ALK* and green for *ROS1*), as previously described (19,20). Slides containing at least 50 tumor cells were considered evaluable and were read independently by two experts blinded to the patient's history and histological findings.

Mutation analysis

Mutation analyses were centralized and performed in the Biosciences Laboratory of IRST IRCCS. *KRAS*, *BRAF*, *ERBB2*, *PIK3CA*, *NRAS*, *ALK*, *MAP2K1*, *RET* and *DDR2* gene status was analyzed by Myriapod®Lung Status kit (Diatech Pharmacogenetics, Jesi, Italy) on MassARRAY® (SEQUENOM® Inc., San Diego, CA, USA). Exons 18 to 23 of the *HER4* gene were evaluated by direct sequencing.

Statistical analysis

The chi-square test was used for group comparison of variables.

Results

Frequency of gene alterations

KRAS, *BRAF* and *HER2* determinations were performed in the entire case series. Conversely, there was only sufficient biological to perform *NRAS*, *PIK3CA*, *MAP2K1*, *ALK*, *RET* and *DDR2* mutation analysis in 901 patients, *EML4-ALK* evaluation in 889 patients and *ROS1* determinations in 733 patients. Overall, characterization of all 11 markers was performed in 657 patients. *HER4* mutation analysis was also carried out in 450 cases. Three hundred and forty-eight

Table 1 Relation between the Different Alterations and the Clinical Pathological Characteristics of Patients

Gene alteration	Total No. gene mutations	Gender (%)			Age, years (%)			Smoking habits* No. (%)			
		Female	Male	P	<70	≥70	P	Never	Former	Current	P
KRAS	348	132 (37.9)	216 (62.1)	0.950	174 (50.0)	174 (50.0)	0.467	29 (11.1)	79 (30.2)	154 (58.8)	<0.001
BRAF	31	13 (41.9)	18 (58.1)	0.633	17 (54.8)	14 (45.2)	0.669	3 (15.0)	6 (30.0)	11 (55.0)	0.955
NRAS	6	4 (67.0)	2 (33.0)	0.204	6 (100.0)	–	0.032	–	2 (50.0)	2 (50.0)	0.656
PIK3CA	16	6 (37.5)	10 (62.5)	0.000	6 (37.5)	10 (62.5)	0.240	1 (10.0)	4 (40.0)	5 (50.0)	0.760
MAPK1/2	5	–	5 (100.0)	0.164	4 (80.0)	1 (20.0)	0.376	1 (20.0)	1 (20.0)	3 (60.0)	1.000
HER2	9	6 (66.7)	3 (33.3)	0.087	4 (44.0)	5 (56.0)	0.746	2 (40.0)	2 (40.0)	1 (20.0)	0.221
EML4-ALK	39	23 (59.0)	16 (41.0)	0.005	28 (71.8)	11 (28.2)	0.015	17 (51.5)	4 (12.1)	12 (36.4)	<0.001
ROS1	14	9 (64.3)	5 (35.7)	0.053	8 (57.1)	6 (42.9)	0.748	6 (66.7)	1 (11.1)	2 (22.2)	0.002

*, percentages refer to the total number of patients with smoking habits information available.

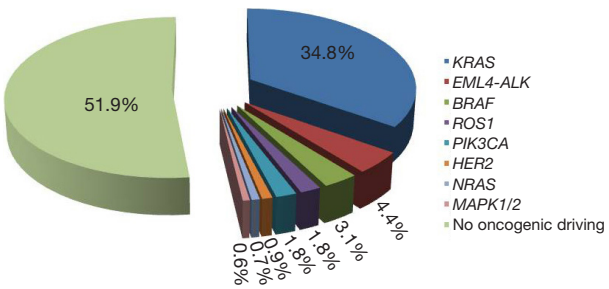

Figure 1 Frequency of gene alterations in the entire case series of EGFR wt patients.

