



Synergistic effect of metformin and EGFR-TKI in the treatment of non-small cell lung cancer

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Abstract: In recent years, the incidence of lung cancer has been increasing, and lung cancer has become the leading cause of cancer-related death. Non-small cell lung cancer (NSCLC) accounts for 85% of all lung cancers. Platinum-containing chemotherapy is the first-line treatment for advanced patients. For patients with epidermal growth factor receptor (*EGFR*) mutation, EGFR-tyrosine kinase inhibitor (EGFR-TKI) is the best treatment choice. In studies, these patients have initially shown excellent response to EGFR-TKI treatment. However, the median progression-free survival (PFS) of NSCLC patients treated with EGFR-TKI is only 10–12 months, so the problem of drug resistance in treatment needs to be urgently solved. Clinical studies have shown that metformin and EGFR-TKI have synergistic effects in the treatment of NSCLC patients. Additionally, patients who are diagnosed with type 2 diabetes mellitus, with *EGFR* mutation have shown synergistic effects. This combination therapy can lead to longer PFS and overall survival (OS). This article reviews the synergistic effect of metformin and EGFR-TKI in the treatment of NSCLC.

Keywords: Epidermal growth factor receptor-tyrosine kinase inhibitor resistance (EGFR-TKI resistance); metformin; non-small cell lung cancer (NSCLC)

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Introduction

Lung cancer is one of the most common malignant tumors and the leading cause of cancer-related death (1). Non-small cell lung cancer (NSCLC) accounts for 85% of all lung cancers (2). For patients with advanced NSCLC, comprehensive treatment is the main treatment plan. Platinum-containing chemotherapy is the first-choice treatment plan for advanced patients. The discovery of epidermal growth factor receptor (*EGFR*) mutation and first-generation application of EGFR-tyrosine kinase inhibitor (TKI) has provided a new method for the treatment of NSCLC (3,4).

First-generation EGFR-TKI mainly includes gefitinib

and erlotinib. Its mechanism centers on preventing the activation of *EGFR* downstream signaling pathway through reversible competition for adenosine-triphosphate (ATP) binding sites, inducing the apoptosis of cancer cells. The IPASS study (5), NEJ002 study (6) and WJTOG3405 study (7) confirmed that progression-free survival (PFS) was significantly prolonged in patients with *EGFR* mutation after gefitinib treatment. These studies established the role of EGFR-TKI in first-line therapy for advanced NSCLC patients with *EGFR* mutation. However, after using the first-generation EGFR-TKI, drug resistance is unavoidable.

The second-generation of EGFR-TKI includes afatinib, dacomitinib, and other treatments. Afatinib is

characterized by a high selectivity and low molecular weight. It is an irreversible *ErbB* family inhibitor. It can inhibit the proliferation and metastasis of cancer cells by binding *ErbB* and blocking signaling pathway (8). In the LUX-Lung 3 study (9), the median PFS in the afatinib group was significantly longer than in the pemetrexed/cisplatin group (11.1 vs. 6.9 months). In the LUX-Lung 6 study (10), the PFS of patients with the exon 19 mutation or L858R mutation treated with afatinib were significantly longer than those treated with gemcitabine/cisplatin (11.0 vs. 5.6 months). Moreover, the afatinib group had a better objective remission rate (ORR) (66.9% vs. 23.0%) and disease control rate (92.6% vs. 76.2%). The LUX-Lung 7 study (11) compared the efficacy of afatinib and gefitinib as the first-line treatment for patients with *EGFR* mutation. The results showed that the median PFS in the afatinib group was slightly longer (11.0 vs. 10.9 months), and there was no significant difference in safety. Based on these studies, the Food and Drug Administration (FDA) approved afatinib as a first-line treatment for advanced NSCLC patients with the *EGFR* exon 19 deletion or the exon 21 mutation (L858R mutation) on July 12th, 2013. However, second-generation EGFR-TKI is expensive, and its binding with wild-type *EGFR* can cause related toxicity. These shortcomings hamper the clinical application of second-generation EGFR-TKI.

