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Commentary

Epithelial transfer variability of the tyrosine kinase inhibitors of epithelial-growth factor receptor: Implications for pharmacokinetic resistance in NSCLC

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Introduction

The discovery of Tyrosine Kinase inhibitors (TKIs) of Epidermal Growth Factor Receptor (EGFR) represented a significant revolution for the treatment of non-small cell lung cancer (NSCLC). In fact, they are administered orally, on an out-patient basis, at fixed dose, and independently of weight, height, sex or age. Moreover, they recognize a specific target (EGFR) on lung cancer cells and for this reason are referred to as "targeted therapy" [1]. Clinical studies and meta-analyses have shown that TKIs of EGFR were more effective than chemotherapy [2–5] so that they represented the new gold standard treatment. Gefitinib and Erlotinib were the first-generation reversible EGFR TKIs approved while Afatinib is a second-generation irreversible EGFR TKI.

Osimertinib is a third-generation irreversible EGFR-TKI, which binds both EGFR activating mutations (Exon 19 deletion, L858R) and the resistance-associated T790M mutation via covalent bonding to cysteine 797 in the ATP-binding site [6]. Initially, it was approved only for T790M-positive NSCLC patients progressing on earlier-generation TKIs [7,8]. Later, it became the preferred choice both for the first-line treatment (regardless of T790M status) and in the adjuvant setting [9].

Nevertheless, clinical trials have reported primary resistance to EGFR TKIs in approximately 20% to 40% of patients.

TKIs have physical chemistry properties that make them insoluble in water [10,11] limiting IV preparation. For this reason, it is important to study the bioavailability of each individual TKI across the gut membrane. It is not yet clear whether this primary resistance is partially attributable to impaired intestinal epithelial transport of these targeted agents [2–4,7].

Absorption Mechanisms

The physicochemical properties of each molecule influence its absorption by the small intestine so that poor uptake from the gut may lead to drug resistance.

The intestinal epithelium cells are polarized into an apical membrane (exposed to the gut contents) and a basolateral membrane across which the compounds go into the blood flow. The transcellular process is considered the main absorption process since the paracellular process is limited by tight junctions between epithelium cells [12,13]. After the uptake across the apical membrane the compound becomes, into the cytosol, exposed to several processes that could limit its efficacy: ATP-binding cassette (ABC) transporters such as P-gp (P-glycoprotein) and BCRP (Breast

Cancer Resistance Protein) are ATP-dependent efflux transporters located on the apical membrane of enterocytes that actively facilitate its efflux back into the gut [14]; CYP3A4 (Cytocrome P450 3A4) is a metabolic enzyme could change the molecular characteristics of the compound favoring or its deactivation braking down [15]; the compound could be sequestered into internal organelles or vesicles [16]. Pharmacokinetically, a high efflux ratio reduces drug bioavailability, resulting in lower plasma concentrations and reduced steady-state levels.

Transwell System

To better investigate the gut absorption of several TKIs, it was used an optimized Transwell system with a Caco-2 cell line originating from a colorectal adenocarcinoma and was cultured in DMEM supplemented with 10% FBS and 20 mM HEPES at 37°C, 5 % CO_2 and 100% humidity. Using a special coating in a transwell, the cell line forms a cellular layer with specific transporter expression at each side [17,18]. Active and passive transport were characterized by culturing the cells at a low temperature (4°C; passive transport) or a physiological temperature (37°C; active and passive transport). The methodology employs TEER measurements for barrier integrity, 5% BSA for stability, and specific inhibitors (Ko143, NaN_3) with LC-MS/MS quantification to differentiate active transport from passive diffusion. The optimization and validation of this model was previously described [18].

This widely used *in vitro* model is considered one of the best representative models. Through this model, it was possible to investigate for each compound the transport from the theoretical gut to the epithelium lining and vice versa and to determine the perfusion coefficients. A perfusion coefficient ratio greater than 1 was indicative that most of the compound was subjected to efflux from the epithelium lining to apical direction; on the contrary, a perfusion coefficient ratio less than 1 was indicative of a predominant uptake direction.