(34.8%), 31 (3.1%), 39 (4.4%), 14 (1.8%), 6 (0.7%), 16 (1.8%), 5 (0.6%) and 9 (0.9%) patients showed an alteration in *KRAS*, *BRAF*, *ALK*, *ROS1*, *NRAS*, *PIK3CA*, *MAPK1/2* and *HER2* genes, respectively (*Figure 1*). Of the 657 patients in whom all the markers were determined, 318 (48%) showed at least one alteration. The different mutations found for each gene are shown in *Figure 2*. Eighty-four percent of *KRAS* mutations were found at codon 12, the majority (39%) being G12C alterations, while 10.3% of mutations involved codon 13. Around half of all *BRAF* mutations (54.8%) were V600E, whereas 45.2% were other exon 15 alterations or exon 11 mutations. In particular, 2 (14%) of the mutated patients with no V600E alteration harbored a different exon 15 mutation (D594G), while 12 (86%) showed an exon 11 alteration, 5 involving codon 466 (2 G466A, 2 G466E, one G466V) and 7 codon 469 (3 G469A, 1 G469E, 3 G469V). All *NRAS* mutations were at codon 61 (3 Q61K, 2 Q61L and one Q61R), whereas *PIK3CA* alterations were found in exon 9 (93.8%) in all but one patient (exon 20). Of the 5 patients carrying a *MAPK1/2* mutation, 2 (40%) had a Q56P alteration whereas 3 (60%) showed a K57N substitution (*Figure 2*). Finally, all *HER2*-mutated patients had an exon 20 insertion. The only mutation found in *HER4* gene was found in a former male smoker and located in exon 19 (G735V). No alterations were found in *ALK*, *RET* or *DDR2* genes.

Eight patients showed overlapping mutations: concomitant *KRAS* mutation and *EML4-ALK* translocation (4 cases); *KRAS* mutation together with *ROS1* rearrangement (1 case); concomitant *KRAS* and *PIK3CA* mutation (2 cases); and concomitant *BRAF* and *PIK3CA* mutation (1 case).

Gene alterations in relation to clinical pathological characteristics of patients

The relation between the different alterations and the clinical pathological characteristics of patients is reported in *Table 2*. *EML4-ALK* translocation was significantly correlated with gender, age and smoking habits (P=0.005, P=0.015 and P<0.001, respectively) and was more frequent in young, non-smoking females. *ROS1* rearrangements were significantly correlated with gender and smoking habits (P=0.053 and P=0.002, respectively) but not with age. Moreover, *KRAS* mutations were significantly more common in current smokers (P<0.001), whereas *NRAS* mutations were only found in patients <70 years of age (P=0.032).

