Epidermal growth factor receptor exon 20 insertion variants in non-small cell lung cancer patients

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Abstract

Epidermal growth factor receptor (*EGFR*) exon 20 insertions occur very rarely among different cancer types, with the highest frequency reported among non-small-cell lung cancer (NSCLC) patients, in particular lung adenocarcinomas (ADCs). As a general rule, exon 20 insertions fall back in the tyrosine kinase domain, and can be clustered into two principal groups represented by in frame insertions and three to 21 bp (corresponding to 1–7 amino acids) duplications within amino acids 762 and 774. The correct identification of these alterations is key for an adequate management of NSCLC patients due to the possibility to selectively treat these patients with specific target therapies. In this context, next generation sequencing (NGS) technology, able to detect several hotspot gene mutations for different patients simultaneously, is the best option due to its higher sensitivity and specificity respect to other molecular techniques. Here we reviewed the principal biological characteristics, the best molecular approach and treatment options for NSCLC patients harbouring *EGFR* exon 20 insertions in 2021.

Keywords: NSCLC; EGFR; exon 20 insertions; NGS; target therapy.

Biology of EGFR ex20ins variants

Epidermal growth factor receptor (*EGFR*) exon 20 insertions occur very rarely among different cancer types (0.35% of AACR GENIE cases). Regarding these rare alterations, the highest frequency has been reported among non-small-cell lung cancer (NSCLC) patients, in particular lung adenocarcinomas (ADCs).[1] Overall, *EGFR* exon 20 insertions are identified in about 1.5-3.0% of NSCLC patients and these mutations are detected in 6% to 10-12% of all *EGFR* mutated NSCLC patients.[2-8] [Figure 1] [Supplementary Table 1]

Briefly, *EGFR* gene is located on chromosome 7 (7p11.2) and encodes for EGFR protein, a transmembrane receptor with tyrosine kinase activity.[9] From a structural point of view, the EGFR protein is characterized by an extracellular ligand-binding domain (or ectodomain) containing four different leucine rich (LR) and cysteine rich (CR) domains, a transmembrane region, and an intracellular domain (amino acids 687 to 955) tyrosine kinase domain where trans-autophosphorylation takes place.[10] As for the other *EGFR* activating mutations (exons 18, 19 and 21), exon 20 insertions fall back in the tyrosine kinase domain.[7]

EGFR exon 20 insertions can be clustered into two principal groups represented by in frame insertions and three to 21 bp (corresponding to 1–7 amino acids) duplications within amino acids 762 and 774.[11] Interestingly, almost all (about 90%) exon 20 insertions are detected within amino acids 767 and 775, whereas the remaining cases are reported in amino acids positions 761 to 766 (C-terminal of the C-helix); however, despite the type and the position, insertions determine an active conformation of C-helix.[2, 3] This phenomenon leads to an internal rotation of C-helix, that ensures a stable dimerization of EGFR monomers and the constitutive signal transduction pathway activation.[12] As a matter of the facts, *EGFR* exon 20 insertions do not directly interest the EGFR receptor ATP-binding pocket domain but induce a wedge at the end of the C-helix determining a constitutive activation of the tyrosine kinase domain.[13] It has been demonstrated that *EGFR* exon 20 insertions determine an increasing

affinity for ATP while reducing the affinity for EGFR tyrosine kinase inhibitors (TKIs), in particular first generation EGFR TKIs.[7, 14]

Overall, preclinical studies highlighted that insertions involving codons 769 to 775 may determine EGFR TKIs resistance whereas more proximal codons may predict EGFR TKIs sensitivity.[15] As a general rule, *EGFR* exon 20 insertions are mutually exclusive with other mutations. However, it has been highlighted the association with other gene alterations, including *EGFR* amplifications and *EGFR* sensitizing mutations.[3-5, 16]