Osimertinib is an irreversible third-generation EGFR-TKI, which is sensitive to the *EGFR* mutation and *T790M* resistance mutation. The AURA 2 study (12) showed that median PFS was 9.9 months, ORR was 70%, and the 1-year overall survival (OS) rate was 81%. The AURA 3 study (13) showed that the osimertinib group had a longer PFS than the pemetrexed/cisplatin group (10.1 vs. 4.4 months), and the side effects were lower than the chemotherapy group. On November 13th, 2015, the FDA approved osimertinib for the treatment of NSCLC patients with the *T790M* mutation during or after EGFR-TKI treatment. In the FLAURA study (14), the median PFS was significantly prolonged in the osimertinib group (18.9 vs. 10.2 months), and the level 3 adverse events were less (34% vs. 45%) in the osimertinib group. This established the position of osimertinib as the first-line drug for patients with *EGFR* mutation. However, the mechanisms of resistance to third-generation EGFR-TKI began to appear, including *HER2* amplification, *KRAS* mutation, and *CMET* amplification.

Combination therapy is also emerging for new drug resistance mechanisms, for example, combination therapy

with EGFR-TKI and pathway inhibitors, including inhibitors of *IGF-1R*, *HER2*, *PI3K*, and *mTOR* (15-18). Compared with EGFR-TKI alone, these combination therapies can improve the therapeutic effect to some extent. However, these inhibitors also have their disadvantages, such as a higher cost and higher toxicity. The disadvantages limit the clinical application of these treatments. Therefore, in the course of clinical treatment, there is an urgent need for a cheap, low-toxicity, high-efficiency treatment for EGFR-TKI resistance, which will bring better curative effect to patients.

Metformin and lung cancer

Metformin is a kind of oral hypoglycemic drug, which has been proven to reduce fasting blood sugar. Since its advent in 1957, it has been used in clinic for more than 60 years (19). Metformin is the preferred oral hypoglycemic agent for patients with type 2 diabetes. It mainly inhibits hepatic gluconeogenesis, reduces hepatic glycogen production, and increases the utilization of glucose by skeletal muscles and fat cells, thereby reducing blood sugar, mainly by activating adenosine 5'-monophosphate-activated protein kinase (AMPK) signaling pathway (20-23).

After analyzing several studies from 2009 to 2013, it was concluded that patients with type 2 diabetes mellitus who use metformin have a lower risk for lung cancer (24). In recent years, experimental studies have shown that metformin can inhibit tumor cell proliferation and improve tumor sensitivity to chemotherapy drugs and small molecule targeted anticancer drugs (25). Therefore, the application of metformin can reduce the incidence of cancer and improve the prognosis of diabetic patients (26). Additionally, studies have further pointed out that in the treatment of type 2 diabetic NSCLC patients with *EGFR* mutations, a combination of metformin and EGFR-TKI has been shown to have a synergistic effect that delays the onset of resistance and results in longer PFS and OS (27) (PFS: 19.0 vs. 8.0 months, $P=0.005$; OS: 32.0 vs. 23.0 months, $P=0.002$) (Figure 1).

Metformin is associated with reversal of EGFR-TKI resistance

Metformin inhibits the IGF-1R pathway and makes drug-resistant cells re-sensitive to EGFR-TKI