Of the 4 patients showing concomitant *EML4-ALK*

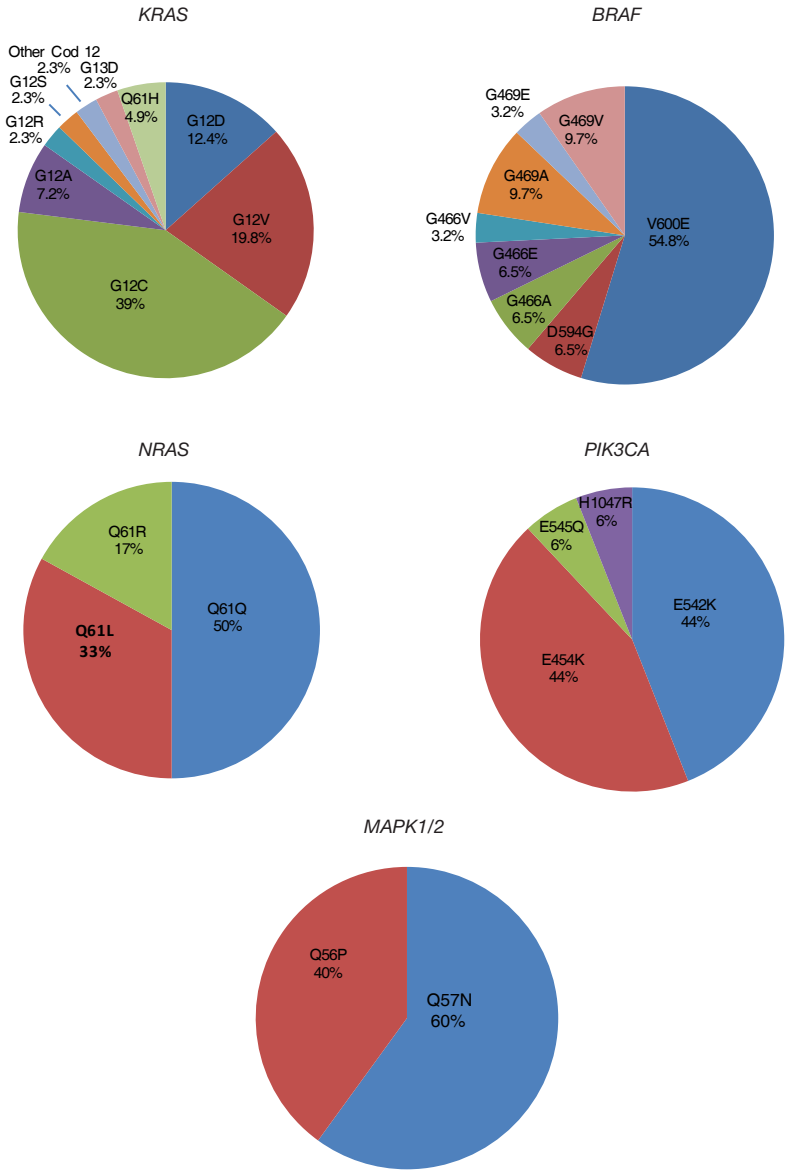

Figure 2 Types of mutations found in the different genes.

translocations and *KRAS* mutations (3 of whom were smokers), 2 were treated with first-line crizotinib and second-line ceritinib. One patient harboring a G12D *KRAS* mutation and with 70% fluorescent *in situ* hybridization (FISH) positivity initially showed stable disease with crizotinib but progressed after 5 cycles, and then again obtained stable disease with ceritinib, relapsing after 4 treatment cycles. Another patient with a G13S *KRAS* mutation and 50% FISH positivity obtained a partial response with crizotinib lasting 6 treatment cycles and another partial response with ceritinib lasting 3 cycles. The only patient showing a concomitant *KRAS* mutation (G12V) and *ROS1* rearrangement was treated with second-line crizotinib but developed severe toxicity that led to treatment suspension before the clinical response could be evaluated.

Discussion

In the present study we report our results on a cohort of 1,000 consecutive NSCLC patients identified as *EGFR* wt by routine diagnostic molecular analysis performed at our

Table 2 Clinical pathological characteristics of analyzed samples

Variables	No. (%)
Overall	1,000
Age, years	
>70	431 (43.0)
≤70	569 (57.0)
Gender	
Male	622 (62.0)
Female	378 (38.0)
Smoking habits	
Current	379 (37.9)
Former	239 (23.9)
Never	145 (14.5)
Missing	237 (23.7)
Histotype	
ADC	793 (79.3)
PDC	178 (17.8)
Other	29 (2.9)
Type of sample	
Histological	639 (64.0)
Cytological	361 (36.0)

ADC, adenocarcinoma; PDC, poorly differentiated carcinoma.

institute (IRST IRCCS). We demonstrated that about half of all the *EGFR* wt patients carried a potentially targetable gene alteration. The frequencies of alterations were as follows: *KRAS*, 35%; *EML4-ALK*, 4.4%; *BRAF*, 3.1%; *ROS1*, 0.7%; *PIK3CA*, 1.8; *NRAS*, 1.8%; *MAPK1/2*, 0.6%; and *HER2*, 0.9%. Such findings are in agreement with literature data (6,15,16,21). A slightly higher frequency of *KRAS* mutations was observed, possibly attributable to the fact that we considered a selected case series of *EGFR* wt patients in whom *KRAS* mutations were more frequent due to the mutual exclusivity of the 2 gene mutations. In accordance with previous authors (22), we observed a higher incidence of *KRAS* mutation and a high prevalence of G12C alterations in current smokers.

With regard to *BRAF* mutation, we saw that almost half of the mutated patients carried non-V600E alterations that were predominantly located in exon 11 at codons 466 and 469. It is known that V600 alterations predict sensitivity to anti-BRAF and anti-MEK combinations (23). Moreover, recent evidence suggests that non-V600 alterations may also be associated with sensitivity to such treatments (24). These results suggest that about 3% of *EGFR* wt patients could benefit from this type of targeted treatment. In agreement with other studies (7), no associations were observed between *BRAF* mutations and clinical pathological characteristics of patients. Around 6% of our patients harbored an *EML4-ALK* (4.4%) or *ROS1* (1.8%) rearrangement, the majority of whom were predominantly young females who had never smoked, as described in other studies (19,25).

