Chapter

Breast Cancer Drug Repurposing a Tool for a Challenging Disease

Jonaid Ahmad Malik, Rafia Jan, Sakeel Ahmed and Sirajudheen Anwar

Abstract

Drug repurposing is one of the best strategy for drug discovery. There are several examples where drug repurposing has revolutionized the drug development process, such as metformin developed for diabetes and is now employed in polycystic ovarian syndrome. Drug repurposing against breast cancer is currently a hot topic to look upon. With the continued rise in breast cancer cases, there is a dire need for new therapies that can tackle it in a better way. There is a rise of resistance to current therapies, so drug repurposing might produce some lead candidates that may be promising to treat breast cancer. We will highlight the breast cancer molecular targets, currently available drugs, problems with current therapy, and some examples that might be promising to treat it.

Keywords: drug repurposing, breast cancer, mechanism, non-oncology drugs, resistance

1. Introduction

Drug discovery is a multifaceted process that aims at identifying a therapeutic agent that can be useful in treating and managing certain medical conditions. This process includes identification of candidates, characterization, validation, optimization, screening, and assays for therapeutic effectiveness. If a molecule achieves acceptable results in these studies, then the molecule has to go through drug development processes and be recruited to clinical trials [1]. Several drug candidates (about 90%) have collapsed in early clinical trials due to unexpected results such as adverse effects or inadequate effectiveness [2, 3]. Drug development is probably among the most complicated and challenging processes in biomedical research. Apart from the already enormous complexities underlying pharmacological drug designs, additional significant challenges arise from clinical, regulatory, intellectual property, and commercial constraints. Such as challenging atmosphere has made the drug development process very sluggish and unpredictable [4]. The process of discovering and developing a new drug is a lengthy and expensive process taking somewhere from 10 to 15 years and costs about US$2–3 billion [1]. Despite massive sums of money being spent on drug development, no substantial rise in the new therapeutic drug agents in a clinical setting has been observed over several decades. Although overall global R&D spending for drug discovery has risen 10-fold from 1975 (the US $4 billion) to 2009 ($40 billion), the number of novel molecular entities (NMEs) approved has stayed essentially constant since 1975 (26 new drugs approved in 1976 and 27 new drugs approved in 2013) [5].

The essential step in discovering new drugs involves the evaluation of the safety and effectiveness of new drug candidates in human subjects, and it consists of four phases. In Phase I clinical trial, the candidate drug's safety is assessed in a small population (20–80 individuals) to establish safe dose range and uncover adverse effects. Phase II involves the examination of intervention for its effectiveness and safety in large populations (a few hundred people). Phase III further involves the assessment of drug efficacy in a large population (several thousand) and compares new drug candidates with standard or experimental treatments. Phase IV is conducted when the intervention is marketed. This study aims to track how well the approved treatment is performing in the general population and gather data on side effects that may arise from broad usage over time. Phase III studies determine whether or not a medication is effective, and if so, FDA clearance is granted. The FDA approves one anticancer treatment out of every 5000–10,000 applicants, and just 5% of oncology medicines entering Phase I clinical trials are approved in the end. Because of the increased cost and time frame for new medication development in recent years, patients with severe illness may die until alternative therapies are available if they develop resistance to current therapy [6]. In searching for an alternative treatment option for managing various diseases, including cancer, the researchers have shifted their focus to drug repurposing strategies.

The drug purposing or drug reprofiling or drug redesigning process explores the therapeutic use of existing clinically approved, off patent drugs with known targets for another indication to minimize the cost of therapy, time, and risk [7]. The huge benefit of drug repurposing is that the efficacy, pharmacokinetics, pharmacodynamics, and toxicity characteristics have previously been explored in preclinical and Phase I investigation. These drug moieties may thus be quickly made to proceed to Phase II and Phase III clinical trials, and hence related developmental costs might be substantially lowered [6, 8]. The failure risk in drug development is low because *in vitro* screening, *in vivo* screening, toxicity profile, chemical optimization, and formulation development have already been accomplished. Therefore, drug repurposing has made the pharmaceutical industry a desirable choice for investors. So the pharmaceutical companies and researchers have begun to make significant investments in drug repurposing, which offers a tremendous benefit over *de novo* drug design and development [9]. Therefore this new approach of drug purposing has reduced the timeline and cost of the drug development, notably in the case of FDA-approved repurposed pharmaceuticals, which will undergo faster clinical

