Potential use of lapatinib in the treatment of head and neck squamous cell carcinoma (Review)

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Abstract. To date, cetuximab is the only anti-epidermal growth factor receptor (EGFR) monoclonal antibody (mAb) approved for the targeted therapy of head and neck squamous cell carcinoma (HNSCC). However, this therapy has left an eloquent number of patients with recurrence and local or/and distance failures, which are associated with Erb-B2 receptor tyrosine kinase 2 (ERBB2) signaling. The dual targeting (EGFR/ERBB2) irreversible covalent tyrosine kinase inhibitor (TKI), afatinib, has exhibited promising results in HNSCC trials; however, its toxicity arises from further biosynthesis for receptor recovery. Conversely, lapatinib, a reversible TKI, is almost as potent as afatinib, and its interaction with the unique inactive conformation of EGFR has been shown to be associated with its selectivity, specificity and long residence time, resulting in efficacy. However, as a monotherapy, lapatinib appears minimally active and hepatotoxicity has been reported with its use. The present review article summarizes some of the research conducted over the years in order to determine the profile of lapatinib along, with its potential for use in the treatment of HNSCC and associated challenges.

Contents

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1. Introduction

Head and neck squamous cell carcinoma (HNSCC) is cancer derived from the mucosal epithelium in the oral cavity, pharynx (naso-, oro-, and hypo-), and larynx (1). Globally, HNSCC ranked as the sixth most common cancer, with 890,000 new cases and 450,000 deaths in 2018 (1), moreover, it was reported that in 2020, the number of new cases of larynx cancer was 184,615; oropharynx cancer was 98,412; and oral cavity cancer was 377,713 (2). The risk factors associated with the disease are smoking, alcohol consumption, systemic condition, socioeconomic condition, oral hygiene, viral infection with oncogenic strains [human papillomavirus (HPV) in particular HPV-16 and HPV-18], a family history of malignancy, diabetes mellitus and heavy metals found in soil (1,3).

The most common treatments for this chronic disease are drug combinations and surgery, chemotherapy (CT), and radiation. However, the existing cytotoxic therapies lack selectivity in target cells (4) and are associated with considerable toxicity in patients with HNSCC (1). The discovery of novel biomarkers/signaling pathways involved in head and neck carcinogenesis provides hope for the development of future novel targeted therapies and strategies (5). Epidermal growth factor receptor (EGFR) is the only proven target for HNSCC as it is overexpressed in >90% of HNSCC cases and is associated with a poor prognosis (6), which is inhibited extracellularly by cetuximab. In March 2006, cetuximab, a chimeric human-murine IgG1 monoclonal antibody (mAb) was the only molecularly targeted HNSCC therapy approved by the United States Food and Drug Administration (FDA) to be used in combination with radiation therapy for locoregionally advanced HNSCC. Moreover, in 2011, it was approved for

the treatment of recurrent and metastatic (R/M) HNSCC (7,8) combined with platinum-based CT or in monotherapy after platinum failure (5). RGFR tyrosine kinase inhibitors (TKIs) have been developed, which are mostly adenosine triphosphate (ATP) analogs, that function by inhibiting the EGFR signaling pathway and by preventing the phosphorylation necessary for downstream signaling (9). These inhibitors have been assessed thoroughly (10).

Despite these multimodalities, the majority of patients with HNSCC usually have a poor prognosis (7,8), and also exhibit recurrence and a risk of local and distant metastasis (11). The clinical responses for cetuximab as a single drug therapy are poor with a short duration of action (12). In due course, the majority of patients treated with cetuximab develop resistance (13). This is considered to be contributed to the extensive crosstalk between Erb-B2 receptor tyrosine kinase 2 (ERBB2), a transmembrane tyrosine kinase receptor, particularly ERBB2-dependent signaling pathways in HNSCC, and the molecular and genetic aberrations present (5). It has been found that increasing concentrations of cetuximab do not affect the autophosphorylation of EGFR or the activity of human epidermal growth factor receptor 2 (HER2)/ERBB2 (14).

