Not All EGFR Exon 20 Insertions Are Created Equal

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There are hundreds of *EGFR* mutations reported in lung cancer, but not all *EGFR* mutations are created equal.[1] Most *EGFR* mutations are deletions in exon 19 (del19) and substitution of arginine for leucine at codon 858 of exon 21 (L858R). *EGFR* del19 and L858R are often referred to as the common (or classic) *EGFR* mutations, accounting for up to 80% to 85% of all *EGFR* mutations.[1] Currently available EGFR tyrosine kinase inhibitors (TKIs), including first-generation gefitinib and erlotinib, second-generation afatinib and dacomitinib, and third-generation osimertinib have been proven to control lung cancer with *EGFR* del19 or L858R mutations in phase 3 trials. EGFR TKIs are currently the standard of care for advanced lung cancer with the common *EGFR* mutations, whereas the secondary *EGFR* T790M mutation contributes to around 50% of the resistance to first- and second-generation TKIs.[2] In contrast, *EGFR* mutations other than del19 or L858R are referred to as uncommon *EGFR* mutations.[1] Among them, exon 20 insertions (ins20) make up the majority, accounting for around 4% of all *EGFR* mutations.[3] The response of ins20 to the first-generation TKIs gefitinib and erlotinib is notoriously poor. The response rate (RR) is about 5%, and the disease control rate is around 15%.[3] Moreover, the required concentrations of the second-generation TKIs, afatinib and dacomitinib, that produce 50% inhibition (50% inhibitory concentraion, IC_{50}) of ins20 are above the achievable plasma concentrations, precluding them as effective therapies.[3] Although afatinib was reported to be effective against some uncommon mutations—G719X, L861Q, and S768I—in the post hoc analysis of LUX-Lung 2, 3, and 6, the RR and median progression-free survival (PFS) for ins20 were only 8.7% and 2.7 months, respectively.[4] The third-generation TKI osimertinib had revealed possible activity against ins20 in vitro[5] and was once the hope for ins20 therapy. However, the clinical RR was only 5% with a median PFS of around 3.6 months.[6] Considering that the number of patients was very limited, a larger study is warranted to evaluate the efficacy of osimertinib in patients with ins20. In general, ins20 are regarded as EGFR TKI–resistant mutations. Consequently, they have been excluded from randomized controlled trials evaluating the efficacy of EGFR TKIs.

There are dozens of different types of ins20 reported.[3] Most insertions in exon 20 occur at the loop following the C-helix, between residues 769 and 775.[7] In 2013, the crystal structure of ins20 D770_N771insNPG was identified for the first time in the laboratory by Drs. Daniel Costa and Susumu Kobayashi at the Beth Israel Deaconess Medical Center.[8] D770_N771insNPG at the end of the C-helix is at the "pivot point" of the C-helix, and sterically inhibits the reorientation of the C-helix to the inactive state. In 2018, a three-dimensional modeling of the structure revealed a steric hindrance of the drug-binding pocket for EGFR TKIs that caused the insensitivity to osimertinib.[9]

Unlike other ins20, A763_Y764insFQEA, occurring in the middle of the C-helix, was reported to be sensitive to first-generation EGFR TKIs in 2013 by the same study

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team.[9] By homology modeling, the structural mechanism of this activating mutation was analyzed in detail. The inserted FQEA shift the adjacent residues in the C-helix toward the N-terminal site. In the resting state, the I759 is then replaced by alanine, disturbing the hydrophobic residues, which stabilize EGFR, in a similar manner as L858R and L861Q. The structural change of A763_Y764insFQEA is very different from those of other exon 20 insertions. Compared with other sensitizing *EGFR* mutations, the IC_{50} of A763_Y764insFQEA to erlotinib was around 0.1 μM, similar to that of L858R but lower than that of G719A, L861Q, and S786I plus V769I.[10] Several case series and case reports have supported it as a first-generation EGFR TKI–sensitive mutation.[11] However, A763_Y764insFQEA is reported to compose only around 8% of all ins20.[11] In addition, it has not attracted much attention, probably because of its rarity. It is worth noting that rare mutations such as A763_Y764insFQEA cannot be detected by hotspot analyses for *EGFR*,[1] such as the authority-approved Roche Cobas EGFR Mutation Test V2 and matrix-assisted laser desorption ionization–time of flight. Direct sequencing of *EGFR* or next-generation sequencing is necessary to detect this mutation; therefore, its prevalence may be underestimated. Additional cases with this rare ins20 may be detected in the era of next-generation sequencing.