No significant differences have been reported in *EGFR* exon 20 insertions among different ethnicity,[2, 4, 5, 17] whereas these mutations seem to occur more frequently in women, in absence of smoking history and in ADC patients, in particular *EGFR* exon 20 p.V769_D770insASV, *EGFR* exon 20 p.H773_V774insNPH, *EGFR* exon 20 p.V774_C775insHV and *EGFR* exon 20 p.D770_N771insSVD.[2, 4, 8] Furthermore, it has *EGFR* exon 20 p.V769_D770insASV seems to occur more frequently in older patients (≥ 65 years) respect to the *EGFR* exon 20 p.A763_Y764insFQEA (< 65 years).[4] Concerning some disease characteristics Cardona *et al* reported that the most common alterations discovered in brain metastasis was the *EGFR* exon 20 p.H773_V774insPH (about one third of patients).[4] Conversely, in the brain metastatic setting, Yang *et al* identified that the *EGFR* exon 20 p.V769_D770insASV was the most common alterations (about 21%).[16]

EGFR ex20ins detection: from sanger sequencing to Next Generation Sequencing

EGFR exon 20 insertions is becoming more and more predictive, due to the approval of different therapeutic approaches and others that are currently under investigation.[18] Thus, despite the low frequency of these alterations, it is crucial their identification in order to avoid leaving any patient behind.

Despite the ability to identify the most common mutated forms of the protein, in particular associated with *EGFR* exon 21 p.L858R and some *EGFR* exon 19 deletions, immunohistochemistry featured a high number of false positive results for *EGFR* exon 20 insertions.[19] Direct sequencing is considered the "gold standard" methodology in molecular predictive pathology laboratories, but it showed several limitations in the detection of *EGFR* exon 20 insertions. In particular, Angulo *et al* were able to generate no false positive results (specificity 100.0%), however they highlighted a high number of false negative results (sensitivity 67.7%).[20] This may be related to the low tumor content within small tissue samples, that may be below the lower limit of detection of direct sequencing (about 20-25%). It is therefore conceivable the adoption of high sensitive molecular techniques, ensuring the identification of clinical relevant gene alterations at low frequencies (at least 1%).[21, 22]

Targeted based assays, such as real time polymerase chain reaction (RT-PCR) based approaches, may be a reliable solution for *EGFR* exon 20 insertions detection. RT-PCR is useful to identify either "common" or "uncommon" *EGFR* mutations, including *EGFR* exon 20 insertions.[23] In particular, the therascreen® EGFR kit, adopting the amplification-refractory mutation system (ARMS) and Scorpions technologies, is able to identify two *EGFR* exon 20 insertions (p.H773dup and p.D770_N771insG).[therascreen® EGFR RGQ PCR Kit Instructions for Use (Handbook), 2013. Available online: http://www.accessdata.fda.gov/cdrh_docs/pdf12/P120022c.pdf].[20] Despite the high specificity, an important limitation of targeted-based approaches is the capacity to detect only known and well characterized mutations. Thus, RT-PCR technology may be limited when low frequent alterations are considered.

In this scenario, next generation sequencing (NGS) assays, able to detect several hotspot gene mutations for different patients simultaneously,[24] is a high sensitivity and specificity valuable tool for molecular assessment of less frequent gene mutations, such as *EGFR* exon 20

insertions. In addition, different from targeted-based approaches, NGS is able to identify either known or unknown mutations within gene panel reference range. Due to the adoption of NGS approaches, it has been demonstrated an increase in detection of *EGFR* exon 20 insertions in patients with advanced NSCLC from 2011 to 2019.[25] Of note, Tuononen *et al* were able to correctly identify an *EGFR* exon 20 p.A767_S768insSVG previously missed by a RT-PCR based approach.[26] In a similar experience, Coleman *et al*, adopting a NGS approach (Foundation One) in a previously *EGFR* wild type patients tested by adopting a RT-PCR approach (Cobas), were able to correctly identify the presence of an *EGFR* exon 20 p.A763_Y764insFQEA. Noteworthy, the patient was treated with osimertinib and demonstrated a terrific intra- and extra-cranial responses.[27] Interestingly, Hwang *et al*, adopting the OncoPanel AMC version3, were able to detect a higher number (twice) of *EGFR* exon 20 insertions than those assessed by PNA clamping method.[28]

