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In Vitro Anticancer Efficacy of Metformin Combination Therapy in Breast Cancer: A Systematic Review

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ABSTRACT

Metformin, a widely used antidiabetic drug, has attracted increasing interest as a potential anticancer agent owing to its ability to modulate cellular metabolism and oncogenic signaling pathways. This systematic review evaluated the in vitro anticancer efficacy of metformin-based combination therapies in breast cancer, focusing on synergistic activity, molecular mechanisms, and subtype-specific responses. A systematic search of the Scopus database was conducted in accordance with PRISMA 2020 guidelines, resulting in the inclusion of 23 eligible studies published between 2016 and May 2026. The studies investigated combinations of metformin with chemotherapeutic agents, targeted therapies, repurposed drugs, and natural compounds across estrogen receptor-positive (ER+), HER2-positive, and triple-negative breast cancer (TNBC) cell lines. Most combinations demonstrated synergistic or potentiating effects, characterized by enhanced apoptosis, cell-cycle arrest, metabolic disruption, and inhibition of tumor-promoting signaling pathways. Mechanistically, recurrent modulation of the AMPK/mTOR, PI3K/Akt, MAPK/ERK, and mitochondrial bioenergetic networks was observed across multiple therapeutic combinations. Several studies also reported reversal of drug resistance, suppression of cancer stemness, and inhibition of epithelial–mesenchymal transition-associated phenotypes. However, the interpretation and translational relevance of these findings are limited by the widespread use of supra-physiological metformin concentrations and considerable methodological heterogeneity among studies. Overall, the available evidence supports metformin as a metabolic sensitiser capable of enhancing the efficacy of diverse anticancer agents in breast cancer models. Future research should prioritize clinically relevant exposure conditions, advanced in vitro models, and predictive biomarker development to facilitate translational application.

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I. INTRODUCTION

Breast cancer remains the most frequently diagnosed cancer and one of the leading causes of cancer-related mortality among women worldwide[1]. Despite substantial advances in screening, early detection, and therapeutic interventions, breast cancer continues to pose a significant global health burden [2]. Current treatment strategies, including surgery, radiotherapy, chemotherapy, endocrine therapy, and targeted therapies, have improved patient outcomes; however, treatment resistance, disease recurrence, metastatic progression, and therapy-related toxicities remain

major clinical challenges [3,4]. These limitations underscore the need for innovative therapeutic approaches that can enhance treatment efficacy while minimizing adverse effects[4].

Drug repurposing has emerged as an attractive strategy in oncology because it leverages the established pharmacokinetic, pharmacodynamic, and safety profiles of

approved drugs, thereby reducing both development costs and timelines[5]. Among repurposed agents, metformin (1,1-dimethylbiguanide hydrochloride), a first-line oral antihyperglycemic drug widely prescribed for type 2 diabetes mellitus, has attracted considerable attention due to its potential anticancer properties[6–10]. Epidemiological studies have suggested that metformin use may be associated with reduced cancer incidence and improved clinical outcomes in several malignancies, including breast cancer, although the clinical evidence remains inconclusive [10–13].

Although epidemiological and preclinical studies have highlighted the anticancer potential of metformin, clinical investigations have yielded inconsistent outcomes[14]. This discrepancy suggests that the therapeutic efficacy of metformin may depend on specific tumor characteristics, metabolic dependencies, and interactions with co-administered therapies[15–17]. Consequently, increasing research efforts have focused on metformin-based combination strategies, which aim to enhance anticancer activity through complementary molecular and metabolic mechanisms[18–20]. In breast cancer, a biologically heterogeneous disease comprising distinct molecular subtypes, experimental studies have reported synergistic effects between metformin and chemotherapeutic agents, targeted therapies, repurposed drugs, and natural compounds[10,21,22]. These findings suggest that the anticancer activity of metformin may be subtype-specific and highly context-dependent, particularly in aggressive phenotypes such as triple-negative breast cancer (TNBC), where effective therapeutic options remain limited[10,21–23].

Over the past decade, a growing body of in vitro evidence has investigated metformin-based combination therapies across diverse breast cancer cell lines and molecular subtypes. However, the available findings remain fragmented, with substantial heterogeneity in experimental models, combination partners, and reported mechanisms of action. As a result, the extent to which synergistic effects are consistently observed across molecular subtypes and therapeutic combinations remains unclear. To date, no systematic review has comprehensively synthesized the in vitro evidence regarding the efficacy, synergistic interactions, subtype-specific responses, and molecular mechanisms of metformin-based combination therapies in breast cancer. This systematic review aims to comprehensively evaluate the in vitro anticancer effects of metformin-based combination therapies in breast cancer, with particular emphasis on synergistic efficacy, molecular mechanisms underlying combination benefits, and subtype-specific responses across diverse breast cancer cell line models.