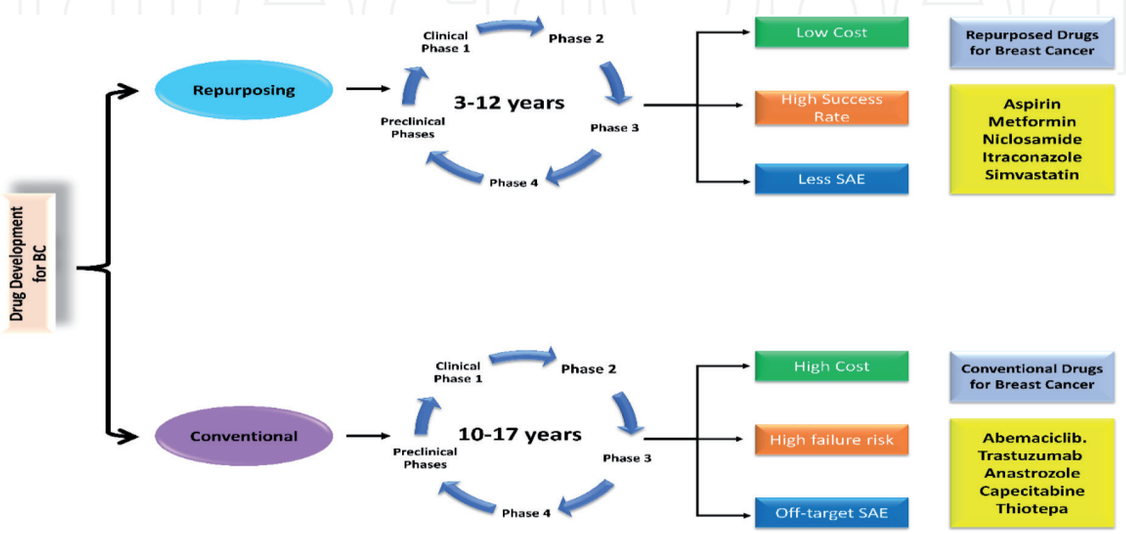

Figure 1.
Outline of developing new drug versus repurposing.