Recently, *EGFR* exon 20 insertion targeting agents, such as poziotinib,[9] mobocertinib (TAK-788),[12] amivantamab (JNJ-61186372, JNJ-6372),[13] DZD9008,[14] and TAS6417/CLN-081[10] have been undergoing clinical trials. *EGFR* exon 20 insertions have become a hot issue again. Seven years after the discovery of A763_Y764insFQEA, Vasconcelos et al.,[15] from the same laboratory, have reported its sensitivity to not only second and third generation but also in-development EGFR TKIs in this issue of *JTO Clinical and Research Report*. The authors collected details from published cohorts to evaluate the effectiveness of TKIs for A763_Y764insFQEA. A total of 11 enrolled patients received a first-generation TKI, two received afatinib, two received osimertinib, and one received mobocertinib (TAK-788). The overall RR was 62.5%, with a median PFS of 5.5 months ($n = 16$), consistent with the previous report on first-generation TKIs.[11] Of note, the patient who received mobocertinib (TAK-788) had a partial response. In the preclinical part of the study, A763_Y764insFQEA was sensitive to afatinib, osimertinib, and poziotinib in vitro. The IC_{50} values to the newer EGFR TKIs were much lower than that to erlotinib, indicating the potential activity against this specific mutation. Using the ratio of IC_{50} of EGFR mutation to IC_{50} of wild-type EGFR, the authors also evaluated the therapeutic window for different TKIs for different exon 20 insertions. A763_Y764insFQEA-driven cells had a low IC_{50} ratio (EGFR mutation to wild-type EGFR) for erlotinib, afatinib, osimertinib, and poziotinib, indicating a wide therapeutic window. Interestingly, although A767_V769dupASV and D770_N771insSVD cells also had a low IC_{50} ratio (EGFR mutation to wild-type EGFR) for poziotinib, this was not the case for H773_V774insH cells. The IC_{50} of H773_V774insH and wild-type EGFR to poziotinib were almost equal. This raises a concern about the capability of poziotinib to inhibit H773_V774insH. There was a similar report for another insertion at the same residue, H773insNPH. The IC_{50} of H773insNPH to poziotinib was higher than those of A767_V769dupASV and D770_N771insSVD.[9] In addition, IC_{50} values of H773_V774insH to erlotinib and afatinib were also the highest among ins20.[8] Cancer cells with insertions at residue 773 seemed to be more resistant to EGFR TKIs. The latest report from the ZENITH20 trial (NCT03318939), just presented in the American Society of Clinical Oncology 2020 Virtual Annual Meeting, that poziotinib RR for the "far loop" ins20 (insertion at residue 773, 774, or 775) was lower than that for the "near loop" ins20 (insertion at residue 767–772), supported this hypothesis.

In contrast, it is debatable whether the different exon 20 targeting agents have similar activities against the same ins20. Although the IC_{50} value of H773_V774insH to poziotinib was the highest among ins20, its IC_{30} to amivantamab (JNJ-61186372, JNJ-6372) was the lowest.[13] In xenograft models, amivantamab seemed to suppress H773_V774insH cells more than D770delinsGY and P772_H773insPNP cells.[13] Because A763_Y764insFQEA was not included in the study, it is not clear whether A763_Y764insFQEA was also very sensitive to amivantamab. TAS6417/CLN-081 revealed good activity against various ins20 cells, including A763_Y764insFQEA, V769_D770insASV (A767_V769dupASV), D770_N771insG, D770_N771insSVD, H773_V774insPH, and H773_V774insNPH.[10] Among them, A763_Y764insFQEA was the most sensitive. To date, the antitumor activity of mobocertinib (TAK-788) and DZD9008 against different ins20 has not been formally reported. We do not know whether there are also "mutation preferences" for the two agents. On the basis of the findings from other TKIs, it is prudent to suggest that different TKIs may have different activities against different ins20. Despite the preclinical evidence, *EGFR* exon 20 insertions have been regarded as a uniform entity in current clinical trials for poziotinib (NCT03318939, NCT03066206, NCT04044170), mobocertinib (TAK-788) (NCT02716116, NCT03807778, NCT04129502), amivantamab (JNJ-61186372, JNJ-6372) (NCT02609776, NCT04077463), DZD9008 (NCT03974022), and TAS6417/CLN-081 (NCT04036682). Consequently, the most sensitive ins20 A763_Y764insFQEA and, probably the most resistant, ins20 H773_V774insH (at least for

poziotinib), may be treated as equal. When interpreting the results of the trials, we should pay particular attention to the responses of different ins20. As we know that not all *EGFR* mutations in lung cancer are created equal, not all *EGFR* exon 20 insertions are created equal as well.

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