The ERBB signaling pathway contributes to oncogenic processes, e.g., cell proliferation, migration, invasion, and progression (7,15). The majority of HNSCC tumors express both EGFR and ERBB2 (1,14). Of note, the co-expression of PDGFRα/HER2 and PDGFRα/p53 has been reported present in 71 patient samples of poorly differentiated oral squamous cell carcinoma, suggesting that the association between these proteins may promote the aggressive behavior of tumors (16). ERBB2/HER2/Neu is known to promote the activation of EGFR (17). ERBB2 harbors no ligand-binding cleft (14); hence, it does not require an activating ligand (18). The deprivation of a ligand contributes to its role as a potent signal amplifier for other ERBB family receptors (14,19), as ERBB2 forms heterodimers with other members of the ERBB family to be activated. In the case of targeting EGFR, compared to EGFR homodimers, heterodimers of EGFR/ERBB2 are more potent signaling complexes (8). These findings have led to the assumption that ERBB2 is a potentially attractive and valid therapeutic target in HNSCC (6,14). Drugs that simultaneously inhibit EGFR and ERBB2 have been suggested to be effective in enhancing the efficacy of cetuximab and preventing or overcoming resistance, as also demonstrated in studies using xenografts derived from HNSCC cell lines (20-22).

To the best of our knowledge, no specific TKIs have been described for ERBB2 (23). The present review article summarized the majority of the research conducted over the past year in order to determine the profile of lapatinib, a known TKI, along with its potential for use in the treatment of HNSCC and associated challenges.

2. TKIs for EGFR and ERBB2 in HNSCC

Antitumor agents that act on multiple ERBB families include lapatinib (Tykerb; GW572016), afatinib (BIBW-2992) (GIOTRIF/GILOTRIF), and its analog, dacomitinib (13,24). The latter two agents are FDA-approved irreversible oral inhibitors of EGFR, ERBB2 and ERBB4, covalently modifying the receptor (25). Although the interaction of afatinib with EGFR results in a longer transit time within the tumor (26), drugs that covalently bind to their targets have always been perceived as being potentially toxic (13,27) in consequence of the intrinsic reactivity of the cysteine-reactive groups, which is toxic due to off-target related effects (28). Moreover, regardless of the superior efficacy of these irreversible inhibitors compared to that of reversible inhibitors, the irreversible inhibition of ERBB requires further biosynthesis for the maintenance of the receptor (27) and is also limited by the emergence of drug resistance (28).

3. Profile of lapatinib

Lapatinib (chemical structure shown in Fig. 1), a reversible TKI, has an equal strength as covalent binders (29), with a gradual dissociative half-life equated to that of gefitinib and erlotinib (30), indicating that covalent target interaction is not essential for effective ERBB2 kinase inhibition (29). Moreover, lapatinib (1,250 mg/day) administered orally in combination with capecitabine (1,000 mg/m^2 twice/day), has been shown to result in the primary outcome of overall survival (31).

The quinazoline core of the lapatinib structure occupies the adenine pocket, although with poor specificity and selectivity for ERBB-type receptors. The bulky 3-fluorobenzyl-oxy moiety further increases its specificity and contributes to the distinction between activation states by occupying the allosteric pocket. This selectivity provides higher access to the hydrophobic pocket adjacent to the nucleotide-binding site in the inactive state of the receptor (32,33). The structure and activity of lapatinib are as follows: i) The pyrimidine group in the quinazoline core is important; ii) a free NH linker at the 4-position of the quinazoline core is optimal; iii) electron-withdrawing lipophilic substituents at the 3-position of the aniline moiety are favorable; and iv) electron-donors at the 6- and 7-positions of the quinazoline are preferred (32).

4. Potential of lapatinib as a dual TKI for EGFR/ERBB2

Lapatinib has been approved by the FDA for patients with ERBB2-positive breast carcinoma (31,34-36). It is a competitive inhibitor of EGFR and ERBB2, previously reported to reduce the proliferation, inhibit multiplication and increase the apoptosis of HNSCC cells and other tumor xenografts expressing EGFR and ERBB2 (13,37). It is currently being evaluated in several phase II trials in HNSCC with the continued investigation as a phase III trial (NCT00424255) (38,39). The IC_{50} values of lapatinib for EGFR and HER have been shown to be 10.8 and 9.2 nM, respectively, compared to afatinib at 0.5 and 14 nM, respectively (13).