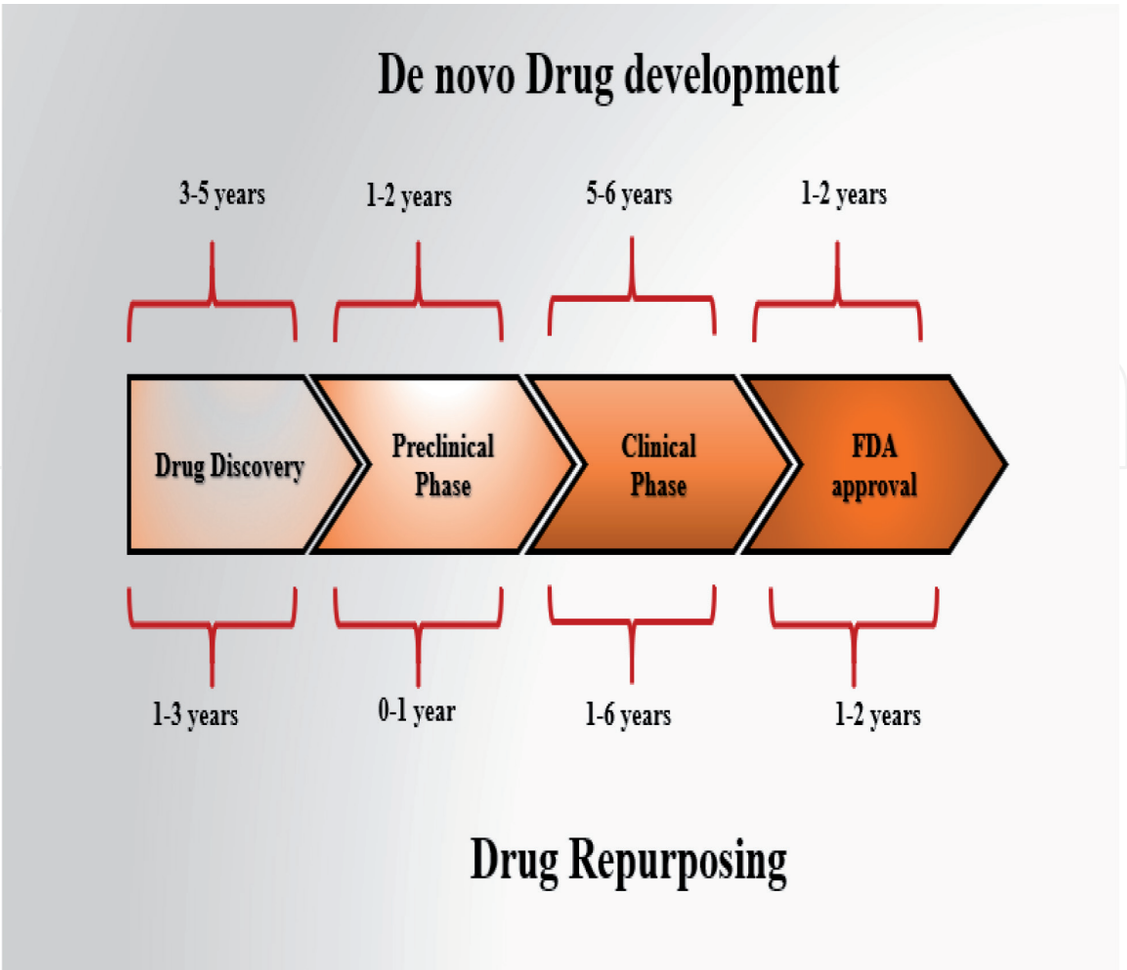

Figure 2.
The time taken by the conventional of process of drug development with respect to drug repurposing. Conventional drug development process takes around 5 years and the same can be minimized to 5 years.

trials because of already well-known safety and toxicological profile [10]. Outline of developing new drug verses repurposing is represented in **Figure 1**.

The development of new drugs for breast cancer like any other cancer is a multistep process that includes drug designing, synthesis, characterization, safety and efficacy assessment, and finally, regulatory approval (**Figure 2**). The overall process is very lengthy and involves significant financial expenditure [11]. Further, the sky-high cost of the therapies and associated side effects make it desirable to look for other approaches to manage cancer effectively. Therefore, concurrently with the synthesis and design of new therapeutic modalities, various strategies should be considered for repurposing various already approved drugs that may target this deadly disease.

2. Non-oncology drugs repurposed for breast cancer (preclinical data)

2.1 Aspirin

Aspirin was originally discovered in 1897 and was first commercialized as an analgesic. It has been utilized as an anti-inflammatory medication and for managing arterial and venous thrombosis [12]. Recent research has sparked interest in the usage of Aspirin for the prevention of various cancers. There are compelling evidences authenticating that regular use of low doses of aspirin results in a significant reduction in the occurrences and mortality of various cancers [13–17]. The

possibility that Aspirin has an anticancer benefit has received considerable interest nowadays, with a lot of research being done to figure out how successful it is in the prevention of colorectal cancer [18], lung cancer [19], gastric cancer [20], prostate cancer [21], and many other cancers including breast cancer. Because of the effect of Aspirin in several biological processes such as inhibitory effect on angiogenesis [22], cancer cell metastasis [23], causing cell apoptosis [24], etc., it is reasonable to predict that Aspirin will be beneficial when employed as an additional alternative treatment option for cancer patients. Aspirin directly inhibits the activity of the enzyme cyclooxygenase (COX-2) and thereby impedes the synthesis of prostaglandin E2 (PGE-2), which leads to cancer cell death [25]. Recent research also suggests that Aspirin may mediate anticancer potential through COX-independent pathways such as inhibition of NFκB [26], downregulation of survivin [20], targeting AMPK-mTOR signaling [27], Wnt signaling cascade [28], etc.

