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Review Article

Pharmacogenomics of 5-Fluorouracil in Oesophageal Carcinoma; Association of Single Nucleotide Polymorphisms with Efficacy & Adverse Effects

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Abstract

Esophageal carcinoma (EC) is a highly fatal cancer. The usual therapy for localized squamous cell carcinoma has traditionally been surgically removing the tumor from its main location. However, recent clinical advancements have led to the development of new therapeutic options. The available choices encompass chemotherapy which involves continuous administration of 5-fluorouracil (5-FU), cisplatin, and simultaneous radiation treatment. To enhance clinical outcomes, future advancements would have to improve the integration of new anticancer medications, the administration of cisplatin, or 5-FU, guided by pharmacokinetics, and the identification of potential responders through genetic profiling of patients before treatment initiation. This review provides a comprehensive summary of the information about the genetic variation in *ABCB1*, *ERCC!*, *TYMS*, *XPD*, and *DYPD* genes that most likely contribute to the pharmacokinetic or genotype-guided administration of 5-FUfor the treatment of oesophageal cancer. The review aims to facilitate personalized treatment strategies for this condition in the future.

Keywords: Esophageal carcinoma, genetic polymorphism, 5-fluorouracil, adverse effects, efficacy

1. Introduction

Esophageal carcinoma (EC) ranked seventh in incidence and sixth in death among various cancers, whereas, death rates for newly diagnosed patients reach up to 50% (Sung et al. 2021, Arnold et al. 2015). Most patients exhibit symptoms in advanced stages, resulting in a significantly low overall survival rate of 20% at the five-year mark (Lagergren et al. 2017, Network 2017, Gao et al. 2014). Several individuals diagnosed with esophageal cancer exhibit complex or unresectable disease (Enzinger and Mayer 2003). The frequency of esophageal squamous cell carcinoma (ESCC) in men is two to three times higher than that in women. Notably, males dominate the esophageal adenocarcinoma (EAC) histological subgroup (Hongo, Nagasaki, and Shoji 2009). Unusual proliferation and infiltration in the squamous or glandular esophageal epithelium cause EC. Since they are often asymptomatic, esophageal cancers have a poor prognosis and are detected late, decreasing the possibility of cancer excision and cure. A considerable number of persons diagnosed with esophageal cancer have either distant metastases or develop a form of neoplasm that cannot be surgically removed (Enzinger and Mayer 2003). Furthermore, Pakistan's rising cancer rates have become a public health issue, necessitating government initiatives. Recent publications show that head and neck cancers account for

37.38% of the reported cases. Male incidence was 24.52% and female incidence was 13.24%. The consumption of tobacco, areca nut, chewable tobacco, snuff, niswar, and other substances influenced these patterns (Mazahir et al. 2006, Goldenberg et al. 2004, Rozi and Akhtar 2007).

Despite progress in understanding esophageal cancer risk factors, developing anti-cancer medicines has been challenging (Koag et al. 2014). Drug effectiveness and toxicity are impacted by genetics as evident in pharmacogenomics investigations. Several genetic studies show how certain genetic differences influence medication pharmacokinetics and pharmacodynamics.

Examining genetic factors and drug-induced phenotypes can enhance the effectiveness of anticancer treatments. This optimization is achieved through the comprehensive gathering of substantial data on genetic variations within a community. Moreover, cancer is a major cause of illness and mortality in affluent countries, and inadequate treatment can be lethal. Individualized oncology is likely to bode well for a cancer patient's response to therapy.

2. Pathophysiology

EC is classified as EAC or ESCC depending on epithelial cell growth. Globally, 84% of esophageal cancer cases are ESCC, and 15% are EAC (Bray et al. 2018, Arnold et al. 2020). In addition, there are small cell/neuroendocrine and infrequent basaloid esophageal tumors (Abnet, Arnold, and Wei 2018, Lagergren et al. 2017). ESCC develops in the upper and mid-esophagus from its squamous epithelial lining. Risk factors for developing ESCC include smoking, alcohol use, eating choices (such as hot drinks, red meat, and nutritional deficiencies), various carcinogens, and poor oral hygiene. Epithelial changes move from normal epithelium to basal cell hyperplasia, low-grade and then high-grade intra-epithelial neoplasia, and invasive carcinoma (Abnet, Arnold, and Wei 2018). EAC occurs in the lower third of the esophagus and is thought to initiate around glandular cells near the stomach (Huang and Yu 2018). Chronic gastroesophageal reflux disease also causes metaplastic changes in the esophageal epithelium. Moreover, Barrett's esophagus occurs when squamous cell epithelium becomes columnar, and low-grade dysplasia, high-grade dysplasia, and invasive cancer may follow (Smyth et al. 2017).