Several studies have reported that the activation of the

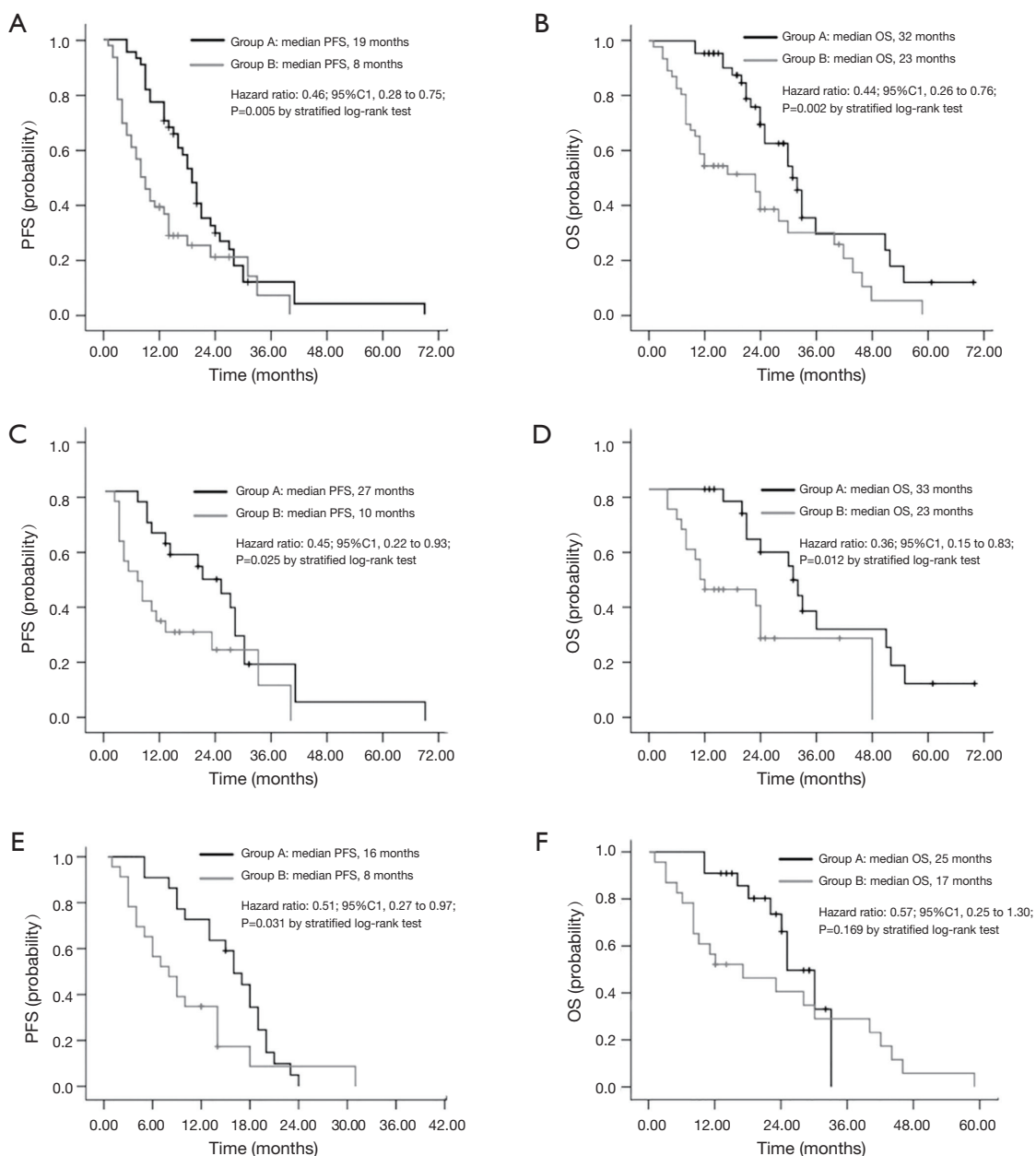


Figure 1 Kaplan-Meier estimates of (A) progression-free survival (PFS) as a whole; (B) overall survival (OS) as a whole; (C) PFS in a subgroup of patients treated with first-line EGFR-TKI; (D) OS in the subgroup of patients treated with first-line EGFR-TKI; (E) PFS in the subgroup of patients treated with second-line EGFR-TKI; (F) OS in the subgroup of patients treated with second-line EGFR-TKI. EGFR-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor.

IGF-1R pathway may lead to a resistance of EGFR-TKI (28-31). IGF-1R is a transmembrane tyrosine-protein kinase receptor expressed on the surface of many types of cells with potential mitogen action. After IGF-1R binds to ligand, phosphorylation of IGF-1R activates RAS/RAF/MAPK and

PI3K/AKT/mTOR pathways promote intracellular mitosis, induce cell proliferation and differentiation, while inhibiting apoptosis (32-34).