Lapatinib blocks the activation of the mitogen-activated protein kinase (MAPK) pathway, phosphoinositide 3-kinase (PI3K)-Akt (protein kinase B) and phospholipase C γ) in the EGFR and HER2 signaling pathways (34). By shutting out the introduction of the phosphate group to the receptor and the subsequent activation of these routes, apoptosis is enhanced, and the growth of the cancer cells is inhibited (13). Furthermore, it has been reported that this drug can inhibit the proliferation of various human cancer cell lines (34,35). Theoretically, lapatinib has the advantage of being a dual TKI (for EGFR/ERBB2) (8).

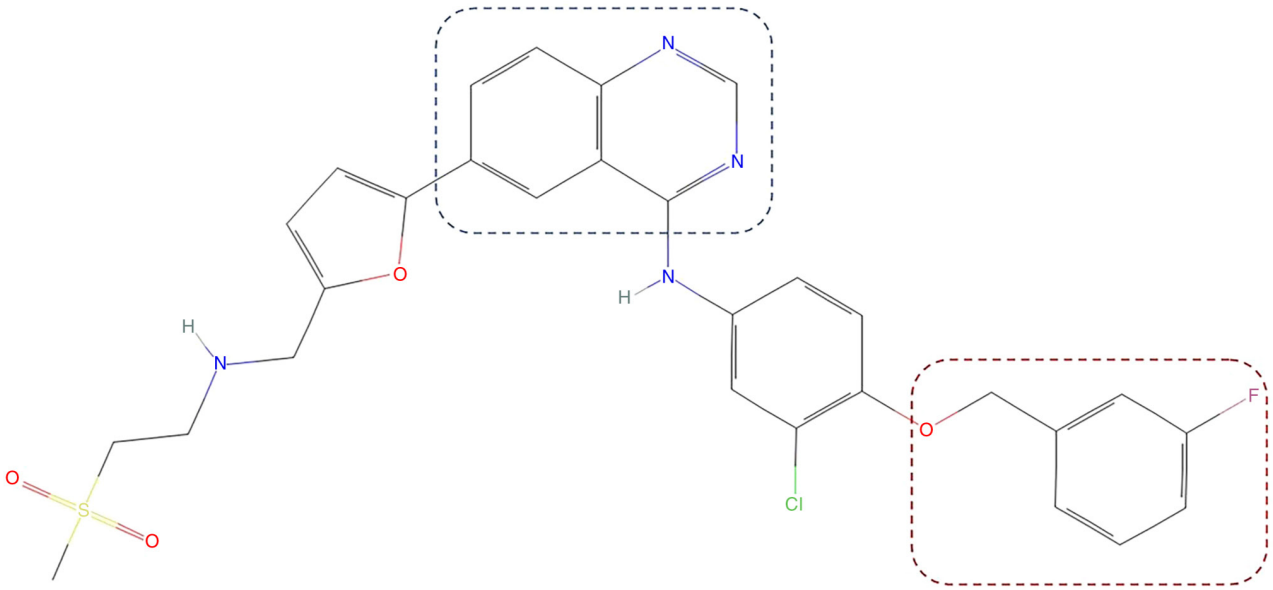

Figure 1. Structure (two-dimensional) of lapatinib (PubChem CID 208908). The quinazoline structure is illustrated in the box with blue dashed lines and 3-fluorobenzyl-oxy in the box with red dashed lines.

Advantages of lapatinib gained from inactive kinase conformation. Although all TKIs function by competitively inhibiting phosphorylation by ATP, these drugs target both active and inactive kinase states (40). The active state is formed when the kinase adopts a conformation almost identical to that used to bind ATP at the intracellular ATP binding site, while the inactive conformations are characterized by an activation loop arrangement, allowing accessible additional allosteric binding site directly linked to the region where ATP is bound. These different states can be utilized in the design of selective antagonists (41).