II. MATERIALS AND METHODS

A. Search Strategy and Information Sources

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines. A comprehensive literature search was performed using the Scopus database, covering the publication period from January 2016 to May 2026. The search strategy was designed to maximize sensitivity while maintaining specificity for the research question concerning metformin-based combination therapies in breast cancer in vitro models. The search string employed was: ((metformin OR "dimethylbiguanide") AND ("breast cancer" OR "breast carcinoma" OR "breast tumor") AND ("in vitro" OR "cell line*" OR "cell culture*") AND (combination OR combin* OR synerg* OR cotreatment OR "co-treatment" OR "co-administration") AND (apoptosis OR cytotoxicity OR proliferation OR viability OR "cell cycle")).

B. Eligibility Criteria

Studies were included if they met the following criteria: (1) peer-reviewed original research articles published between 2016 and 2026; (2) indexed within the Scopus database and written entirely in English with full-text availability; (3) conducted exclusively or primarily in vitro using established breast cancer cell lines; (4) evaluated the therapeutic efficacy of metformin administered in combination with either conventional chemotherapeutic agents, targeted therapies, repurposed drugs, or natural compounds; and (5) reported clear quantitative anticancer outcomes with sufficient data available for extraction.

Studies were excluded if they fell under any of the following categories: (1) secondary literature including reviews, systematic reviews, meta-analyses, and protocol papers; (2) non-peer-reviewed or incomplete formats such as conference abstracts, editorials, letters to the editor, or book chapters; (3) studies utilizing purely animal models (in vivo) or clinical trial settings without any in vitro experimental components; (4) investigations that did not involve breast cancer models or evaluated metformin solely as a monotherapy; (5) articles lacking measurable or quantifiable anticancer outcomes; and (6) any publications written in languages other than English.

C. Study Selection Process

The study selection process was conducted systematically following PRISMA 2020 guidelines. The initial database search identified 313 records from Scopus. Prior to screening, 194 records were removed for various reasons, leaving 119 records for title and abstract screening. Following this initial screening phase, 77 records were excluded based on predefined criteria, resulting in 42 reports sought for full-text retrieval. All 42 reports were successfully retrieved and assessed for eligibility. During full-text assessment, 19 reports were excluded: 3 were not conducted in breast cancer cell lines, 5 evaluated metformin

monotherapy only, 2 utilized non-human models exclusively, 5 had insufficient outcome data, and 4 involved ineligible interventions. Ultimately, 23 studies met all eligibility criteria and were included in the final synthesis.

D. Data Extraction and Synthesis

Data were extracted systematically from each eligible study using a standardized extraction form. The following variables were recorded: first author and publication year, breast cancer cell lines utilized, cell subtype characteristics, co-administered agents, metformin and co-agent concentrations, treatment duration, combination analysis methodology, synergistic efficacy outcomes (including Combination Index values where reported), apoptosis and cell cycle markers, molecular signaling pathways investigated, additional biomarkers assessed, main conclusions, research limitations, and digital object identifiers (DOIs).

The Combination Index (CI) method, based on the Chou-Talalay theorem, was the predominant approach for quantifying drug interactions. CI values < 1 indicated synergism, CI = 1 indicated additivity, and CI > 1 indicated antagonism. Studies were thematically categorized into three groups based on the class of co-administered agent: (A) chemotherapeutic and targeted agents, (B) repurposed drugs, and (C) natural compounds.