First-generation EGFR-TKI erlotinib increases the level of EGFR/IGF-1R heterodimers in NSCLC

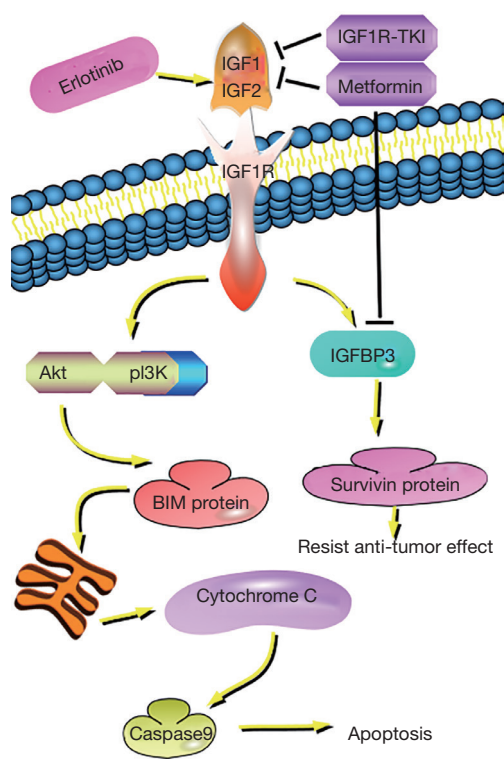


Figure 2 IGF-1R inhibitors and metformin inhibit the expression of IGFBP3 and also inhibit the PI3K/AKT pathway. Inactivation of the AKT signaling pathway leads to a significant increase in BIM protein, which promotes mitochondrial release of cytochrome C, whereas cytochrome c activates caspases, which mediate apoptosis through mitochondria.

cell membranes, activates IGF-1R and its downstream signaling medium IGFBP3 (28,30), stimulates surviving protein synthesis, and counteracts the anti-tumor effect of erlotinib (28). IGF-1R inhibitors can inhibit the expression of IGFBP3 (15,30). While IGF-1R also activates the PI3K/AKT pathway (35,36). The AKT signaling pathway plays an important role in various biological activities such as cell proliferation and apoptosis (33,34), while increasing the kinase activity of AKT and promoting cell transformation can make cells resistant to TKI (37). Inactivation of the AKT signaling pathway leads to a significant increase in BIM protein (38,39). BIM protein promotes mitochondrial release of cytochrome C, whereas cytochrome C activates caspases, which mediate apoptosis via mitochondria (40,41).

IGF-1R inhibitors, such as α -IR3, AG-1024, or R1507 interact with EGFR-TKI to enhance TKI-induced

cell growth inhibition and apoptosis (42). Moreover, knockdown of IGF-1R by siRNA can also enhance the sensitivity of EGFR-TKI resistant cells to EGFR-TKI (43). Studies have also shown that metformin can also inhibit IGF-1R signaling in cancer cells (44-46). In short, metformin can restore the sensitivity of EGFR-TKI-resistant cells to EGFR-TKI by inhibiting the IGF-1R pathway, and inhibit the expression of IGFBP3 (30), down-regulate AKT, and enhance BIM-mediated synergistic anti-tumor effects (43) (Figure 2).

Metformin inhibits IL-6 and TGF- β signaling pathways to reverse epithelial-mesenchymal transformation (EMT) and overcome TKI resistance

EMT refers to the biological process of epithelial cells transforming into mesenchymal phenotype cells through specific procedures, which is an important biological process for epithelial-derived malignant tumor cells to acquire migration and invasion ability. It is characterized by the loss of the polarity of epithelial cells and the loss of connection with the basement membrane, which enhances the motility of cancer cells and increases invasion, proliferation and metastasis (47,48). EMT and EGFR-TKI are closely related to lung cancer cell sensitivity (49,50). *In vitro* studies showed that the resistance of mesenchymal phenotype to EGFR-TKI was higher than that of the epithelial phenotype (51). AXL is a marker of EMT and is up-regulated in NSCLC patients with EGFR-TKI resistance, with AXL's activation being an important cause of EGFR-TKI resistance (52).