Lapatinib has a high affinity for the inactive conformation of wild-type EGFR (42) and is selective to the inactive conformation of the ERBB2 kinase in which the Cα-helix is orientated apart from the nucleotide-binding pocket without the formation of a salt bridge between glutamic acid-lysine residues. Lapatinib occupies the EGFR kinase CDK/Src-like inactive conformation (43-45), with its benzyl ether substituent (41), a voluminous 3-fluorobenzyl-oxy group due to the presence of an extra hydrophobic pocket created by the outwardly displayed helix αC in the CDK/Src-like conformation. Molecular modeling has revealed that the binding of lapatinib to the active kinase results in a steric hindrance, which explains the preferential interaction of this drug with the inactive kinase conformation (44). From the current perspective, there are at least three advantages of targeting inactive kinase conformation for HNSCC, which are i) the potential to target multiple kinases concomitantly; ii) to function directly at the location of intracellular signaling; and iii) the capability to cross the blood-brain barrier (45).

5. Kinase structure

Due to the conserved kinase structure, several small molecules that bind to the ATP binding site reveal a degree of promiscuity and interaction with multiple kinases (28,46). The kinase active state (Fig. 2A) is rigid and highly conserved, and this characteristic that leads to a selective focus on the active kinase using conventional ATP-mimetic antagonists is formidable. On the contrary, kinase-inactive states (Fig. 2B) are structurally diverse and dynamic, demonstrating that inhibitors of these states should be highly critical and specific. The neighboring areas not occupied by ATP are more variable; thus, TKIs may be directed towards the substrate-binding site or allosteric sites to achieve specificity and selectivity (47). More specifically, these allosteric locations provide the prospect of extremely selective inhibitors (42). These structures are regulated by a number of pivotal elements within the kinase catalytic pocket and the main modulators, including the αC-helix, the DFG-Asp motif and the activation loop or commonly referred to as the A-loop (48).

The A-loop is flexible, consisting of 20-30 residues, and is indicated by a conserved aspartic acid-phenylalanine-glycine (DFG) motif, positioned at the beginning of the C-terminal domain and the terminus of the amino domain (46,47). The A-loop along with a glycine-rich loop dictates the accessibility of the catalytic site and modulates the active (open) and inactive (close) conformation of the enzyme (37). The A-loop is very flexible in conformation, which is associated with its important function in regulating the enzyme, as the phosphorylation process maintains the conformation and allows strong activity. However, in a confined alteration state, such as the DFG flip, the A-loop regularly encounters a small root mean square deviation in its shape of ~20 Å. In the activation of the kinase (where the C-helix rotates), the A-loop extends apart from the C-helix, thereby revealing the substrate-binding domain (49).

The DFG motif is a protected sequence of three amino acids (aspartic acid-phenylalanine-glycine), which builds the ATP binding domain of the enzyme and is responsible for the appropriate direction of the Asp to coordinate with two magnesium ions, and harboring the C-helix commencing