A study was conducted by Dai et al. reported that Aspirin possesses antiangiogenic and anti-metastatic potential in MDA MB 23 cell line by directly binding to the enzyme heparinase. The results were further confirmed *in vivo* experimentation [23]. Heparinase is an endo-β-D glucuronidase that is specific to heparin sulfate. It dissolves heparin sulfate chains of proteoglycans on the cell surface and extracellular matrix (ECM) that consequently contributes to the degradation of the extracellular matrix that further assists tumor invasion and metastasis [29]. Further, heparin also facilitated the release of angiogenic factor, vascular endothelial growth factor (VEGF) blocked by aspirin-mediated heparin inhibition [23]. Breast cancer cell lines (MDA MB 231 and MCF-7) showed a dose-dependent inhibitory effect on growth after treatment with Aspirin. The Aspirin further restricts the migration of these cells by preventing epithelial to mesenchymal transition through suppression of various mesenchymal markers such as vimentin and increasing expression of various epithelial markers such as Keratin-19 and E-cadherin.

Further inhibitory effect of TGF-β/SMAD4 signaling, as evident from decreasing the production of SMAD proteins, also contributes to the anti-metastatic potential of Aspirin [30]. In another study, Choi et al. demonstrated the effect of Aspirin in the MCF-7 cell line. It was observed that Aspirin alters the complex formation between Bcl-2 and FKBP38 and leads to the nuclear translocation of Bcl-2 and phosphorylation that causes its activation, contributing to its inhibitory effect on MCF-7 cell proliferation and also triggers apoptosis in cell lines [31]. In combination with exemestane, Aspirin showed synergy in inhibition of cell proliferation. Significant arrest in the G0/G1 phase was observed along with a more detrimental effect on COX-1 and Bcl-2 expression than individual therapy [32]. In addition, when combined with tamoxifen (which is used as a drug of choice for the estrogen receptor positive BC), it downregulates the level of cyclinD1. Subsequently, it arrests the cell cycle in phase G0/G1. In the same study, authors also reported that Aspirin inhibits the ER + ve BC cells growth and overcomes the resistance to tamoxifen in MCF-7/TAM cell line. Study demonstrated a new way to treat ER + ve BC in combination therapy of Aspirin and tamoxifen [33].

2.2 Metformin

Metformin (1,1-dimethyl biguanide hydrochloride) is a well-recognized biguanide derivative and has a long history of usage in managing type 2 diabetes (T2D). Because of the outstanding ability to lower plasma glucose levels, metformin has become the primary drug for managing T2D [34]. The drug was firstly approved in 1958 in the United Kingdom, and this decade-old drug is in the WHO's list of essential medicines [35]. Metformin belongs to the category of successful repurposed drugs and advanced into the clinical trials Phase 3/4 for its use in the

prostate, oral, breast, pancreatic, and endometrial cancers [6]. Various preclinical and clinical examinations have demonstrated the effectiveness of metformin in the treatment of various malignancies such as pancreatic cancer [36], gastric cancer [37], blood cancer [38], etc. A meta-analysis study on diabetic patients with breast cancer concluded that patients who were treated with metformin and neoadjuvant therapy had a higher pathological complete response rate (24%) compared with patients not undergoing metformin treatment (8%) [39]. Another meta-analysis study demonstrated 65% survival improvement when compared with control [40]. Metformin has increased the survival opportunity in type 2 diabetic patients suffering from invasive breast cancer [41]. Study also suggested that patients on metformin demonstrate improved in the survival and response to treatment [40]. The metformin uptake is mediated by the OCT1 in BC cells [42], which is reported to play important role in the BC cells as an anticancer activity [43]. Upon entry into the cells, it leads to increase apoptosis, anti-proliferative, anti-angiogenic, which seems to be mediated by the mTOR, Akt/MAPK pathway [44]. Study conducted by Shi et al., established that metformin can also inhibit the expression of the COX-2, suggested the potential of metformin in combination with others COX-2 inhibitor [45]. Low cost and stability of metformin make it a good candidate for the treatment of cancers when compared with available treatment options [46].