3. Staging of Esophageal Carcinoma

After diagnosis, it is essential to do staging to determine the appropriate course of therapy. The TNM categorization system is utilized for this purpose (Amin et al. 2017). Although tumor location does not alter staging, it does influence the treatment plan. Locations include the cervical, upper, middle, and lower esophagus depending on the tumor epicenter. Furthermore, upper-third tumors occur between the thoracic inlet and the azygos vein, middle esophageal tumors between the azygos and the inferior pulmonary vein, and lower esophageal carcinomas from the inferior pulmonary vein to the stomach, including the esophagogastric junction, and cancers above the thoracic inlet are classified as cervical cancers. Other staging tools include endoscopic ultrasonography (EUS), chest, abdomen, pelvic, and neck computed tomography (CT) images, and fluorodeoxyglucose-positron emission tomography (FDG-PET) or FDG-CT Rice, Patil, and Blackstone 2017). These staging modes reinforce each other and provide different information. The T classification for ESCC may be determined with 79% accuracy using EUS (Luo et al. 2016). The ability to distinguish between T1a (invasion of the lamina propria and muscularis mucosa) and T1b (invasion of the submucosa) using EUS may not be universally feasible. To obtain accurate T classification and

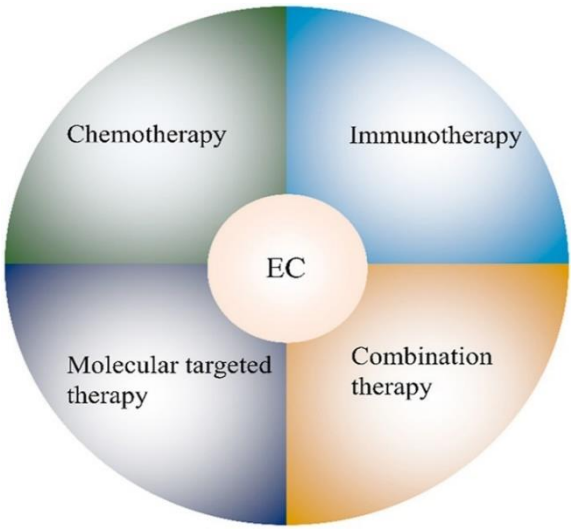

Figure 1: Various approaches for the treatment of EC.

exclude penetration into the muscularis propria, it may be essential to perform either endoscopic mucosal resection (EMR) or endoscopic submucosal dissection (ESD)(Rice, Patil, and Blackstone 2017). It is recommended that endoscopic resection be taken into consideration for the precise evaluation of the depth of invasion in nodular lesions that are equal to or smaller than 2cm (Waters and Reznik 2022). The determination of clinical nodal classification (N) involves the use of EUS, with or without fine-needle aspiration (FNA), as well as CT, and FDG-PET scanning. The sensitivity of EUS in the absence of biopsy might be as low as 20%, whereas its diagnostic accuracy can vary between 64% and 71% (Luo et al. 2016, Kutup et al. 2007). EUS-guided FNA has been shown to enhance diagnostic accuracy and so should be promoted (Cummings et al. 2021). Moreover, imaging techniques are commonly employed for the identification of distant metastasis. The utilization of FDG-PET/CT has been found to enhance overall accuracy when compared to the use of CT alone (Rice, Patil, and Blackstone 2017). It is advisable to do a confirmatory tissue biopsy before classifying a patient as M1 (Amin et al. 2017). However, some conditions may benefit from adjunctive staging. Patients with upper and middle-third cancers should have routine bronchoscopy with biopsyThe utilization of endobronchial ultrasound (EBUS) and narrow-band imaging allows for the detection of occult invasion. The management of lower-third cancers has demonstrated success through laparoscopy, encompassing both biopsy and peritoneal cytology. This method is widely employed in medical institutions (Convie et al. 2015). The inclusion of a repeat staging laparoscopy following induction therapy should be taken into consideration, either as an independent surgery or as an initial step in a planned resection (Cardona et al. 2013).