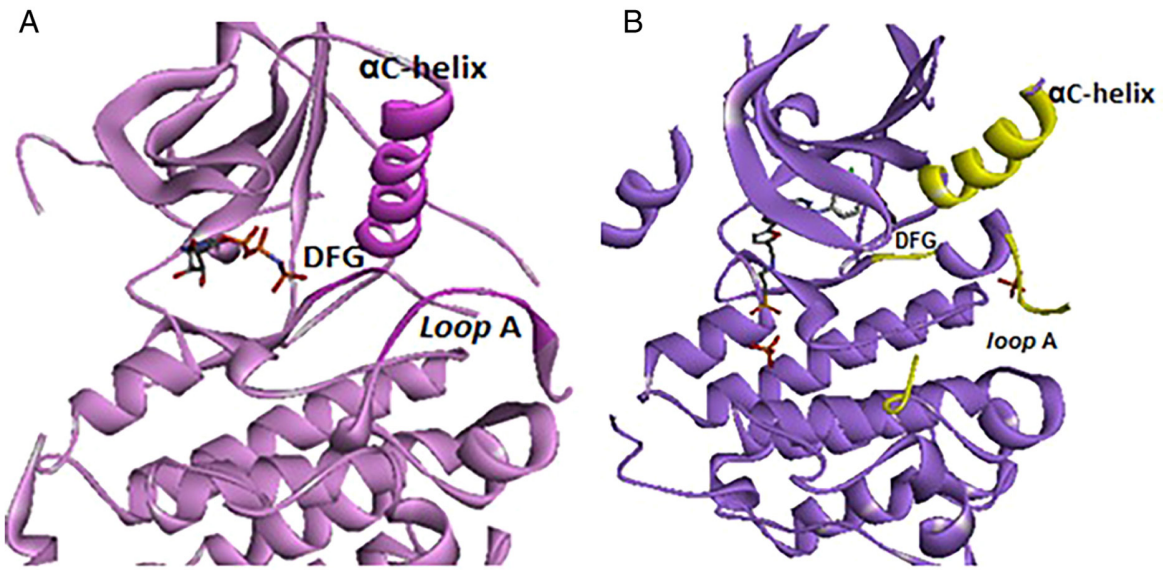

Figure 2. Structure (three-dimensional) of (A) active (PDB ID: 2ITX) and (B) inactive kinase (PDB ID: 1XKK). In the inactive kinase, the DFG is positioned inward while the αC-helix is facing outward.

the glutamic acid-lysine residues salt bridge. Magnesium cations are required to build covalent coordination with the phosphates during the transfer to the substrate (47). In the inactive kinase state, the DFG-out conformation distinguishes it from the active structure in that it features a flipped-DFG (by ~180˚), in which Phe832, instead of Asp831, is observed in the ATP-binding domain. The aspartic acid slightly diverges from the ATP binding domain by ~5 Å, leading to the DFG-out conformation, which opens a new allosteric pocket directly linked to the ATP binding domain. Moreover, the protonation of the aspartic acid residue results in a DFG flip and favors the DFG-out conformation, and the DFG flip is coupled with the large-scale motion of the αC helix (46). Potent antagonists of the kinase can be achieved, particularly if the DFG-out structure of this enzyme is thoroughly studied (30).

The αC-helix is the most conserved structure positioned in the amino terminus along with five-stranded β-sheets. The carboxylate-terminus of the helix is anchored to the center by the β4-loop via the β5 strand to the hinge region, whereas its amino-terminus binds with the A-loop. The amino-terminus of the αC-helix has to be pointed correctly to facilitate the phosphate transfer to the substrate by the conserved Lys-Glu salt bridge. The conserved Glu residue is located on the αC-helix, and disruption of the salt bridge is a strong indicator of protein kinase inactivity. The αC-helix along with the catalytic loop (C-loop), the A-loop, and the glycine-rich nucleotide-binding loop (P-loop), characterize the active site in the cleft between the β strand-rich N-lobe and the helical C-lobe. The A-loop, P-loop, and αC-helix modulate the activity of the kinase domain by regulating the accessibility of the active site to binding and coordinating both ATP and the substrate tyrosine. As a relatively rigid structural element, the motion of the αC helix has the potential to stimulate a marked disruption to the system and may play a general and critical role in the activation/deactivation transitions of different kinases possessing the Src/CDK-like inactive conformation, in which the αC-helix is positioned outward and the N-terminal segment of the A-loop takes a helical form (50).

Upon the activation of the enzyme, the αC-helix rotates ~90 degrees to orientate the glutamate residue, and the A-loop extends away from the C-helix, thereby revealing the substrate-binding domain. Rotating the N-terminus of the C-helix in a suboptimal position for catalysis ('C-helix-out') resulted in an inactive state of the kinase (50).

A long residence time leads to efficacy. A long residence time (slow tight binding), higher potency, and cellular efficacy have been found to be the requirements for selective inhibitors of this enzyme (50,51). The unusual inactive kinase conformation significantly prolongs the off-rate (30), enabling dosing frequency and levels to be reduced with low drug concentrations (52).