TKI Comparisons

When the gut absorption of different EGFR TKIs was analyzed, using Transwell system, important differences were identified.

Gefitinib showed a perfusion coefficient in the uptake direction of 0.57 µm/s and in the reverse direction of 0.19 µm/s, with a net efflux ratio of 0.33. Higher concentrations of Gefitinib into the gut inhibited the absorption effect suggesting that the passage of the compound was a combination of an active and passive transport mechanism although the uptake diffusion was predominantly active. Erlotinib showed a perfusion coefficient in the uptake direction of 0.48 µm/s and in the reverse direction of 0.79 µm/s with a net efflux ratio of 1.66. Therefore, these experiments showed consistent differences between first generation TKIs of EGFR in terms of gut absorption. Afatinib showed a perfusion coefficient in the uptake direction of 1.5 µm/s and in the reverse direction of 10.9 µm/s with a net efflux ratio of 7.3. Similarly to Erlotinib, Afatinib reported a higher basolateral to apical perfusion rate than apical to basolateral perfusion rate. Therefore, it was observed among EGFR TKIs a large variation in the net intestinal absorption from the apical to basolateral side: in particular erlotinib and Afatinib showed an efflux significantly greater ratio than 1 indicating either a poor apical-basolateral transfer or a high reversed reflux to the apical (gut) [11].

Unfortunately, we don't have sufficient *in vitro* data about the gut absorption of Osimertinib, likely due to its recent approval and its demonstrated superiority in phase III studies compared to first- and second-generation EGFR TKIs.

A comparison between the absorption properties of EGFR TKIs and clinical response rates is shown in **Table 1.**

An interesting study reported the plasma concentration analyses, using mass spectrometry, of a patient with advanced EGFR-mutated adenocarcinoma of the lung who received Osimertinib 80 mg/day orally achieving a partial response. Subsequently, the patient was subjected to extensive intestinal resection resulting in short bowel syndrome. A malabsorption of Osimertinib was documented with a consequent plasma concentration of the drug below the expected plasma concentration. The optimal plasma concentration was obtained increasing the dose of Osimertinib at 120 mg/day [19].

Clinical Implications

If we consider the activity of TKIs of EGFR reported in the clinical trials, the response rate ranged between 75% to 80% [2–4,7]. We could postulate that interindividual genetic characteristics of single patients may affect the gut absorption of the TKIs through a variability in the uptake or efflux processes [20]. Furthermore, it remains uncertain whether an individual's intestinal absorption capacity is static or can undergo dynamic changes over time. Multiple physiological and clinical variables such as gastric pH fluctuations, gastrointestinal transit alterations, aging, disease progression, and concomitant medications can influence oral drug exposure within the same patient, yet the temporal evolution of absorption capacity remains poorly defined [21]. Administration of TKIs is usually recommended under fasting conditions although the effect of food on intestinal absorption appears to differ depending on the drug: increased absorption in the case of Gefitinib and Erlotinib and decreased in the case of Afatinib [21,22].

The majority of advanced NSCLC patients treated with TKIs of EGFR necessarily experience progression of disease. Several mechanisms of acquired resistance have been described including

Table 1. TKIs of EGFR: absorption properties versus clinical response rates.

TKI [ref]	Perfusion coefficient µm/s			Response Rate %
	Uptake	Reverse direction	Net efflux ratio	
Gefitinib [2]	0.57	0.19	0.33	74
Erlotinib [3]	0.48	0.79	1.66	58
Afatinib [4]	1.5	10.9	7.3	67
Osimertinib [7]	MD	MD	MD	80

TKI: Tirosine Kinase Inhibitor; EGFR: Epidermal Growth Factor Receptor; MD: Missing Data

secondary mutations of EGFR or activation of other pathways such as MET, HER2, BRAF, RAS [23,24]. In about 20% of the cases the mechanism of resistance is not identified so that we may hypothesize that in some cases a secondary pharmacokinetic resistance mechanism may be involved. To overcome the pharmacokinetic resistance, plasma pharmacokinetic assays could be helpful although they are rarely applicable in routine clinical practice. A better understanding of the conditions that can alter the intestinal absorption might help identify which patients would benefit from pharmacokinetic monitoring. Moreover, it can be postulated that engineering new compounds with a lower net efflux ratio may reduce this pharmacokinetic resistance.