2.3 Itraconazole (ITC)

Itraconazole, a triazole antifungal drug, is a well-tolerable agent that is extremely effective against a wide range of fungal infections. Itraconazole is a highly potent and effective antifungal agent due to its active metabolite, hydroxyitraconazole, which also has significant antifungal action [47]. Itraconazole blocks ergosterol synthesis in the fungal cell membrane by inhibiting the enzyme 14α-demethylase and suppressing their growth [48]. It has emerged as a potent anticancer agent because of its ability to overcome chemoresistance prompted by P glycoproteins, altering various signaling pathways such as hedgehog (Hh) signaling cascade, Wnt/β-catenin pathway in cancer cells, and also preventing angiogenesis and lymphangiogenesis [49]. Itraconazole has been shown to have the ability to eliminate cancer cells by disrupting Hh signaling [50]. In invertebrates, the Hh signaling cascade is responsible for the regulation of complicated developmental processes. However, aberrant activation of this pathway plays a crucial role in carcinogenesis and cancer maintenance and contributes to chemoresistance, thus, targeting this pathway offers the potential therapeutic possibility [51]. Itraconazole was able to exhibit cytotoxicity in breast cancer cell lines by influencing mitochondrial membrane potential through induction of apoptosis, decreasing expression of Bcl-2, and enhancing the caspase activity. Itraconazole also promoted autophagic cell death via elevation of LC3-II expression, degradation of P62/SQSTM1, formation of autophagosomes. Hedgehog signaling is an important regulator of apoptosis and autophagy. Hence, inhibition of this signaling by Itraconazole results in cytotoxicity, tumor shrinkage, apoptosis, and autophagy in breast cancer both in *in vitro* and *in vivo* investigations [50, 52]. Anticancer activity is also reported in esophageal cancer, mediated by downregulating the HERK/AKT pathway [53]. A pilot study with 13 participants demonstrated that increased levels of Itraconazole in plasma were associated with the increased level of thrombospondin-1, angiogenesis inhibitor.

Additionally, the level of other growth factors such as fibroblast growth factor (FGF) and placenta-derived growth factor also decreased without any direct association with the Itraconazole [54]. When administered in combination with other cytotoxic agents, Itraconazole increased the response rate [55]. Researchers

are trying various ways to enhance the anti-neoplastic activity of itraconazole. One such example is the development of the modified lipid nanoparticles having Miltefosine (subtherapeutic dose), called M-ITC-LNC (Membrane additive itraconazole with lipid nanoparticles (Miltefosine). The results from the cytotoxicity studies demonstrated that the anticancer activity and selectivity significantly increased in MCF-7 BC cells compared with the ITC-solution and ITC-LNC without modification [56]. In another study, itraconazole was co-delivered with the doxorubicin by liposome (coated with the Pluronic P123), resulting in the increased anti-neoplastic activity in BC [57]. The combination of the verapamil and ITC with 5-FU decreased cell survival and proliferation.

Moreover, ITC and 5-FU are more effective in the treatment of BC [58]. Administration of the Itraconazole with erlotinib (tyrosine kinase inhibitor) increased the AUC and C_{max} by 10.8 and 2.78-fold, respectively, without any SAE [59]. Abovementioned all the studies reveal the potential of Itraconazole alone or in combination with other anticancer agents to treat BC.

2.4 Simvastatin

Simvastatin belongs to the class of statins and is a well-explored hydroxy-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor that reduces cholesterol biosynthesis initially used to reduce cholesterol biosynthesis marketed in 1988 [60]. Clinical data suggest that statins are effective in BC management. Statins amplify tumor cell death and radiosensitivity in various cell lines, inhibit invasion and proliferation, and show anti-metastatic activity. Clinical trials conducted on breast cancer (inflammatory and TNBC) patients also favored these observations by representing improved mortality benefits for patients on statins [61, 62]. In the same context, Simvastatin is the most explored statin to explore the role of statins in cancer. Simvastatin targets the transcription factor NFκB that reduces the expression level of anti-apoptotic protein Bcl-xL, concomitantly inhibits the expression of anti-proliferative and proapoptotic tumor suppressor PTEN and hence inhibiting the growth of breast cancer cells. The elevation of PTEN expression results in the suppression of Akt phosphorylation. Akt activity is upregulated in many cancers by increasing cancer cell survival, inhibiting apoptosis, and increasing proliferation. Therefore, Simvastatin substantially decreased Akt phosphorylation concurrently with the reduction in expression of anti-apoptotic protein by dysregulation of NFκB, thus showing the anticancer activity against BC [63]. On administration of Simvastatin, the expression of PTTG1 (pituitary tumor-transforming gene 1) was also reduced in a dose-dependent manner in the MDA-MB-231 cell line. PTTG1 is the important gene involved in the invasion and metastasis of BC [64]. In the same cell line (MDA-MB-231), Simvastatin leads to fragmentation of the cell's nuclei, subsequently inducing apoptosis. It also enhanced the level of ROS in a dose-dependent manner, which causes oxidative stress and further DNA damage [65]. Apoptotic effects were due to the increased expression of miR-140-5p in a dose-dependent manner mediated by the activating transcription factor NRF1 [65]. Apart from the MDA-MB-231 cell line, Simvastatin effects were also explored in other breast cancer cell lines such as T47D, BT-549, and MCF-7, showing apoptotic inducer anti-proliferative activity [66, 67]. In *in vivo* studies with DMBA (dimethyl-Benz(a)anthracene) induced breast cancer rat model, Simvastatin reduced the tumor volume by around 80% [68]. Karimi et al. also explored its activity in breast cancer mice model and reported improved mortality and tumor volume compared with control [69]. Although Simvastatin's lipophilic nature makes it a good candidate for the BC treatment, the researcher tried to develop nano formulations to improve the delivery in a targeted specific manner and reduce the non-target side