The structural determinants of a long residence time include the hydrogen bonds between aspartyl protease inhibitors and the flap elements, 'back pocket' engagement by ATP-competitive protein kinase inhibitors and 'side pocket' engagement where the drug occupies the back cleft. A detectable residence time generally requires at least one of these factors: An indispensable hydrogen bond with the hinge region and hydrophobic complementarities with the front pocket. This combination of hinge and front pocket inhibitor interactions may hinder breathing motions between the N and C lobes complex, which are normally required to enable nucleotidebinding and release (52). The narrow opening between N- and C-lobes and the occlusion of the active site by the A-loop in inactive states have affected some circumstances, e.g., the drug-receptor association and dissociation, and the residence time of the drug-receptor complex (53).

A significant linear association between the efficacy and residence time has been noted, as numerous marketed drugs slowly dissociate from their targets, emphasizing the potential importance of drug-target complex residence time, for *in vivo* drug activity (54).

Appropriateness for HNSCC. One kinase differentiates from the other kinase by the diversity of incoming signals that strike on the catalytic domain, and a rich variation in the mechanisms converting inactive forms of the kinase to active ones (43). In HNSCC, EGFR is mostly overexpressed due to gene amplification, instead of an EGFR activating mutation, which is extremely rare in HNSCC. This also applies to ERBB2 as the mutations of this protein have not been evaluated as a predictive biomarker in selecting patients with HNSCC likely to receive advantages from anti-ERBB2 treatment (13). In a prospective cohort study of 82 patients who had not been treated with EGFR molecular targeting therapy, the Western blotting analysis revealed that o-EGFR was observed in 67 of 82 patients, while p-EGFR was detected in 14 of 82 patients. Moreover, 13 patients with p-EGFR stratified by f-EGFR had survived without recurrence longer than patients without f-EGFR (55), implying that they are not a common cause of its oncogenesis (8) and unlikely to be useful as predictive biomarkers for EGFR targeted therapy (10). The oncogenic EGFR mutants greatly favor their active over inactive kinase conformations, while the wild-type EGFR catalytic domain monomer is mostly present in an inactive conformation. This results in a distinct shift toward the active conformation for all of the mutants and suggests that inactive kinase conformation may be rationally feasible in targeting EGFR and ERBB2 for HNSCC.

6. Challenges associated with the use of lapatinib

Although lapatinib has been shown to exhibit clinical activity, particularly in HPV-positive patients (53) and in locally advanced HNSCC (56), as a monotherapy, lapatinib appears minimally active (37), as it has not yielded successful results in a phase II trial (trial no. NCT00490061 in combination with radiotherapy), actual enrollment 17 of stage III-IV HNSCC participants, with terminated status) (14) and has shown lack of activity in R/M HNSCC (54). A phase III (trial no. NCT00424255) trial did not reveal any beneficial effects of lapatinib, and lapatinib exhibited additional toxicity (diarrhea, rash, and cardiac events) compared with the placebo when it was used in combination with radiation or cisplatin therapy in patients with surgically treated high-risk HNSCC (57). In another study, apoptosis induction did not result in a significant difference compared with placebo and the change in the apoptotic index was not notable, despite the observation of tumor shrinkage in some patients with locally advanced HNSCC (54).

7. Conclusions and future perspectives

Lapatinib, as an anticancer drug that functions by inhibiting tyrosine kinase and multiple ERBBs, has a high affinity for the inactive conformation of wild-type EGFR. This capability to interact with inactive kinase structure has the advantage to attenuate tight binding, prolong the off-rate and enable dosing frequency, leading to a higher potency (low therapeutic dose).

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Authors' contributions

TS and JL were principally responsible for the conception and design of the study. CSH and JL contributed to collecting and selecting the references, and writing of the original version of the manuscript. JL and SJ were responsible for reviewing and revising the manuscript. SM contributed to revising the manuscript, collecting the references, and improving the quality of figures. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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