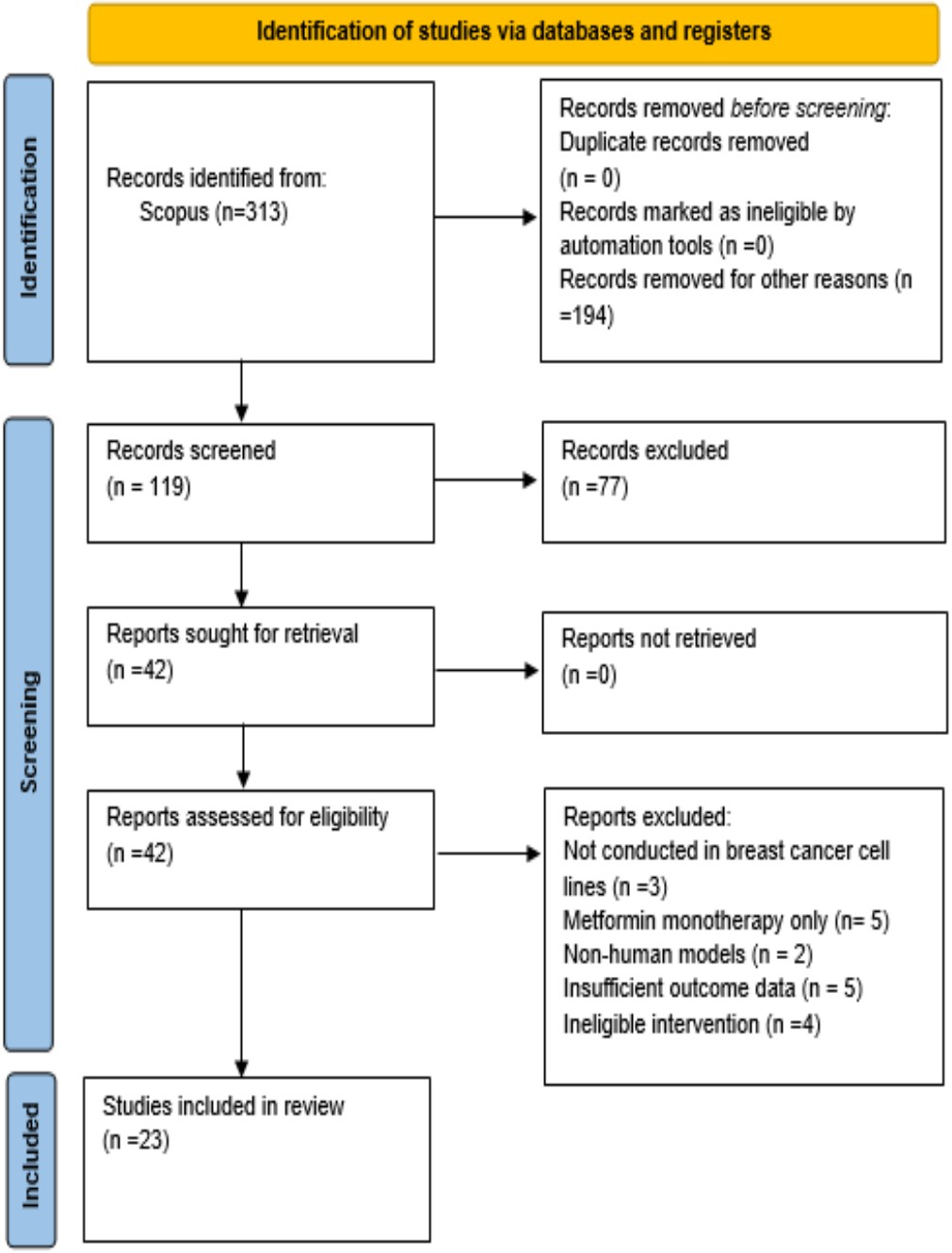

Figure 1. PRISMA 2020 Flow Diagram of the Study Selection Process

III.RESULTS

A. Study Characteristics and Overview

The systematic search and selection process yielded 23 studies meeting all eligibility criteria for inclusion in this review. The included studies were published between 2016 and 2026, representing the most current body of evidence on metformin combination therapy in breast cancer. The studies employed a diverse array of breast cancer cell line models spanning all major molecular subtypes: ER-positive (MCF-7, T47D), HER2-positive (SKBR3, SK-BR-3, BT-474, HCC1954, ZR75, MDA-MB-453), and TNBC (MDA-MB-231, MDA-MB-468, HCC1806, BT-549, Hs578T, 4T1). Treatment durations ranged from 24 hours to 14 days, with 48–72 hours being the most commonly employed exposure period.

B. Combination Therapy Categories

Chemotherapeutic and Targeted Agents*:* Among chemotherapeutic and targeted agents, metformin generally enhanced anticancer efficacy through synergistic modulation of proliferative, metabolic, and survival pathways. In TNBC models, combinations with cisplatin and doxorubicin increased cytotoxicity and restored treatment sensitivity by disrupting DNA repair mechanisms, suppressing P-glycoprotein-mediated drug resistance, depleting intracellular ATP, and promoting apoptosis [24,25]. Similarly, targeted therapies including neratinib, alpelisib, BMS-754807, alisertib, and vorinostat demonstrated synergistic interactions characterized by suppression of HER-family signalling, PI3K/mTOR, ERK, STAT3, and Wnt/β-catenin pathways, accompanied by cell-cycle arrest, apoptosis induction, metabolic stress, and inhibition of cancer stemness-related phenotypes [26–30]. Notably, the metformin–BMS-754807 combination exhibited synergistic activity in 85% of TNBC cell lines evaluated, whereas the metformin-alpelisib combination substantially reduced ALDH1-positive populations and tumorsphere formation, indicating suppression of cancer stem cell characteristics [27,28].

Additional evidence highlighted the role of metabolic targeting as a recurrent mechanism of synergy. Metformin enhanced the activity of alisertib, vorinostat, and BMS-754807 through ATP depletion, mitochondrial dysfunction, glycolytic suppression, and AMPK activation, while combinations with everolimus and OBHS further demonstrated that modulation of mTOR signalling and cellular metabolism can potentiate antiproliferative responses across molecular subtypes [31,32]. Although the metformin–everolimus combination produced additive rather than synergistic effects, it consistently reduced cellular bioenergetic activity and viability, supporting the importance of metabolic vulnerability as a therapeutic target [31]. Collectively, these findings indicate that metformin functions as a metabolic sensitiser capable of amplifying the efficacy of diverse chemotherapeutic and targeted agents through coordinated disruption of oncogenic signalling and energy homeostasis.