How to report EGFR ex20ins variants

Due to the different biological effects on EGFR protein and treatment responsiveness to the plethora of different available therapies, a pivotal post-analytical issue is to report, in an adequate and intelligible manner for clinicians, the genomic alterations identified by molecular analysis. As a matter of the facts, it is key an efficient and exhausting communication between molecular pathologists and clinicians to avoid any misinterpretations, that may lead to an inadequate treatment of advanced NSCLC patients and a report must contain all relevant information for the correct management of cancer patients. In particular, a report should include patient's unique identifiers, such as name, surname, date of birth, and identification number. In addition, information regarding the ward of service, the date of shipment, the sample type (including the number of specimens) and the name of the physician who requested the molecular test, should be reported.

In the first part of the report, information regarding some pre-analytical data (such as fixation issues, etc.), should be included. The molecular status of the requested biomarkers must be reported.[29, 30] Following the Association for Molecular Pathology (AMP), American Society of Clinical Oncology (ASCO), and College of American Pathologists (CAP) guidelines, mutations, including *EGFR* exon 20 insertions, should be reported according to Human Genome Variation Society (HGVS) as follow: gene, exon, p. annotation, and c. annotations (e.g. EGFR exon 20 p. A763_Y764insFQEA c. 2284-5_2290dup).[30]

Finally, the report should include a clinical interpretation of the detected variants, methodological data, regarding the test employed, the reference range, the limit of detection (LOD), and run NGS parameters.[30]

Another important element to juggle the complexity of *EGFR* exon 20 insertions and to facilitate their clinical interpretation, is the collegial discussion within molecular tumor boards (MTBs).[31-33] MTBs involve different healthcare figures who discussing together on how manage difficult patients.[31] In addition, MTBs should adopt tools, such as OncoKB and the European Society for Medical Oncology Scale for Clinical Actionability of Molecular Targets (ESCAT) to better interpret less familiar molecular results.[34-37]

Current standard of care for EGFR exon 20 insertion NSCLC patients

Chemotherapy

Considering the low sensitiveness to the EGFR TKIs currently available for clinical use, platinum-based doublet chemotherapy represents the standard upfront treatment for *EGFR* exon 20 insertion advanced NSCLC patients, as recommended by international and national guidelines.[38, 39]

Although some clinical characteristics are similar to patients harboring common *EGFR* mutations, the prognosis is dramatically worse. Real world data report a median overall

survival (OS) of 16.2 months and 25.5 months, with an estimated 5-year survival rate of 8% and 19%, for NSCLC patients harboring exon 20 insertions and classical *EGFR* mutations, respectively.[40] An American study recently reported encouraging responses as well as longer median OS (20 *versus* 12 months, p=0.007) and time-to-treatment discontinuation (TTD) (7 *versus* 4 months, p=0.02) to platinum chemotherapy in 106 *EGFR* exon 20 insertion NSCLC patients as compared to a similar cohort without targetable driver alterations.[41] In another study including 84 NSCLC patients with exon 20 insertion, first-line pemetrexed-containing regimens were associated with improved survival outcomes compared to other approaches (progression-free survival, PFS: 6.2 *versus* 2.7 months; p<0.001; OS: 28.0 *versus* 15.4 months; p=0.009),[42] suggesting a potential sensitiveness of *EGFR* exon 20 insertion to pemetrexed, as already observed in other molecular NSCLC subsets, such as *ALK*, *ROS1* and *RET* rearranged tumors.[41] A Chinese retrospective real-world study including *EGFR* exon 20 insertion NSCLC patients, showed significantly longer median PFS in favour of platinum-based chemotherapy as compared to all-generation EGFR TKIs both in first (6.4 *versus* 2.9 months, p<0.001) and second-line setting (4.0 *versus* 2.0 m; p=0.342).[16] In their meta-analysis Burnett *et al* partially confirmed these data, showing a trend towards greater PFS and OS for chemotherapy compared to TKI in the first-line treatment setting.[43]

Overall, these data confirm the efficacy of platinum-based chemotherapy in EGFR exon 20 insertion NSCLC, suggesting pemetrexed-containing regimens as the preferred option. Conversely, the activity of EGFR TKIs plus chemotherapy combinations remains still unknown, since the majority of ongoing randomized clinical studies have not included this subgroup of patients.