4. Treatment Regimens

Chemotherapy is the primary treatment approach for treating EC since it has shown substantial effectiveness in suppressing tumor development and decreasing the probability of distant metastasis. Cisplatin, 5-FU, and doxorubicin (Dox) are commonly used chemotherapy drugs for treating EC.

4.1. 5-Fluorouracil

Patients diagnosed with ESCC were treated initially with 5-FU as the primary treatment

method. However, when the dosage increases, there is a simultaneous increase in toxicity, drug resistance, and side effects. Hence, 5-FU monotherapy for the treatment of ESCC is insufficient. At the moment, combinations of 5-FU are utilized to improve its therapeutic efficacy and reduce its adverse effects, as indicated in the table below (He et al. 2021).

4.2. Doxorubicin

Dox, a common chemotherapy treatment, produces reactive oxygen species, which damage the DNA. In EC treatment, Dox may induce significant adverse effects. However, some studies indicate that mixing orange peel extract, and naringin reduces Dox's deleterious effects in EC stem cell-derived xenograft mice (Tajaldini et al. 2020).

4.3. Paclitaxel and Docetaxel

Radiochemotherapy, which involves the administration of paclitaxel, is an additional treatment option for the advanced EC. The simultaneous use of carbon ion beam irradiation and docetaxel has a synergistic effect against EC, indicating a potentially beneficial treatment strategy for patients with locally advanced ESCC (Kitabayashi et al. 2006).

4.4. Combination Therapy

Combination therapy is likely to be the most effective way to treat ESCC while minimizing side effects and drug resistance. The combination of lapatinib and paclitaxel has a synergistic impact that inhibits MAPK and AKT downstream signaling molecules and significantly reduces phosphorylated epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor 2 (HER2) activation. As a result, the combination therapy reduces cell proliferation, stops cells from migrating and invading, and enhances apoptosis (Guo et al. 2018).

4.5. Molecular Targeted Therapy

Selective inhibition of EGFR, HER2, and vascular endothelial growth factor (VEGF) is the main molecular targeted treatment for EC. Genetic alterations in these important signaling pathways, including the insulin-like growth factor 1 receptor (IGF1R), HER2, EGFR, and UFO (AXL) are frequent in EC. Linsitinib, cetuximab, and R428 can specifically inhibit IGF1R-, EGFR-, and AXL-related signaling cascades. Modulation of these signaling pathways affects neoplastic cell proliferation, survival, migration, invasion, and differentiation, slowing tumor growth (He et al. 2021).

4.5.1. Drugs Targeting EGFR

The EGFR, commonly referred to as erythroblastic leukemia viral oncogene homolog 1 (ERBB-1), is a constituent of the ERBB receptor tyrosine kinase family. It is a transmembrane protein receptor, encompassing an extracellular domain responsible for ligand binding, a transmembrane domain, and an intracellular domain with kinase activity (Ciardiello and Tortora 2003). The EGFR dimerizes its extracellular domain after interacting with its ligand (EGF). The intracellular domain dimerizes, activating the kinase domain via self-phosphorylation. This activation modulates downstream molecules, regulating cell proliferation, differentiation, and invasion. Most esophageal cancers overexpress EGFR, more often in ESCC than EAC (Hanawa et al. 2006, Kawaguchi et al. 2007). Drugs targeting EGFR are divided into two groups, the first group includes monoclonal antibodies like cetuximab and nimotuzumab that specifically bind to the extracellular region of EGFR. By doing so, they prevent ligand binding and receptor activation. The second group includes small molecule EGFR intracellular tyrosine kinase inhibitors (TKIs) like gefitinib and afatinib. Trastuzumab, a recombinant humanized monoclonal antibody that targets the extracellular domain of HER2, treats advanced EC (Sanchez-Vega et al. 2019, He et al. 2021).