Repurposed Drugs*:* In the realm of repurposed medications and alternative therapeutic agents, metformin has been shown to significantly boost anticancer efficacy by influencing cellular metabolism, apoptosis, and pathways related to stress responses. Synergistic or potentiating effects were reported in combinations with simvastatin, propranolol, iron chelators, ciclopirox olamine, azacitidine, aspirin, melatonin, and a glimepiride-metformin adduct, resulting in reduced proliferation, increased apoptosis, and suppression of aggressive tumour phenotypes [33–40]. Mechanistically, these combinations frequently converged on AMPK activation, mTOR inhibition, mitochondrial dysfunction, and modulation of apoptosis-related proteins, including Bcl-2 family members, caspases, and cell-cycle regulators [37,38,40].

Several combinations additionally targeted tumour adaptation and progression pathways. Metformin combined with simvastatin suppressed the ET-1/ETBR/HIF-1α axis and inhibited angiogenesis, whereas combinations with propranolol, iron chelators, and ciclopirox olamine disrupted cellular bioenergetics through modulation of mitochondrial function and metabolic stress [33–36]. Notably, azacitidine and the glimepiride-metformin adduct enhanced growth inhibition through coordinated regulation of AMPK/mTOR signalling and cell-cycle progression, while melatonin and aspirin further potentiated antiproliferative responses through apoptosis-related and stress-associated pathways [37–40]. Taken together, these results demonstrate that metformin acts as a potent metabolic sensitizer, enhancing the efficacy of various structurally distinct compounds by simultaneously disrupting survival pathways and tumor metabolic plasticity.

Natural Compounds and Other Biological/Targeted Interventions*:* Metformin also demonstrated synergistic or potentiating effects when combined with natural compounds and targeted therapeutic strategies. Combinations with formononetin, silibinin, curcumin, and D-limonene enhanced antiproliferative activity through induction of apoptosis, suppression of pro-survival signaling, and modulation of cell-cycle- and angiogenesis-related pathways. These effects were associated with downregulation of Bcl-2, hTERT, Cyclin D1, and VEGF, together with inhibition of ERK signaling and activation of p53-related responses [41–44]. Although the magnitude of synergy varied among cell lines, particularly for curcumin, the findings collectively support the ability of metformin to enhance the activity of bioactive natural compounds through complementary molecular mechanisms.

Additional evidence highlighted the role of metabolic and signaling pathway modulation in potentiating anticancer responses. Combinations with 2-deoxyglucose and targeted therapies, including tamoxifen, trastuzumab, and anti-ROR1 antibodies, enhanced growth inhibition through disruption of cellular bioenergetics, apoptosis induction, and modulation of PI3K/AKT/mTOR, RAS/MAPK, and WNT-associated signaling pathways [45,46]. Collectively, these findings

further support the concept that metformin functions as a metabolic sensitiser capable of amplifying the efficacy of structurally diverse agents through coordinated regulation of survival signaling, energy metabolism, and tumour adaptation mechanisms.

C. Consolidated Data Synthesis Table

Table 1. Summary of Metformin Combination Therapy Studies in Breast Cancer Cell Lines