EGFR-TKIs

EGFR exon 20 insertion NSCLC patients have been generally excluded from randomized clinical trials assessing first-, second- and third-generation EGFR TKIs activity in metastatic disease. Actually, the available clinical data suggest very low efficacy of the first-generation TKIs gefitinib and erlotinib, with a overall response rate (ORR) of 5% and a disease control rate (DCR) of 15%.[44] As regards second-generation TKIs, preclinical studies reported achievable plasma concentrations of afatinib, dacomitinib and neratinib, below the 50% inhibitory concentrations in most of *EGFR* exon 20 insertions, allowing to consider them clinically ineffective.[44] A post-hoc analysis combined data from LUX-Lung 2, LUX-Lung 3 and LUX-Lung 6 trials, to test the activity of afatinib in patients with uncommon *EGFR* mutations, accounting for 12% (75 out of 600) of the overall included population. Specifically, the activity of afatinib appeared dramatically low in the subgroup of NSCLC patients harboring *EGFR* exon 20 insertions (ORR: 8.7%; median PFS: 2.7 months; median: OS 9.2 months).[45] Conversely the retrospective analysis by Yang *et al*, showed an ORR of 24.3% and a median duration of response (DOR) of 11.9 months in a cohort of *EGFR* exon 20 insertions advanced NSCLC patients treated with afatinib. Four out of the 70 EGFR TKIs-treated patients remained on treatment for more than 3 years, suggesting the potential efficacy of this TKI against specific exon 20 insertions variants.[46]

The activity of osimertinib, in this setting is still unclear. Preclinical data demonstrated tumor growth inhibition in murine models receiving such EGFR TKI,[47] leading to investigate the efficacy of this third-generation inhibitor in the clinical setting too. To date, clinical evidence are quite disappointing, showing low ORR of 5% and median PFS of 3.6 months, in a cohort of 17 NSCLC harboring *EGFR* exon 20 insertions.[48] Similar data come from a Korean phase II study evaluating the activity of osimertinib in *EGFR* exon 20 insertion NSCLC patients who failed prior standard chemotherapy. Median PFS and DCR at 6 months were 3.5 months and 31.1%, respectively.[49] Conversely, a Chinese study demonstrated promising antitumor

activity of osimertinib in *EGFR* exon 20 insertion NSCLC patients reporting a ORR of 67.7% (4/6 patients achieved partial response) and a median PFS of 6.2 months.[50] The EA5162, single-arm, phase II trial evaluated an alternative schedule of osimertinib at double dose of 160 mg in pretreated *EGFR* exon 20 insertion NSCLC patients, reporting a ORR of 25%, a DCR of 85%, and a median PFS of 9.7 months, along with a tolerable safety profile.[51]

Differently from the majority of *EGFR* exon 20 insertions, the A763_Y764insFQEA variant, accounting for about 11% of overall exon 20 insertions, seems to be sensitive to different generation EGFR TKIs, as observed in both preclinical models and clinical setting.[52] These data suggest, once again, that *EGFR* exon 20 insertions are quite heterogeneous and further dedicated studies are needed to specifically explore the activity of different EGFR TKIs against single *EGFR* exon 20 insertion variants.[53]

Immune checkpoint inhibitors

Clinical evidence regarding immune checkpoint inhibitors (ICIs) efficacy in *EGFR* exon 20 insertions NSCLC patients are currently limited since these patients have been largely excluded from the majority of ICI-based randomized clinical trials. Similarly, to common *EGFR* mutations, tumor mutational burden (TMB) has been reported to be lower than *EGFR*-wild type NSCLC, likely reflecting non-smoking habits. Conversely, the programmed death-ligand 1 (PD-L1) expression levels seems to be intermediate between that reported in the non-mutant/wild-type disease and the NSCLC samples harboring classical *EGFR* mutations, depending on different *EGFR* exon 20 insertion variants.[41, 54, 55] In general, anti-PD-1/PD-L1 immunotherapeutic agents have not demonstrated to be an effective strategy in the subgroup of exon 20 insertions.