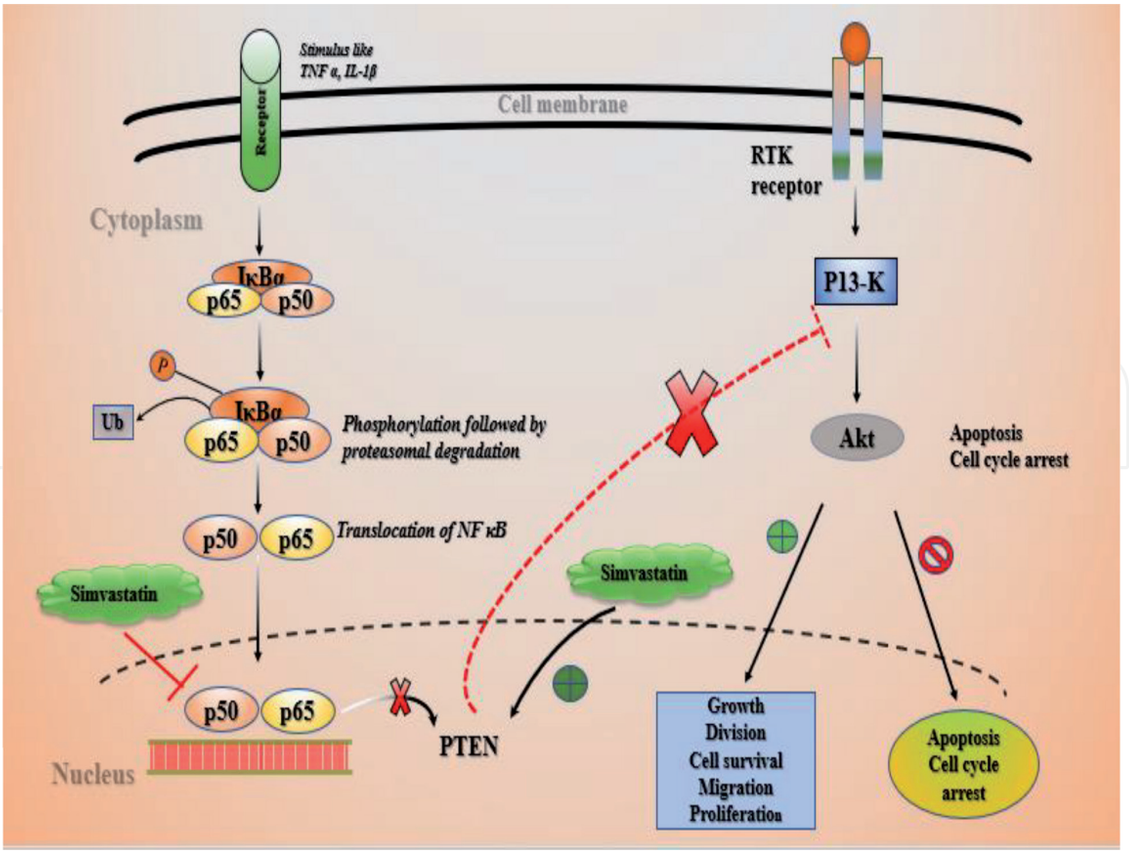

Figure 3.
Simvastatin acts via blocking p50–65 leading to activation of PTEN, which inhibits PI3K-Akt axis leading to inhibition of cell growth, division, survival, migration, and proliferation.

effects. Detailed mechanism of cell growth inhibition, division, survival, migration, and proliferation by Simvastatin is presented in **Figure 3**.