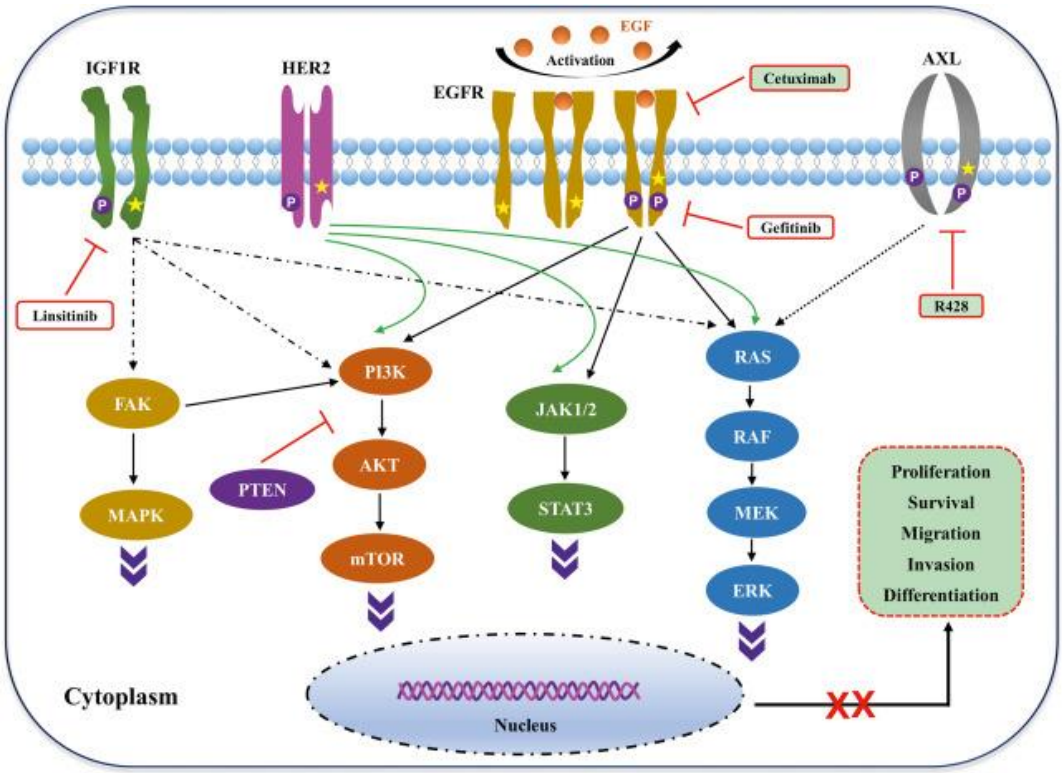

Figure 2: The figure illustrates a schematic diagram depicting the molecular targeted therapy approach for the treatment of EC. (He et al. 2021).

4.5.2. Drugs Targeting Other Molecules

Epithelial–mesenchymal transition is aided by *AXL* overexpression during tumor development (Antony et al. 2016). In EAC, excessive *AXL* expression promotes the AKT/β-catenin pathway, leading to upregulation of *c-MYC*, which results in epirubicin resistance. Suppressing *c-MYC* expression restores epirubicin sensitivity in *AXL*-dependent drug-resistant cells. Notably, combining epirubicin with the *AXL* inhibitor, R428, inhibited cell proliferation and tumor development (Hong, Maacha, and Belkhiri 2018). Hepatocyte growth factor (HGF) activates transmembrane receptor tyrosine kinase cellular-mesenchymal epithelial transition factor (c-MET), which participates in carcinogenesis by promoting cell proliferation, apoptosis, and angiogenesis. HGF/MET signaling pathway components such as MET, cyclin D1, and CDK4 are inappropriately expressed in ESCC cell lines and tissues (Jiang et al. 2016). R428 and carbotinib, AXL/c-MET specific inhibitors, reduced ESCC cell and xenograft tumor growth (Yang et al. 2019). Therapeutic targets include the mammalian target of rapamycin (mTOR), a phosphoinositide 3-kinase (PI3K) protein kinase, and polo-like kinase 1 (plk1), a serine/threonine protein kinase. Small interfering RNA (siRNA) or PLK1 inhibitors reduce mTOR activity in ESCC. Particularly, rapamycin and the PLK1 inhibitor, BI 2536, synergistically disrupt the mTOR complex (mTORC1/mTORC2) cascade and

Table 1: 5-FU Combination Therapies.