Choudhury *et al* have recently reported that the duration of ICI treatment was similar for metastatic NSCLC patients with *EGFR* exon 20 insertion and without targetable alterations

(HR 1.75, p=0.05).[41] Negrao *et al* reported higher PFS (HR 0.45, p=0.002) and OS (HR 0.2, p<0.001) in a small patients cohort of *EGFR* exon 20 insertion compared to classical *EGFR*-mutant NSCLC receiving ICIs, highlighting the importance of further studies on immune biological mechanisms and ICIs efficacy in these molecularly defined subgroup.[56] Similarly to the aforementioned study, Lau *et al* demonstrated that *EGFR* exon 20 insertions and *HER2* mutations subgroups presented significant ICIs benefit, with a 6-month PFS rate of 33% compared to 4% in the classical *EGFR*-mutant patients.[57]

Overall, the small sample size of *EGFR* exon 20 insertion NSCLC patients included in the aforementioned studies precludes any definitive conclusions and the current international guidelines do not recommend the use of first-line single-agent immunotherapy in NSCLC patients harboring any activating *EGFR* mutation (including exon 20 insertions), regardless of PD-L1 expression levels.[38] As regards immune-chemotherapy regimens, although the combination of carboplatin, paclitaxel, bevacizumab and atezolizumab, demonstrated antitumor activity in the subgroup analysis of the Impower 150 study dedicated to the *EGFR*-mutant population, the small number of *EGFR* exon 20 insertion patients included do not allow definitive conclusions.[58, 59]

New treatment horizon for EGFR ex20ins

Novel therapeutic strategies in patients affected by *EGFR* exon 20 insertions NSCLC are urgently needed, considering that, to date, chemotherapy remains the most appropriate treatment both in terms of quality and quantity of available evidence.[16, 42, 60] Several new EGFR TKIs and bispecific antibodies are currently under evaluation in this peculiar subgroup of NSCLC in order to provide novel therapeutical insights and to ideally identify a new standard of care [Table 1].

Poziotinib. Poziotinib, a TKI able to target the small kinase pocket created by the exon 20 insertions, demonstrated both *in vitro* and clinical activity.[14] Preliminary results showed a very promising ORR of 58% in NSCLC harboring *EGFR* exon 20 mutations/insertions.[61] Nevertheless, a phase 2 clinical trial (ZENITH20) investigating poziotinib in previously treated NSCLC patients with *EGFR* exon 20 insertions, reported a lower ORR of 14.8% (being the primary endpoint of the study) with a median PFS of 4.2 months, although the DCR was 68.7%, and responses lasted for a median of 7.4 months. Almost all patients experienced any grade treatment-related adverse events (TRAEs), with the most common grade 3 being diarrhea (25%), rash (28%), stomatitis (9%), and paronychia (6%).[62] Despite this trial did not achieve the primary endpoint, ZENITH20 is continuing the enrolment with three new cohorts and dosing adjustment. The improved understanding of those underlying mechanisms leading to poziotinib resistance may allow to improve the expected outcomes, although poziotinib toxicity profile requires special attention.

Mobocertinib. Mobocertinib (TAK-788) is a potent TKI specifically designed to selectively target *EGFR* exon 20 insertions.[63] In April 2020, Mobocertinib received breakthrough therapy designation from the Food and Drug Administration (FDA) for pre-treated patients affected by metastatic NSCLC harboring *EGFR* exon 20 insertions based on the ORR (43%) and favorable median PFS (7.3 months) observed in a phase 1/2 trial, recently published *in extenso*.[64] In January 2021, at World Conference on Lung Cancer (WCLC), results from the EXCLAIM (extension cohort of the phase 1/2 trial; n = 96) and from a pooled platinum-pretreated patient population (PPP cohort; n = 114) from the phase 1/2 study and EXCLAIM were presented. Mobocertinib was administered at the 160 mg once daily oral dose. The safety profile of mobocertinib was reported as manageable and similar to other EGFR TKIs, although almost all patients experienced TRAEs, with grade ≥3 occurring in more than 40% of patients (mainly diarrhea). Adverse events (nausea, diarrhea, decreased appetite and stomatitis) led to