Other alterations that are potential targets for treatment are present in lung adenocarcinoma, e.g., 1.8% of our patients carried a *PIK3CA* mutation. Although there are still no drugs capable of inhibiting the growth of *PIK3CA* mutated cells, such mutations would seem to confer resistance to TKIs (26,27), making their determination of clinical importance.

We observed a slightly lower percentage of *HER2* mutations with respect to that described in the literature (28) but similar (1.7%) to the findings of Mazières *et al.* (8). in a large case series. However, the relatively low sensitivity of the Sanger sequencing we used for the detection of *HER2* mutations may partly explain our results. No significant associations were found between *HER2* mutation and gender, age or smoking habits.

We also observed 8 patients with overlapping mutations, 4 of whom showed concomitant *EML4-ALK* and *KRAS* alterations. Of these, 2 underwent treatment with anti-ALK agents, one obtaining a partial response. In agreement with other authors, we have already seen that the presence of a *KRAS* mutation in patients with *EML4-ALK* translocation can confer resistance to treatment with crizotinib (29,30). Although the low number of double-mutated patients in our study does not permit us to draw any definitive conclusions about this, there were seem to be sufficient evidence to warrant *KRAS* status being taken into consideration in *EML4-ALK* translocated patients treated with anti-ALK agents.

In conclusion, although the frequency of single gene alterations in our study was low, about half of the patients with *EGFR* wt lung adenocarcinoma analyzed showed a potentially targetable alteration. Anti-ALK agents have already been approved for use as first-line treatment of *EML4-ALK*-translocated tumors. However, larger, randomized clinical trials are needed to verify the usefulness of targeted agents in tumors harboring other specific alterations.

Acknowledgements

The authors would like to thank Cristiano Verna for editorial assistance.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The study was approved by our institutional Review Board (No. 675 of 3.09.2013) and all patients gave written informed consent.

References

1. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 2009;361:947-57.
2. Rosell R, Carcereny E, Gervais R, et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. Lancet Oncol 2012;13:239-46.
3. Yang JC, Wu YL, Schuler M, et al. Afatinib versus cisplatin-based chemotherapy for EGFR mutation-positive lung adenocarcinoma (LUX-Lung 3 and LUX-Lung 6): analysis of overall survival data from two randomised, phase 3 trials. Lancet Oncol 2015;16:141-51.
4. Solomon BJ, Mok T, Kim DW, et al. First-line crizotinib versus chemotherapy in ALK-positive lung cancer. N Engl J Med 2014;371:2167-77.
5. Mazières J, Zalcman G, Crinò L, et al. Crizotinib therapy for advanced lung adenocarcinoma and a ROS1 rearrangement: results from the EUROS1 cohort. J Clin Oncol 2015;33:992-9.
6. Kris MG, Johnson BE, Berry LD, et al. Using multiplexed assays of oncogenic drivers in lung cancers to select targeted drugs. JAMA 2014;311:1998-2006.
7. Chen D, Zhang LQ, Huang JF, et al. BRAF mutations in patients with non-small cell lung cancer: a systematic review and meta-analysis. PLoS One 2014;9:e101354.
8. Mazières J, Peters S, Lepage B, et al. Lung cancer that harbors an HER2 mutation: epidemiologic characteristics and therapeutic perspectives. J Clin Oncol 2013;31:1997-2003.
9. Gautschi O, Milia J, Cabarrou B, et al. Targeted therapy for patients with BRAF-mutant lung cancer: results from the european EURAF cohort. J Thorac Oncol 2015;10:1451-7.
10. Hyman DM, Puzanov I, Subbiah V, et al. Vemurafenib in multiple nonmelanoma cancers with BRAF V600 mutations. N Engl J Med 2015;373:726-36.
11. Kris MG, Camidge DR, Giaccone G, et al. Targeting HER2 aberrations as actionable drivers in lung cancers: phase II trial of the pan-HER tyrosine kinase inhibitor dacomitinib in patients with HER2-mutant or amplified tumors. Ann Oncol 2015;26:1421-7.
12. Lu X, Peled N, Greer J, et al. MET Exon 14 mutation encodes an actionable therapeutic target in lung adenocarcinoma. Cancer Res 2017;77:4498-505.
13. Suryavanshi M, Shah A, Kumar D, et al. MET amplification and response to MET inhibitors in stage IV lung adenocarcinoma. Oncol Res Treat 2017;40:198-202.
14. Ding L, Getz G, Wheeler DA, et al. Somatic mutations affect key pathways in lung adenocarcinoma. Nature 2008;455:1069-75.
15. Sholl LM, Aisner DL, Varella-Garcia M, et al. Multi-institutional oncogenic driver mutation analysis in lung adenocarcinoma: the Lung Cancer Mutation Consortium experience. J Thorac Oncol 2015;10:768-77.
16. Yeung SF, Tong JH, Law PP, et al. Profiling of oncogenic driver events in lung adenocarcinoma revealed MET mutation as independent prognostic factor. J Thorac Oncol 2015;10:1292-300.
17. Schrock AB, Frampton GM, Suh J, et al. Characterization of 298 patients with lung cancer harboring MET exon 14 skipping alterations. J Thorac Oncol 2016;11:1493-1502.
18. Vaishnavi A, Capelletti M, Le AT, et al. Oncogenic and drug-sensitive NTRK1 rearrangements in lung cancer. Nat Med 2013;19:1469-72.
19. Bergethon K, Shaw AT, Ou SH, et al. ROS1 rearrangements define a unique molecular class of lung cancers. J Clin Oncol 2012;30:863-70.
20. Kwak EL, Bang YJ, Camidge DR, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. N Engl J Med 2010;363:1693-703.
21. Daoud A, Chu QS. Targeting novel but less common driver mutations and chromosomal translocations in advanced non-small cell lung cancer. Front Oncol 2017;7:222.
22. Chapman AM, Sun KY, Ruestow P, et al. Lung cancer mutation profile of EGFR, ALK, and KRAS: meta-analysis and comparison of never and ever smokers. Lung Cancer 2016;102:122-34.