In the same series, Sed et al. used nanoparticles made of superparamagnetic iron oxide to Simvastatin delivery with enhanced anticancer activity in the PC-3 cell line. This action is mediated by inducing apoptosis and cell cycle arrest in the G2 phase [70]. Researchers from another lab developed poly D, L-lactide-co-glycolide (PLGA) with cholic-acid-based nanoparticles for Simvastatin release in a sustained and controlled manner for breast adenocarcinoma treatment. These nanoparticles showed maintainable and more efficiently inhibit tumor growth than normal Simvastatin [71]. Other formulations such as nanocapsule [72], nanoemulsions [73], liposomes [74], and immunoliposome [75] for Simvastatin were developed with increased anticancer activity in breast cancer cells. In a randomized placebo-controlled study, Simvastatin shows a better anticancer profile with the carboplatin and vinorelbine in metastatic breast cancer [76]. Consistency in the results from both clinical and preclinical studies suggests the vast potential of Simvastatin in treating breast cancer either alone or in combination. Moreover, the development of nanoformulations also provided advantages such as enhanced cytotoxicity, lower side effects, targeted delivery over the conventional available treatment options for BC.

2.5 Niclosamide

Niclosamide, an FDA-approved anthelminthic drug used to manage tapeworm infection, has been used almost from the last half of the century and included in the WHO's list of essential medicines. Recent research suggests that niclosamide has a wide range of therapeutic uses other than treating parasitic infection. Niclosamide's

Drug	Experimental model	Mechanism of action	Observation	Original indication	References
Aspirin	B16F10, MDA-MB-231, MDA-MB-435 xenograft mode MCF-7, MDA-MB-231 MCF-7, MDA-MB231	Inhibition of heparinase Inhibition of TGF-β/SMAD4 signaling pathway ↓EMT Apoptosis	↓Metastasis, ↓angiogenesis ↓Mesenchymal markers (vimentin, Snail, TWIST)	NSIAD	[23, 30, 31]
Itraconazole	MCF-7 and SKBR-3 breast cancer cell	Antiproliferative effect via inhibition of hedgehog signaling cascade ↑Apoptosis ↑Autophagy ↑Cell cycle arrest	↓Tumor size ↑Caspase 3 ↓Bcl-2	Antifungal drug	[50]
Niclosamide		↓EM T ↑Apoptosis Inhibition of stat signaling	↓Snail ↓Vimentin ↓Tumor growth ↑Caspase 3, ↓Bcl-2, ↓surviving, ↓Mcl-1 expression	Anthelminthic drug	[80, 81]
Simvastatin	MDA-MB-231, T47D, BT-549 and MCF-7 (*in vitro*) DMBA model (*in vivo*)	↓PTTG1, ↓Bcl-xL ↑ROS ↑miR-140-5p Inhibition of Akt and DNA damage	Anti-proliferative, induce apoptosis, and increased survival	Anti-hypercholesterolemic drug	[63–67]
Metformin	MDA-MB-231, MCF-7	Via mTOR, Akt/MAPK pathway COX-2 inhibition	Apoptosis, anti-proliferative, anti-angiogenic	Anti-diabetic drug	[44, 45]

Table 1.
Summary of the repurposed drugs for BC discussed in the chapter.

clinical application diseases include type 2 diabetes, endometriosis, neuropathic pain, bacterial and viral infections, including cancer [77]. The anticancer benefits of niclosamide have been shown in many malignancies such as colon cancer, lung cancer, prostate cancer in humans, as well as breast cancer by suppressing various cancer related pathways such as Wnt Notch, mTOR, STAT, and NFκB [78, 79]. The combinational treatment of niclosamide with cisplatin overcomes the resistance to cisplatin and induces an inhibitory effect on proliferation *in vitro* and reduced tumor size *in vivo*.