Future Directions

The microbiome, is an emerging and widely studied factor in cancers. Unfortunately, up to now the potential influence of gut microbiome in the absorption of EGFR TKIs has not been extensively studied and future studies are warranted. Microbiome alterations can alter drug absorption either through direct structural modification of the drug or by varying the expression of intestinal transporters and CYP3A4 [25]. After the gut absorption into the blood flow other pharmacokinetic characteristics of single compounds can determine) their efficacy. Interesting preliminary data reported from clinical samples showed that for some compounds, the concentration in the whole blood was significantly higher than that found in the plasma. Both Gefitinib and Erlotinib showed a high red cells uptake but in particular in the case of Erlotinib the whole blood concentration of the compound resulted 100-fold higher than plasma concentration. This observation deserves further investigations taking into account that pharmacokinetic studies are usually limited to plasma samples.

Conclusions

Although clinical studies have clearly shown the superiority of EGFR TKIs compared to chemotherapy, approximately 20 to 40% of patients experience primary resistance to these compounds. This primary resistance might be partially attributable to pharmacokinetic resistance driven by altered or reduced gut absorption. Therefore, investigating the intestinal absorption of EGFR TKIs and the mechanisms that can modify it may further improve clinical outcomes for EGFR- mutated lung cancer patients.

Abbreviations

TKIs: Tyrosine Kinase inhibitors; IV: Intravenous; ABC: ATP-Binding Cassette; P-gp: P-glycoprotein; BCRP: Breast Cancer Resistance Protein; EGFR: Epidermal Growth Factor Receptor; NSCLC: Non-Small Cell Lung Cancer