Combination	Type of Carcinoma	Effect produced	Studies Conducted	References
β-Carotene and 5-FU	ESCC	Induction of apoptosis, downregulation of BCL-2 and PCNA, up-regulation of BAX and caspase-3. Decreases the protein levels of CaV-1, p-AKT, p-NF-κB, p-mTOR, and p-P70S6K in ECA109 cells.	*In vitro* and *in vivo*	(Zhang et al. 2016)
CA3 and 5-FU	EAC	The transcriptional pathway of YAP/TEAD is impeded by the presence of CA3. The concurrent administration of a combination treatment regimen leads to a notable reduction in the levels of YAP1, SOX9, and Ki67 expression in mouse models.	*In vitro* and *in vivo*	(Song et al. 2018)
BAY1143572 and 5-FU	EAC	The concurrent administration of these therapies results in a decrease in the expression of MCL-1.	*In vitro* and *in vivo*	(Tong et al. 2019)
Hesperetin and 5-FU	ESCC	The combination therapy demonstrates efficacy in inducing cellular death, resulting in the downregulation of BCL-2 and the up-regulation of BAX, cleaved caspase-3, and cleaved caspase-9.	*In vitro* and *in vivo*	(Wu et al. 2018)
Puerarin and 5-FU	ESCC	The concurrent use of these substances greatly hampers the process of cell proliferation and triggers programmed cell death.	*In vitro* and *in vivo*	(Wang et al. 2014)
ABT-263 and 5-FU	ESCC	The concurrent administration of multiple therapies exhibits a synergistic effect by enhancing programmed cell death and suppressing the expression of genes associated with stem cell properties.	*In vitro* and *in vivo*	(Chen et al. 2015)

activate S6 and 4E-BP1 (Liu et al. 2018) to produce higher antitumor effects. Another study found that inhibiting only mTORC2 strongly inhibited EC cell growth (Lu et al. 2020). The PI3K/AKT/mTOR/PKM2 signaling pathway was also inhibited by metformin, a type 2 diabetes medication. In ECA109 xenografts, metformin dramatically suppressed tumor growth, thus, opening the possibility of treating EC with conventional medications, which are meant to treat other disorders. CD147 exhibits high expression in EC tissues, as demonstrated previously (Li et al. 2017). Another drug, Matuzumab activates effector cells and hinders CD147, thereby mitigating the progression of EC both in vitro and in vivo (Wang et al. 2019).

4.6. Immunotherapy

Antigen-presenting cells, particularly dendritic cells, can recognize inflammation caused by tumor cell surface antigens. These antigen-presenting cells then deliver the antigens to T or B lymphocytes, triggering an adaptive immune response. However, tumor cells have developed several evasion strategies against immune responses. The main treatments for EC are immune checkpoint inhibitors and tumor vaccinations (Zhao, Yu, and Meng 2019). Typically utilized pharmaceutical agents include pembrolizumab, nivolumab, toripalimab, and camrelizumab (Hong and Ding 2019).

5. Genetic Variations and the Efficacy & Adverse Effects of 5-FU

EC remains a devastating malignancy with a high mortality rate. While advancements in treatment modalities have been made, chemotherapy remains a cornerstone of therapy. However, its success is often hampered by variable drug responses and severe adverse

Table 2: Summary of Pharmacogenetic Studies Highlighting the Polymorphisms Associated with Efficacy and Adverse Effects of 5-FU treatment

Gene	SNPs	Sample Size	Population	Disease	Effect/association	Reference(s)
ABCC2	rs12762549	158	Japanese	EC	Neutropenia	(Nomura et al. 2020)
ABCB1	rs1045642	158	Japanese	EC	Neutropenia	(Nomura et al. 2020)
ABCC2	rs717620	239	Japanese	EC	Hematological Toxicity	(Fujita et al. 2022)
ERCC1	rs3212986	143	Italian	EC	Positive predictive marker	(Rumiato et al. 2013)
SLC23A2	rs2681116	49	Japanese	EC	Predict clinical response	(Minegaki et al. 2014)
	rs13037458	49	Japanese	EC	Predict long-term survival	(Minegaki et al. 2014)
	rs4987219	49	Japanese	EC	Severe Acute Leukopenia	(Minegaki et al. 2014)
	rs1110277	49	Japanese	EC	Stomatitis	(Minegaki et al. 2014)
DPYD	rs2297595	128	Caucasian	EC	Adverse effects	(Gross et al. 2008)
TYMS	rs151264360	57	Japanese	EC	Hyponatremia	(Arakawa et al. 2018)
XPD	rs13181	57	Japanese	EC	Hyponatremia	(Arakawa et al. 2018)
DPYD	rs3918290	227	Caucasians	EC	Life-threatening adverse effects	(Saif 2013)
	rs67376798	227	Caucasians	EC	Life-threatening adverse effects	(Saif 2013)
DPYD	rs115232898	Case report	African American	CC	Life-threatening adverse effects	(Saif et al. 2014)
DPYD	rs3918290, rs115232898	23	Caucasians, African Americans, South-Asians	EC	Mucositis, cytopenia, hypotension, respiratory distress,	(Saif et al. 2007)
DPYD	rs3918290	2038	Netherlands	EC	Fatal adverse effects	(Deenen et al. 2016)