EGFR-TKI treatment causes IL-6 to activate IL-6R in an autocrine manner, thus causing IL-6R/JAK1/STAT3 signaling to activate (53). The IL-6R signaling pathway is more strongly activated in TKI-resistant cells compared to sensitive cells (54). Activation of signaling pathways by IL-6 is a key factor in the development of EMT in tumor cells (55). Also, studies have reported that TGF- β is an important driver of the EMT genetic program (48,56), and can induce activation of the IL-6 axis signaling pathway in lung cancer cells (48,57). Therefore, TGF- β and IL-6 are considered to be important targets for overcoming EGFR-TKI resistance in lung cancer cells (48,57-59).

Studies have shown that metformin can impair the TGF- β -induced mesenchymal state in a variety of pathological processes. It can hinder TGF- β -promoted EMT processes (56), reduce IL-6 secretion, thereby inhibiting signaling in the IL-6R/JAK1/STAT3 pathway (55,60), and

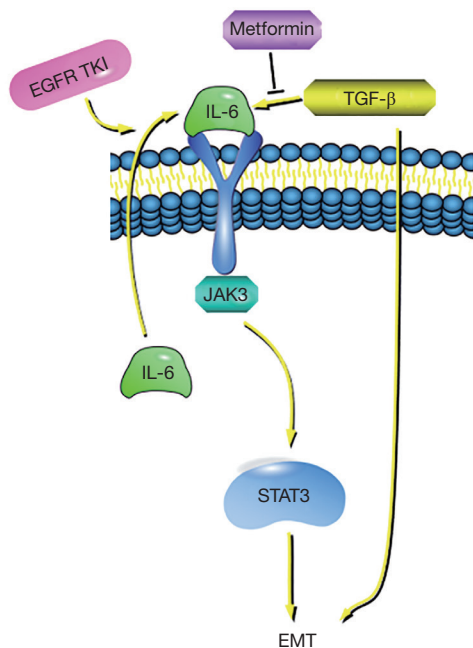


Figure 3 Treatment with EGFR TKI causes IL-6 to activate IL-6R in an autocrine manner, causing IL-6R/JAK1/STAT3 signaling, which is a key factor in EMT in tumor cells. An important driver of the TGF- β (EMT) genetic program, and can induce activation of the IL-6 axis signaling pathway in lung cancer cells. Metformin blocks the TGF- β -promoted EMT process, thereby reducing IL-6 secretion, inhibiting IL-6R/JAK1/STAT3 signaling, and restoring the sensitivity of drug-resistant cells to EGFR-TKI. EMT, epithelial-mesenchymal transformation; EGFR-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor.

restore the sensitivity of drug-resistant cells to EGFR-TKI. Also, some experiments have found that when IL-6 was added to EGFR-TKI drug-resistant cell lines pretreated with metformin, the effect of metformin disappeared. The cell lines resumed their resistance to EGFR-TKI, and re-activated the IL-6 signaling pathway (54). This suggests that metformin can overcome TKI resistance by reversing the EMT process and inhibiting the IL-6 signaling pathway, making cells re-sensitive to EGFR-TKI (61). Therefore, reversing the EMT process and preventing IL-6 signaling pathway transduction may be an effective way to improve the response of EGFR-TKI therapy (Figure 3).

Metformin and EGFR-TKI inhibit tumor cell growth through the LKB1-AMPK-mTOR pathway

Metformin and gefitinib have synergistic effects in LKB1 wild-type NSCLC cells and show significant anti-proliferative and pro-apoptotic activities, which are dependent on the LKB1 mutation status (61). Algire and colleagues reported that (62) cancer cells lacking LKB1 protein expression did not respond to metformin *in vitro*. LKB1 gene alterations are more often detected in NSCLC compared to small cell lung cancer, and the frequency of LKB1 genetic alterations is higher in cancer cell lines with *KRAS* mutations (63).