mobocertinib discontinuation in 17% and 10% of PPP and EXCLAIM cohort, respectively. In terms of activity, ORR was 26% per independent review committee (IRC) and 35% per investigator assessment in the PPP cohort and 23% per IRC and 32% per investigator assessments in the EXCLAIM. In both groups, median PFS was 7.3 months.[65] A first-line phase 3 study (EXCLAIM-2) to evaluate mobocertinib *versus* chemotherapy is currently ongoing (NCT04129502).

Amivantamab. Amivantamab (JNJ-372) is a novel bispecific IgG1 antibody targeting both EGFR and MET, which demonstrated preliminary activity in lung cancer preclinical model with *EGFR* mutations and *MET* amplification.[66] Afterwards, amivantamab demonstrated activity in NSCLC patients with several *EGFR* mutations, including C797S, exon 20 insertion and *MET* amplification.[67] In March 2020, based on the very promising results obtained in 39 patients with advanced NSCLC harboring *EGFR* exon 20 insertions from CHRYSALIS (phase 1 study), amivantamab received breakthrough therapy designation from the FDA.[68] The updated results of this trial were recently presented at WCLC 2020, reporting a good safety profile with grade ≥3 TRAEs occurring in 16% of patients (n = 114 safety population), mainly rash, diarrhea and paronychia, rarely leading to treatment-related discontinuation (4%). Of note, any grade infusion-related reactions occurred in 66% of patients, in most of the cases (94%) with the first infusion without impacting on the possibility to continue with subsequent treatments. A robust and persistent activity was described (ORR 40%, median DOR 11.1 months), with median PFS and median OS of 8.3 months and 22.8 months, respectively. Interestingly, the antitumor activity was observed in all patient subgroups and across different insertion regions of *EGFR* exon 20.[69] Based on amivantamab preliminary data, combination approaches are under evaluation in clinical trials. The PAPILLON (NCT04538664) is a phase 3 ongoing study designed to assess the efficacy of amivantamab plus chemotherapy *versus* chemotherapy alone in patients with advanced NSCLC harboring *EGFR* exon 20 insertions,

with PFS as primary endpoint and ORR, DOR and OS as secondary endpoints.[70] Another ongoing trial is the phase 3 MARIPOSA study evaluating first-line amivantamab + lazertinib *versus* osimertinib *versus* lazertinib in *EGFR*-mutant NSCLC (NCT04487080).[71]

Tarloxotinib. Tarloxotinib was designed as a hypoxia-activated prodrug, able to release tarloxotinib-effector (tarloxotinib-E), a potent and irreversible pan-ErbB (EGFR, HER2, and HER4) TKI.[72] In 2018, preliminary data obtained in murine xenograft models suggested that tarloxotinib was able to overcome intrinsic *EGFR* exon 20 mutation resistance to standard EGFR TKIs.[73] Afterwards, this novel mechanism of action (hypoxia-activated prodrug) was further validated in a series of patient-derived cancer models and in a lung adenocarcinoma patient with an *ERBB2* exon 20 insertion experiencing a dramatic tumor response with tarloxotinib.[72] The first results of the RAIN-701 multi-cohort phase 2 study suggested the activity of tarloxotinib in the cohort of NSCLC harboring *HER2* exon 20 activating mutations (n = 11 - cohort B) with a ORR of 22% and a DCR of 67%. A less promising activity was described in cohort A (n = 11) enrolling patients affected by NSCLC harbouring an *EGFR* exon 20 insertion with disease stability and progression reported in 6/11 (55%) and in 5/11 (45%) of patients, respectively.[74]