AUTHOR	CO-AGENT	CELL LINES	SUBTYPE	SYNERGY (CI)	KEY PATHWAYS	PRIMARY OUTCOMES
Kheraldine et al., [26]	Neratinib	SKBR3, HCC1954, ZR75, MDA-MB-453	HER2+	CI < 1	HER1-3, IGF-1R, ERK1/2, AKT, mTOR↓	Apoptosis, invasion inhibition
Wang et al., [32]	OBHS (SERM)	MCF-7, T47D	ER+	CI < 1	PI3K/Akt/GLUT 1, EMT markers	G_0/G_1 arrest, metabolic reprogramming
Lee et al., [24]	Cisplatin	Hs 578T, MDA-MB-231	TNBC	Enhanced	ERK, RAD51↓	DNA damage, resistance reversal
Cheng et al., [35]	Iron chelators	MDA-MB-231, MDA-MB-231-BR	TNBC	CI < 1	Complex I, AMPK	Proliferation inhibition
Gu et al., [30]	SAHA	MDA-MB-231, BT-549, HCC1806, Hs578T	TNBC	CI < 0.9	FGFR4-JAK1-STAT3↓	PARP cleavage, glycolysis↓
Zhang et al., [29]	Alisertib	MDA-MB-231, 4T1	TNBC	Potentiated	OXPHOS, Aurora A	ATP depletion, mitotic failure
Xin et al., [41]	Formononetin	MCF-7	ER+	CI < 1	p-ERK1/2↓	Bcl-2↓, apoptosis
Chatran et al., [42]	Silibinin	T47D	ER+	CI < 1	hTERT, Cyclin D1↓	Viability reduction
Wokoun et al., [45]	2-DG	HCC1806, MDA-MB-231	TNBC	Enhanced	Glycolysis↓	MMP collapse, PARP cleavage
Rico et al., [34]	Propranolol	4T1, MDA-MB-231, MCF-7	TNBC/ER+	Potentiated	AMPK↑, OCR↓	Anti-metastatic
Wawszczyk et al., [39]	Melatonin	MCF-7, SKBR3	ER+/HER2+	CI=0.68/0.63	P53, AKT, mTOR	DNA fragmentation
Wu et al., [36]	Ciclopirox	HCC1806, MDA-MB-231, Hs578T, MDA-MB-468	TNBC	CI < 1	NR	S-phase arrest, apoptosis
Hosseini et al., [37]	Azacitidine	MDA-MB-231	TNBC	Strong	AMPK↑, mTOR↓	CCND1/ELAVL1/ EIF4EBP1↓
Liu et al., [33]	Simvastatin	MCF-7, MDA-MB-231	Mixed	CI=0.5/0.625	ET-1/ETBR/HIF1α↓	Angiogenesis inhibition
Long et al., [38]	Glimepiride adduct	CAL-148, MDA-MB-453, MDA-MB-231, MCF-7	Mixed	Enhanced	AMPK↑, P53/P21↑	G1/S arrest, Ki67↓
Li et al., [25]	Doxorubicin	MCF-7, MCF-7/ADR	Resistant	CI < 1	AMPK/mTOR	Pgp↓, resistance reversal
Falah et al., [43]	Curcumin	EMT6/p, MCF-7, T47D	Mixed	CI=0.76-1.06	Trp53↑	VEGF↓, angiogenesis
Shi et al., [27]	Alpelisib	SK-BR-3, BT-474	HER2+	CI < 1.0	PI3K/mTOR, STAT3, Wnt↓	Stemness suppression
Zhao et al., [40]	Aspirin	4T1	TNBC	Enhanced	TGF-β1	Apoptosis regulation
Ariaans et al., [31]	Everolimus	MCF-7, MDA-MB-231, T47D	Mixed	Additive	mTOR↓	Metabolic targeting
Salim et al.,	D-Limonene	MCF-7	Luminal A	CI=0.22	PI3K/AKT/mTO	Strong apoptosis

[44]					R	
Mahmoudi et al., [46]	Tam/Tras/Anti-ROR1	MCF-7, SKBR-3, MDA-MB-231	All	Enhanced	PI3K/AKT/mTOR, MAPK, WNT	Targeted therapy enhancement
Xue et al., [28]	BMS-754807	13 TNBC lines	TNBC	CI < 1 (85%)	Skp2-p27, PI3K/mTOR	p27 stabilization, Ki67↓

IV. DISCUSSION

A. Signal Transduction Pathways and Metabolic Reprogramming

***Modulation of the AMPK/mTOR and PI3K/Akt Axis*:** A common mechanistic theme emerging from the included studies is the convergence of metformin-based combination therapies on the AMPK/mTOR and PI3K/Akt signalling networks, which regulate cellular growth, metabolism, and survival. Across HER2-positive, ER-positive, and TNBC models, metformin consistently potentiated the efficacy of co-administered agents by disrupting energy homeostasis and suppressing key oncogenic pathways. This effect was observed in combinations with neratinib, alpelisib, azacitidine, simvastatin, and the glimepiride-metformin adduct, and was associated with inhibition of proliferative signalling, induction of cell-cycle arrest, and promotion of apoptosis [26,27,33,37,38].

Importantly, metformin enhanced targeted therapy responses through mechanisms extending beyond direct growth inhibition. In HER2-positive models, combinations with neratinib and alpelisib concurrently suppressed multiple oncogenic pathways, including PI3K/mTOR, ERK, STAT3, and Wnt/β-catenin signalling, while reducing stemness-associated markers such as ALDH1, Nanog, and Sox2 [26,27]. Similarly, in TNBC models, metformin synergised with BMS-754807 by restoring cell-cycle regulation through modulation of the Skp2–p27 axis, whereas its combination with doxorubicin reversed resistance-associated phenotypes through ATP depletion and suppression of P-glycoprotein activity [25,28]. Collectively, these findings support the role of metformin as a metabolic sensitiser that enhances the activity of diverse therapeutic agents through coordinated inhibition of growth-promoting signalling pathways and reinforcement of cellular stress responses. The recurrent involvement of AMPK/mTOR and PI3K/Akt signalling across molecular subtypes suggests that these pathways represent central mediators of the synergistic anticancer effects observed in metformin-based combination therapies.