23. Planchard D, Smit EF, Groen HJ, et al. Dabrafenib plus trametinib in patients with previously untreated BRAFV600E-mutant metastatic non-small-cell lung cancer: an open-label, phase 2 trial. Lancet Oncol 2017;18:1307-16.
24. Noeparast A, Teugels E, Giron P, et al. Non-V600 BRAF mutations recurrently found in lung cancer predict sensitivity to the combination of Trametinib and Dabrafenib. Oncotarget 2016;8:60094-108.
25. Pillai RN, Ramalingam SS. The biology and clinical features of non-small cell lung cancers with EML4-ALK translocation. Curr Oncol Rep 2012;14:105-10.
26. Chen JY, Cheng YN, Han L, et al. Predictive value of K-ras and PIK3CA in non-small cell lung cancer patients treated with EGFR-TKIs: a systemic review and meta-analysis. Cancer Biol Med 2015;12:126-39.
27. Wu SG, Chang YL, Yu CJ, et al. The Role of PIK3CA mutations among lung adenocarcinoma patients with primary and acquired resistance to EGFR tyrosine kinase inhibition. Sci Rep 2016;6:35249.
28. Pillai RN, Behera M, Berry LD, et al. HER2 mutations in lung adenocarcinomas: a report from the Lung Cancer Mutation Consortium. Cancer 2017;123:4099-105.
29. Ulivi P, Chiadini E, Dazzi C, et al. Nonsquamous, non-small-cell lung cancer patients who carry a double mutation of EGFR, EML4-ALK or KRAS: frequency, clinical-pathological characteristics, and response to therapy. Clin Lung Cancer 2016;17:384-90.
30. Mengoli MC, Barbieri F, Bertolini F, et al. K-RAS mutations indicating primary resistance to crizotinib in ALK-rearranged adenocarcinomas of the lung: report of two cases and review of the literature. Lung Cancer 2016;93:55-8.

Cite this article as: Chiadini E, Canale M, Delmonte A, Dazzi C, Casanova C, Capelli L, Mariotti M, Papi M, Gamboni A, Puccetti M, Bravaccini S, Dubini A, Calistri D, Crinò L, Ulivi P. Frequency of actionable alterations in *EGFR* wild type non-small cell lung cancer: experience of the Wide Catchment Area of Romagna (AVR). J Thorac Dis 2018;10(8):4858-4864. doi: 10.21037/jtd.2018.07.22