At the cellular level, metformin interferes with mitochondrial respiration and interferes with cellular energy metabolism, resulting in an increased intracellular ratio of AMP:ATP. This can lead to the activation of AMPK by LKB1 (64), a serine/threonine-protein kinase regulator of cellular metabolism (65). Some *in vitro* and *in vivo* studies have shown that AMPK activation inhibits mTOR signaling (66-70) and inhibits tumor cell proliferation (71-73). mTOR is an important target protein downstream of AMPK and is involved in the regulation of protein synthesis, cell cycle and apoptosis. When mTOR activity is decreased, phosphorylation of downstream S6K and 4E-BP leads to the inhibition of mRNA translation and the reduction of protein synthesis while playing an anti-tumor role (66-70,74). Thus, metformin and EGFR-TKI inhibit tumor cell growth via the LKB1/AMPK/mTOR pathway (Figure 4).

The use of metformin produces adverse reactions

Domestic reported that the incidence of gastrointestinal reactions of metformin was 15% (Grade I) (75). The symptoms of the reactions are a lack of appetite, nausea, vomiting, abdominal pain, diarrhea, etc., and the incidence rate is 20-30% (76). If a serious gastrointestinal reaction occurs after increasing the dose, the previous lower dose can be tried again, and the dosage subsequently increased according to patient tolerance (77). In addition, sustained release preparations may also be selected to reduce gastrointestinal symptoms in patients. Multiple studies (78-80) have shown that long-term use of metformin can cause a decrease in vitamin B12

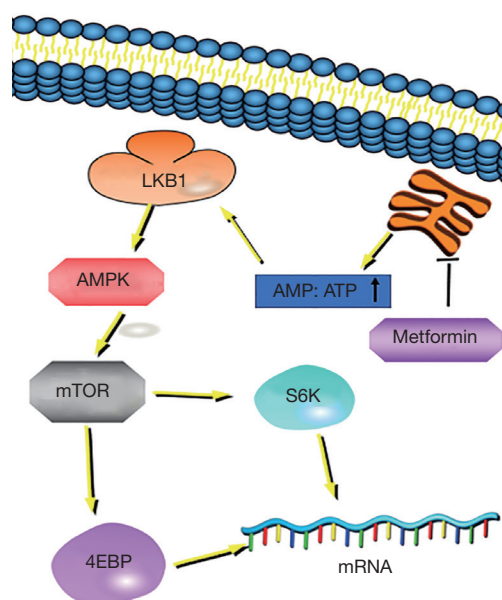


Figure 4 Metformin increases the intracellular proportion of AMP: ATP by destroying mitochondrial respiration, which leads to LKB1 activating AMPK and inhibiting mTOR signal transduction, phosphorylation of downstream S6K and 4E-BP, which results in blocked translation of the mRNA and reduced protein synthesis, thus playing an anti-tumor role.

levels. Therefore, it is recommended that if patients need long-term use of metformin, vitamin B12 should be appropriately supplemented during the treatment. Treatment of type 2 diabetes includes metformin alone, with little or no hypoglycemia, but a few special patients may have hypoglycemia (6.5%); the most serious and rare adverse reaction is lactic acidosis, which has a low incidence of about 3/100,000, but the mortality rate can be as high as 60% (81).

Conclusions

Drug resistance is a major problem in the *treatment of EGFR-mutant NSCLC* patients. The second-generation and third-generation EGFR-TKI drugs can bring about certain effects, but they will eventually become resistant. Moreover, combination therapy can have a certain therapeutic effect, but because of its high price and high toxicity, it has been limited in clinical application.

The discovery of the role of metformin in the treatment of lung cancer can provided new ideas in the treatment of EGFR-mutant lung cancer patients. It has been found that

metformin can inhibit the IGF-1R pathway and make drug-resistant cells re-sensitive to EGFR-TKI. It can inhibit IL-6 and TGF- β signaling pathways to reverse EMT, overcome TKI resistance, and can also be combined with EGFR-TKI. In application, using the LKB1/AMPK/TOR pathway to inhibit tumor cell growth can bring better PFS and OS to lung cancer patients with the *EGFR* mutation.

However, although metformin has a certain role in the treatment of EGFR-mutant NSCLC patients, we should also be wary of the adverse reactions caused by metformin, including gastrointestinal reactions, hypoglycemia, etc., the dosage of metformin should be carefully controlled, or another drug intervention should be applied.

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Footnote

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

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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