***MAPK/ERK and Tyrosine Kinase Inhibitions*:** Beyond AMPK/mTOR modulation, suppression of MAPK/ERK-associated signaling emerged as a recurrent mechanism underlying the synergistic activity of metformin-based combinations. Across multiple breast cancer subtypes, metformin enhanced the efficacy of co-administered agents by disrupting receptor tyrosine kinase and ERK-dependent survival pathways, thereby promoting apoptosis and limiting tumour progression. This effect was particularly evident in HER2-positive models, where metformin combined with neratinib simultaneously inhibited HER-family receptors, IGF-1R, and downstream ERK signaling, resulting in reduced invasion, colony formation, and angiogenic potential [26].

Similar pathway convergence was observed with formononetin and vorinostat. Metformin enhanced formononetin-induced apoptosis through suppression of phosphorylated ERK1/2 and downregulation of Bcl-2, while sensitising TNBC cells to vorinostat through inhibition of FGFR4-mediated signaling involving the JAK1/STAT3, AKT, and ERK pathways [30,41]. In addition, combinations with propranolol and targeted therapies demonstrated that modulation of metabolic stress and RAS/MAPK-related signaling can further potentiate anticancer responses across ER-positive, HER2-positive, and TNBC models [34,46]. Collectively, these findings suggest that inhibition of MAPK/ERK-associated survival networks represents an important complementary mechanism through which metformin enhances the efficacy of diverse anticancer agents.

***Mitochondrial Targeting and Energetic Exhaustion*:** Another recurring mechanistic theme identified across the included studies was the exploitation of metabolic vulnerabilities through mitochondrial dysfunction and energetic stress. As a mitochondrial complex I inhibitor, metformin enhanced the activity of diverse therapeutic agents by disrupting oxidative phosphorylation (OXPHOS), reducing ATP production, and limiting the metabolic flexibility required for cancer cell survival. This effect was consistently observed across combinations targeting distinct molecular pathways, suggesting that energetic exhaustion represents a common mechanism underlying therapeutic synergy.

Evidence supporting this concept was observed in combinations with alisertib, 2-deoxyglucose (2DG), iron chelators, and propranolol. These combinations induced ATP depletion, mitochondrial dysfunction, AMPK activation, and suppression of cellular proliferation, while simultaneously impairing adaptive metabolic responses that normally support tumour survival under stress conditions[29,34,35,45]. Collectively, these findings suggest that metformin acts as a metabolic sensitiser that enhances anticancer efficacy by driving energetic collapse, thereby exposing a broadly exploitable vulnerability across breast cancer subtypes.

B. Overcoming Resistance and Suppressing Tumor Aggressiveness

***DNA Repair Interference and Chemoresistance Reversals*:** Resistance to chemotherapy remains a major

obstacle in breast cancer treatment, particularly in TNBC, where therapeutic options are limited. Several studies suggest that metformin may enhance treatment sensitivity by disrupting molecular mechanisms that support resistance. In TNBC models, metformin potentiated cisplatin activity through suppression of RAD51-mediated homologous recombination repair and induction of DNA damage, as evidenced by increased phospho-H2AX levels [24]. These effects were accompanied by inhibition of ERK signaling and reduced migratory capacity, indicating that interference with DNA repair may also contribute to suppression of aggressive tumour behaviour.

Complementary findings were observed in doxorubicin-resistant MCF-7/ADR cells, where metformin restored drug sensitivity through inhibition of P-glycoprotein-mediated efflux, ATP depletion, and increased oxidative stress [25]. Together, these findings suggest that metformin may reverse resistance-associated phenotypes by simultaneously targeting DNA repair capacity and the metabolic requirements that sustain drug-resistant cells.

***Cancer Stemness Suppression*:** Cancer stem cells (CSCs) contribute to tumour recurrence, metastasis, and therapeutic resistance, making them an important target for combination therapy. Among the included studies, the metformin-alpelisib combination demonstrated the most comprehensive suppression of CSC-related characteristics in HER2-positive breast cancer models. This effect was evidenced by reductions in ALDH1-positive cell populations, inhibition of tumoursphere formation, and downregulation of stemness-associated transcription factors including Nanog and Sox2 [27]. Notably, these changes occurred alongside suppression of PI3K/mTOR, ERK, STAT3, and Wnt/β-catenin signaling pathways, suggesting that metformin may enhance targeted therapies by disrupting multiple networks involved in CSC maintenance and survival.

***EMT Reversal and Metastatic Suppression*:** Cancer Another recurring theme across the included studies was the suppression of metastatic and aggressive phenotypes. In ER-positive breast cancer models, the metformin-OBHS combination reversed EMT-associated features through downregulation of Vimentin and Snail1 and restoration of E-cadherin expression, indicating a shift toward a less invasive epithelial phenotype [32]. These effects were accompanied by modulation of PI3K/Akt/GLUT1 signaling and alterations in cellular metabolism, suggesting a close relationship between metabolic reprogramming and EMT regulation.