effects. Increasingly, research is focusing on the role of genetic factors in influencing these outcomes.

This review highlights the potential of pharmacogenetics in optimizing 5-FU therapy for EC. 5-FU is a widely employed chemotherapeutic agent, but individual responses and toxicities can vary significantly. Studies have identified several genetic polymorphisms that influence 5-FU metabolism and impact its efficacy and safety. For example, *ABCB1* and *ABCC2* have been linked to severe neutropenia following 5-FU and cisplatin treatment. Similarly, *ERCC1* rs3212986 polymorphism may be a prognostic marker for neoadjuvant cisplatin/5-FU therapy. Additionally, single nucleotide polymorphisms (SNPs) in *SLC23A2*, *DPYD, TYMS, and XPD* have also been associated with clinical

outcomes, adverse effects, and risk of severe toxicity, respectively (Table 2). These findings suggest that personalized medicine approaches, incorporating pharmacogenetic testing, can significantly improve EC treatment. Tailoring 5-FU dosage based on individual genetic profiles can optimize efficacy while minimizing the risk of life-threatening adverse effects. This can lead to improved patient outcomes and quality of life. Several other studies have demonstrated the potential of pharmacogenetic testing in guiding 5-FU therapy. For instance, (Minegaki et al. 2014) identified *SLC23A2* SNPs as potential predictors of clinical response and long-term survival, while (Gross et al. 2008) found that a common *DPYD* polymorphism strongly impacts fluoropyrimidine-related side effects. In addition, (Saif et al. 2014) studies highlighted the importance of *DPYD* testing in identifying individuals at risk of severe toxicity and advocated for alternative therapy options or dose modifications for carriers of this variant. Furthermore, (Deenen et al. 2016) demonstrated the cost-effectiveness of upfront *DPYD**2A* testing, emphasizing its potential for optimizing healthcare resource allocation. These studies collectively support the integration of pharmacogenetics into clinical practice to personalize 5-FU therapy and improve EC treatment outcomes.

Despite the encouraging discoveries, there are constraints. A significant number of studies are conducted on a small scale and need further confirmation through analysis of more expansive datasets. Moreover, the practical use of pharmacogenetic testing necessitates the use of standardized techniques and standards. Subsequent investigations should prioritize the validation of current discoveries, investigate other genetic variations, and formulate all-encompassing clinical protocols for the integration of pharmacogenetics into regular medical practice.

6. Conclusions and Research Gap

We found that convincing data supporting the significance of pharmacogenetics in enhancing the effectiveness of 5-FU treatment for EC is available. By integrating genetic testing into clinical practice, we may customize treatment methods, enhance patient results, and eventually alleviate the impact of this debilitating illness. Although several polymorphisms have been recognized as possible indicators of both responsiveness and toxicity, there is still a scarcity of data for many variations, especially in ethnically varied groups. Validation of current findings and discovery of further genetic markers pertinent to 5-FU therapy in esophageal cancer necessitates the implementation of extensive, meticulously planned research projects. Further investigation is required to establish evidence-based recommendations for integrating genetic data into treatment planning and dose modifications. It is crucial that different ethnic and social groups are provided with fair and equal access to pharmacogenetic testing to decrease discrepancies in healthcare results. Research is required to overcome obstacles to entry and formulate tactics for adopting pharmacogenetic testing in underprivileged populations.

Conflict of Interest

The authors declare that they have no competing interests.

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Study Approval

Not Applicable.

Consent Forms

Not Applicable.

Authors Contributions

AA conceptualized the study, SIR, AA, and ZB performed the literature search, SFS and AA analyzed the studies and wrote the initial manuscript. All authors contributed to the final manuscript and helped in revisions.

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