Further, niclosamide prevented the epithelial-mesenchymal transition (EMT) by suppressing mesenchymal markers such as snail and vimentin. The inhibitory effect on EMT and prevention of stem-like phenotype of TNBC by Niclosamide operate by disabling various abnormal signaling pathways such as Akt, ERK, and Src [80]. The niclosamide acts as a potent inhibitor of STAT signaling by preventing cancer cell proliferation, invasion, and metastasis by decreasing the phosphorylation of STAT3 that otherwise was found in 35% of breast cancer tissues. Furthermore, STAT3 promotes the expression of several key downstream genes involved in proliferation, cell survival, and angiogenesis in breast cancer [81]. Human monocyte cells were reduced to HUVECs in the presence of niclosamide. Niclosamide also inhibited VCAM-1 and ICAM1 protein expression in HUVECs. Niclosamide decreased HUVEC proliferation, migration, and development of cord-like structures. *In vivo*, niclosamide inhibits VEGF-mediated angiogenesis [77]. Niclosamide inhibited Wnt/Frizzled 1 signaling, mediated by the increased degradation of the Wnt co-receptor LRP-6 (low-density lipoprotein receptor-related protein 6) [82–84]. Osada et al. determined that on the administration of niclosamide, there was a decrease in Dvl2 expression, which further impeded the downstream signaling (β-catenin) [85]. Londoño-Joshi et al. reported that niclosamide administration also reduced levels of LRP6 and β-catenin in breast cancers [86]. In combination with doxorubicin, niclosamide induces apoptosis and synergistically increases breast cancer cell death. This action is mediated by Wnt/β-catenin pathway downregulation and arrest of the cell cycle by Niclosamide in G0/G1 while both doxorubicin and niclosamide increased ROS production, thus showing cytotoxicity [87, 88]. Niclosamide also showed synergistic anticancer activity with 8-quinolinol [89]. When niclosamide is administered with cisplatin, it could inhibit the invasion and cell stemness of breast cancer cells, mediated by downregulation of anti-apoptotic protein Bcl2 [90]. In a recently published study, albumin-bound niclosamide (nab-Niclo) (Albumin-based nanoparticle transport systems) was found to inhibit cell growth, induce cell death, mitochondrial dysfunction, and increase oxidative stress with DNA damage. This nab-Nicolo was appeared more effective than normal Niclosamide for BC treatment [91]. Taken together, all the data suggest that niclosamide alone and in combination with other drugs could be used for the normal BC and resistance BC all repurposed drugs for BC discussed in this chapter summarized in **Table 1**.

3. Conclusion

Drug discovery is a multifaceted process that aims at identifying a therapeutic agent that can be useful in treating and managing various ailments. This process includes identification of candidates, characterization, validation, optimization, screening, and assays for therapeutic effectiveness. As the mortality due to cancer is progressively increasing, we need effective therapy to treat breast cancer patients or improve survival. When any pharmaceutical organization starts developing a novel chemical entity for the BC, its cost and attrition rate are very high. Drugs

repurposing is how we can minimize the cost and attrition rate by using the already marketed drugs for a new use. Drug repurposing against breast cancer is one of the best alternatives to treat progressive ailments. In the above discussion, we have discussed various drugs that can be repurposed against breast cancer. It will be a game-changing scenario in the treatment of breast cancer. Certain challenges need to be rectified. However, there is a need for optimization of models and more screening of drugs at preclinical stages.

4. Future prospective

To tackle all the challenges associated with the drug development process for breast cancer, scientists need to shift their interest to the alternative drug development, that is, drug repurposing. All the BC repurposed drugs discussed in the book chapter show impressive results that suggest exploring more new non-cancerous drugs for cancerous use [92]. Using the drugs repurposing approaches alone and in combination with other drugs will also reduce the side effects associated with high doses. It will also reduce the cost of the drug development process, ultimately patient compliances and burden. Patients who could not afford the treatment due to the high cost can take treatment and improve survival. As the safety is already studied of drugs that seem a novel interest in the repurposing for BC, the chances of failure at the clinical level will also be less. With the advancement in drug repurposing, there is still a need to develop a valuable model of different types of cancers that mimic cancer. The development of such a model provides the actual clue for drug repurposing. So far, the advantages we discussed, there are some challenges associated with the drugs repurposing such as patent issue, regulatory consideration, inequitable prescription that need to be overcome so, more and more pharma companies show their interest in drug repurposing. It is expected that drug repurposing will achieve the milestone that is currently not possible with the conventional available treatment for cancers in the future. Furthermore, new nanoformulations need to be developed for the targeted and specific delivery of repurposed anticancer drug to avoid the off-target side effects.

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