Similarly, metformin combined with simvastatin inhibited the ET-1/ETBR/HIF-1α signaling axis and reduced angiogenic potential in both MCF-7 and MDA-MB-231 cells [33]. Given the established role of HIF-1α in hypoxic adaptation, angiogenesis, and metastatic progression, these findings suggest that metformin-based combinations may suppress tumour aggressiveness not only through direct cytotoxicity but also through modulation of pathways that facilitate invasion, dissemination, and adaptation to hostile microenvironments.

C. Limitations and Translational Considerations

Despite the promising anticancer activity observed across the included studies, several limitations should be considered when interpreting these findings. The most important limitation is the widespread use of metformin concentrations that substantially exceed clinically achievable plasma levels. While therapeutic plasma concentrations in patients are generally within the micromolar range, many studies employed metformin at millimolar concentrations, often between 1 and 40 mM, raising concerns regarding the clinical relevance of the observed anticancer effects [26,30,41]. Consequently, although the identified molecular mechanisms provide valuable insights into potential therapeutic targets, the extent to which these effects can be reproduced under clinically relevant exposure conditions remains uncertain.

Another important limitation is the considerable heterogeneity of experimental designs across studies, including differences in metformin concentrations, treatment durations, breast cancer subtypes, and synergy assessment methods. Such variability complicates direct comparisons between studies and limits the identification of optimal therapeutic conditions. Furthermore, most investigations relied on conventional monolayer cell cultures, which inadequately capture the metabolic complexity and cellular interactions that influence drug responses in tumours. Future in vitro research should prioritise clinically relevant drug exposures, standardized synergy evaluation frameworks, and advanced models such as three-dimensional spheroids, organoids, and co-culture systems. Greater emphasis should also be placed on identifying predictive biomarkers of response, particularly those related to metabolic dependencies, PI3K/Akt/mTOR signalling, and cancer stemness, to facilitate the rational development of metformin-based combination therapies.

CONCLUSION

This systematic review demonstrates that metformin-based combination therapies frequently enhance anticancer activity across multiple breast cancer subtypes through synergistic modulation of metabolic and oncogenic signaling pathways. The most frequently reported mechanisms involved AMPK/mTOR and PI3K/Akt pathway regulation, suppression of MAPK/ERK signaling, and induction of mitochondrial dysfunction and energetic stress. In addition to improving antiproliferative efficacy, several combinations showed potential to overcome drug resistance, suppress cancer stemness, and inhibit aggressive tumour phenotypes. Nevertheless, the widespread use of supra-physiological metformin concentrations and methodological heterogeneity limit direct clinical translation. Future studies should focus on clinically relevant exposure conditions, standardized experimental frameworks, and biomarker-

guided approaches to better define the therapeutic potential of metformin-based combination strategies in breast cancer.

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REFERENCES

I. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 Countries. CA Cancer J Clin 2021;71:209–49. https://doi.org/10.3322/caac.21660.

II. Freihat O, Sipos D, Kovacs A. Global burden and projections of breast cancer incidence and mortality to 2050: a comprehensive analysis of GLOBOCAN data. Front Public Heal 2025;13:1622954. https://doi.org/10.3389/fpubh.2025.1622954.

III. Wang J, Wu S-G. Breast Cancer: An Overview of Current Therapeutic Strategies, Challenge, and Perspectives. Breast Cancer (Dove Med Press 2023;15:721–30. https://doi.org/10.2147/BCTT.S432526.

IV. Eapen JV, George P, Thomas S, Antony J. Integrative strategies in breast cancer therapy: Conventional approaches, emerging advances, and future challenges. ASPET Discov 2025;1:100012. https://doi.org/https://doi.org/10.1016/j.aspetd.2025.100012.

V. Recino A, Rayner MLD, Rohn JL, Della Pasqua O, Recino A, Rayner MLD, Rohn JL, Carter B, Cupani A, Dahlmann-Noor A, Foltynie T, Furman J, Hobbiger S, Lugarà E, Mole SE, Orlu M, Phillips JB, Spingardi P, Pasqua O Della. Therapeutic innovation in drug repurposing: Challenges and opportunities. Drug Discov Today 2025;30:104390. https://doi.org/https://doi.org/10.1016/j.drudis.2025.104390.

VI. Foretz M, Guigas B, Viollet B. Metformin: update on mechanisms of action and repurposing potential. Nat Rev Endocrinol 2023;19:460–76. https://doi.org/10.1038/s41574-023-00833-4.

VII. Zeljković V, Bogavac M, Dekić M, Minić S, Mahmutović E, Kunkin V, Marković Z, Karaman M. Molecular Insights into the Interaction Between Metformin and Caffeine: The Time-Dependent Antagonism and Modulation of p53 Signaling in Cancer Cells. Molecules 2026;31. https://doi.org/10.3390/molecules31111877.