Other agents. In a broad spectrum of preclinical models, TAS6417 (CLN-081) emerged as a strong inhibitor of the most common *EGFR* mutations (exon 19 deletion and L858R) and the most potent against cells harboring *T790M* mutations.[75] TAS6417 targets *EGFR* exon 20 insertion mutations, while sparing the wild-type form of the receptor, and selectivity indexes (wild-type EGFR/mutant EGFR ratio of inhibition) favored TAS6417 *versus* poziotinib and osimertinib, due to its wider therapeutic window.[76] The interim results of a phase I/II clinical trial (NCT04036682) demonstrated TAS6417 anti-tumor activity at the lowest doses tested in five NSCLC patients with *EGFR* exon 20 insertions both refractory and naïve (to mobocertinib and/or poziotinib), with a manageable safety profile.[77]

Other potentially interesting agents in this field are BDTX-189, an orally available allosteric ErbB inhibitor currently under investigation in patients with advanced solid tumors harboring allosteric *EGFR/HER2/HER3* mutations or *EGFR/HER2* exon 20 insertions (NCT04209465; MasterKey-01), and DZD9008 (NCT03974022), an EGFR TKI targeting *EGFR* or *HER2* exon 20 insertions and other activating mutations.[78]

Finally, a novel HER3-directed antibody drug conjugate composed of monoclonal antibody patritumab, a tetrapeptide-based linker, and a topoisomerase I inhibitor payload (patritumab deruxtecan U3-1402) demonstrated antitumor activity in *EGFR*-mutated NSCLC with different TKI resistance mechanisms, including one patient developing an exon 20 insertion as mechanism of resistance to standard EGFR TKI.[79]

Conclusions

In conclusion, despite *EGFR* exon 20 insertions occur very rarely among different cancer types, the correct identification of these alterations is crucial to the proper management of NSCLC patients. However, several questions related to the preferred technology detection method and the best treatment option for NSCLC patients harboring *EGFR* exon 20 insertions are still open. In order to try to shed some lights on these points, the Atlas group composed by clinicians specifically involved in thoracic oncology field on the Italian territory has given its following opinion:

Q: Which is the preferred technology for EGFR exon 20 insertions detection in advanced NSCLC setting?

A: As previously stated, despite the low frequency of these alterations, it is fundamental the correct identification of *EGFR* exon 20 insertions for NSCLC patients' adequate management, due to the possibility of target treatments.[18] In this scenario, NGS technology, able to detect several hotspot gene mutations for different patients simultaneously,[24] is the best option due

to its higher sensitivity and specificity respect to other molecular techniques. Of note, NGS is able to overcome the limitation of target-based approaches enabling the identification of either known or unknown mutations within gene panel reference range. In fact, it has been reported an increase in the detection rate of *EGFR* exon 20 insertions in patients with advanced NSCLC from 2011 to 2019.[25]

Q: Which are the major challenges clinicians face in everyday clinical practice for treatment decisions in EGFR exon 20 insertion advanced NSCLC patients?

A: In clinical practice, first of all, it is necessary to have a clear report of the type of *EGFR* exon 20 insertion, in order to define the correct therapeutic strategy. Regarding treatment decision, in the majority of the cases the clinician is assessing a patient that, despite affected by an oncogene-addicted disease, should be treated, at this moment, with chemotherapy, with the additional necessity to evaluate in first-line the addition of immunotherapy. Mobocertinib and amivantamab represent new potential treatment options in pre-treated patients, paying attention to the safety profile. Phase III trials in first-line with these agents combined with chemo-immunotherapy are ongoing. Finally, brain metastatic disease can be a particularly challenging situation in this subgroup of *EGFR* mutated patients, in which radiotherapy could play a fundamental role, as we still do not have definitive data about the intracranial activity of these new molecules.

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Figure legend.

Figure 1. *EGFR* exon 20 insertions (within codons 756 and 774) fall back in the tyrosine kinase domain. In particular, two principal groups of insertions can be identified: in frame insertions and three to 21 bp (corresponding to 1–7 amino acids) duplications within amino acids 762 and 774.