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020 Jan;70(1):7–30.
2. Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med. 2009 Sep 3;361(10):947–57.
3. Rosell R, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E, 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 Mar;13(3):239–46.
4. Schuler M, Wu YL, Hirsh V, O'Byrne K, Yamamoto N, Mok T, et al. First-Line Afatinib versus Chemotherapy in Patients with Non-Small Cell Lung Cancer and Common Epidermal Growth Factor Receptor Gene Mutations and Brain Metastases. J Thorac Oncol. 2016 Mar;11(3):380–90.
5. Douillard JY, Ostoros G, Cobo M, Ciuleanu T, McCormack R, Webster A, et al. First-line gefitinib in Caucasian EGFR mutation-positive NSCLC patients: a phase-IV, open-label, single-arm study. Br J Cancer. 2014 Jan 7;110(1):55–62.
6. Leonetti A, Sharma S, Minari R, Perego P, Giovannetti E, Tiseo M. Resistance mechanisms to osimertinib in EGFR-mutated non-small cell lung cancer. Br J Cancer. 2019 Oct;121(9):725–37.
7. Soria JC, Ohe Y, Vansteenkiste J, Reungwetwattana T, Chewaskulyong B, Lee KH, et al. Osimertinib in Untreated EGFR-Mutated Advanced Non-Small-Cell Lung Cancer. N Engl J Med. 2018 Jan 11;378(2):113–25.
8. Wu YL, Tsuboi M, He J, John T, Grohe C, Majem M, et al. Osimertinib in Resected EGFR-Mutated Non-Small-Cell Lung Cancer. N Engl J Med. 2020 Oct 29;383(18):1711–23.
9. Mok TS, Wu YL, Ahn MJ, Garassino MC, Kim HR, Ramalingam SS, et al. Osimertinib or platinum–pemetrexed in EGFR T790M–positive lung cancer. N Engl J Med. 2017 Feb 16;376(7):629–40.
10. Da Silva CG, Honeywell RJ, Dekker H, Peters GJ. Physicochemical properties of novel protein kinase inhibitors in relation to their substrate specificity for drug transporters. Expert Opin Drug Metab Toxicol. 2015 May;11(5):703–17.
11. Honeywell RJ, Kathmann I, Giovannetti E, Tibaldi C, Smit EF, Rovithi MN, et al. Epithelial Transfer of the Tyrosine Kinase Inhibitors Erlotinib, Gefitinib, Afatinib, Crizotinib, Sorafenib, Sunitinib, and Dasatinib: Implications for Clinical Resistance. Cancers (Basel). 2020 Nov 10;12(11):3322.
12. Laksitorini M, Prasasty VD, Kiptoo PK, Siahaan TJ. Pathways and progress in improving drug delivery through the intestinal mucosa and blood-brain barriers. Ther Deliv. 2014 Oct;5(10):1143–63.
13. Salama NN, Eddington ND, Fasano A. Tight junction modulation and its relationship to drug delivery. Adv Drug Deliv Rev. 2006 Apr 20;58(1):15–28.
14. Szakács G, Váradi A, Ozvegy-Laczka C, Sarkadi B. The role of ABC transporters in drug absorption, distribution, metabolism, excretion and toxicity (ADME-Tox). Drug Discov Today. 2008 May;13(9-10):379–93.
15. Cummins CL, Jacobsen W, Benet LZ. Unmasking the dynamic interplay between intestinal P-glycoprotein and CYP3A4. J Pharmacol Exp Ther. 2002 Mar;300(3):1036–45.
16. Duvvuri M, Krise JP. Intracellular drug sequestration events associated with the emergence of multidrug resistance: a mechanistic review. Front Biosci. 2005 May 1;10:1499–509.
17. J Honeywell R, Hitzerd S, Kathmann I, J Peters G. Transport of six tyrosine kinase inhibitors: active or passive?. ADMET and DMPK. 2016 Mar 31;4(1):23–34.
18. Honeywel RJ, Fatmawati C, Buddha M, Hitzerd S, Kathman I, Peters GJ. Adaptation of a human gut epithelial model in relation to the assessment of clinical pharmacokinetic parameters for selected tyrosine kinase inhibitors. ADMET and DMPK. 2015 Mar 31;3(1.):51–67.
19. Longuespée R, Kunz J, Fresnais M, Foerster KI, Burhenne J, Thomas M, et al. Therapeutic drug monitoring of osimertinib in non-small cell lung cancer and short bowel syndrome: A case report. Br J Clin Pharmacol. 2024 Jan;90(1):344–9.

20. Szakács G, Váradi A, Ozvegy-Laczka C, Sarkadi B. The role of ABC transporters in drug absorption, distribution, metabolism, excretion and toxicity (ADME-Tox). Drug Discov Today. 2008 May;13(9-10):379–93.

21. Vinarov Z, Abdallah M, Agundez JAG, Allegaert K, Basit AW, Braeckmans M, et al. Impact of gastrointestinal tract variability on oral drug absorption and pharmacokinetics: An UNGAP review. Eur J Pharm Sci. 2021 Jul 1;162:105812.

22. Ling J, Fettner S, Lum BL, Riek M, Rakhit A. Effect of food on the pharmacokinetics of erlotinib, an orally active epidermal growth factor receptor tyrosine-kinase inhibitor, in healthy individuals. Anticancer Drugs. 2008 Feb;19(2):209–16.

23. Sun D, Zhu Y, Zhu J, Tao J, Wei X, Wo Y, et al. Primary resistance to first-generation EGFR-TKIs induced by MDM2 amplification in NSCLC. Mol Med. 2020 Jul 1;26(1):66.

24. Du Y, Karatekin F, Wang WK, Hong W, Boopathy GTK. Cracking the EGFR code: Cancer biology, resistance mechanisms, and future therapeutic frontiers. Pharmacol Rev. 2025 Sep;77(5):100076.

25. Halley A, Leonetti A, Gregori A, Tiseo M, Deng DM, Giovannetti E, et al. The Role of the Microbiome in Cancer and Therapy Efficacy: Focus on Lung Cancer. Anticancer Res. 2020 Sep;40(9):4807–18.