VIII. Luo Q, Hu D, Hu S, Yan M, Sun Z, Chen F. In vitro and in vivo anti-tumor effect of metformin as a novel therapeutic agent in human oral squamous cell carcinoma. BMC Cancer 2012;12:517. https://doi.org/10.1186/1471-2407-12-517.

IX. Shah S, Mansour HM, Aguilar TM, Lucke-Wold B. Advances in Anti-Cancer Drug Development: Metformin as Anti-Angiogenic Supplemental Treatment for Glioblastoma. Int J Mol Sci 2024;25. https://doi.org/10.3390/ijms25115694.

X. Barbosa F, Cunha A, Barbosa J, Faria J, Queirós O. The Dual Role of Metformin: Repurposing an Antidiabetic Drug for Cancer Therapy. Appl Sci 2025;15. https://doi.org/10.3390/app152111576.

XI. Alim R, Akalanka HMK. Metformin and its derivatives in breast cancer: from glycaemic control to tumor-intrinsic pathways. Breast Cancer Res 2025;28:13. https://doi.org/10.1186/s13058-025-02194-4.

XII. Galal MA Al-Rimawi M HADHASAA. Metformin: A Dual-Role Player in Cancer Treatment and Prevention. 2024. https://doi.org/10.3390/ijms25074083.

XIII. Feng J-L, Qin X. Metformin and cancer-specific survival among breast, colorectal, or endometrial cancer patients: A nationwide data linkage study. Diabetes Res Clin Pract 2021;175:108755. https://doi.org/https://doi.org/10.1016/j.diabres.2021.108755.

XIV. Galal MA, Al-Rimawi M, Hajeer A, Dahman H, Alouch S, Aljada A. Metformin: A Dual-Role Player in Cancer Treatment and Prevention. Int J Mol Sci 2024;25. https://doi.org/10.3390/ijms25074083.

XV. Amengual-Cladera E, Morla-Barcelo PM, Morán-Costoya A, Sastre-Serra J, Pons DG, Valle A, Roca P, Nadal-Serrano M. Metformin: From Diabetes to Cancer-Unveiling Molecular Mechanisms and Therapeutic Strategies. Biology (Basel) 2024;13. https://doi.org/10.3390/biology13050302.

XVI. Khan SS, Rangraze IR, Wali AF, Jhancy M, Attia RA, Elshamly HAH, Adam S, Elbeshbeishy RAM. Repurposing Metformin in Precision Oncology: Mechanistic Insights, Biomarker-Guided Strategies, and Translational Imperatives. Medicina (B Aires) 2025;61. https://doi.org/10.3390/medicina61091577.

XVII. Lv H, Gong H, Zhao R, Gao X, Liu W, Zhao L, Sun R. From basics to clinics: New opportunities for metformin in tumor metabolic intervention and treatment. Biomed Pharmacother 2025;191:118507. https://doi.org/https://doi.org/10.1016/j.biopha.2025.118507.

XVIII. Shamahmood MN, Miri A, Yousefi MJ, Mezginejad F. Therapeutic potential of metformin-

based combination therapy in acute myeloid leukemia. Discov Oncol 2025;17:12. https://doi.org/10.1007/s12672-025-04205-4.

XIX. Zhu L, Yang K, Ren Z, Yin D, Zhou Y. Metformin as anticancer agent and adjuvant in cancer combination therapy: Current progress and future prospect. Transl Oncol 2024;44:101945. https://doi.org/10.1016/j.tranon.2024.101945.

XX. Thyagarajan A, Gajjar V, Sahu RP. Are metformin-based combination approaches beneficial for non-small cell lung cancer: evidence from experimental and clinical studies. Mil Med Res 2025;12:61. https://doi.org/10.1186/s40779-025-00649-5.

XXI. Deng J, Peng M, Wang Z, Zhou S, Xiao D, Deng J, Yang X, Peng J, Yang X. Novel application of metformin combined with targeted drugs on anticancer treatment. Cancer Sci 2019;110:23–30. https://doi.org/10.1111/cas.13849.

XXII. Peng M, Darko KO, Tao T, Huang Y, Su Q, He C, Yin T, Liu Z, Yang X. Combination of metformin with chemotherapeutic drugs via different molecular mechanisms. Cancer Treat Rev 2017;54:24–33. https://doi.org/https://doi.org/10.1016/j.ctrv.2017.01.005.

XXIII. Hosseini SA, Valadbeigi H, Khoshnazar SM, Khoshnood S, Falahi S, Rezaee D, Hematian A, Haddadi MH. Metformin as a Multifaceted Therapeutic Agent for Gastrointestinal Diseases: Mechanisms, Clinical Efficacy, and Future Directions. Pharmacol Res Perspect 2026;14:e70257. https://doi.org/https://doi.org/10.1002/prp2.70257.

XXIV. Lee JO, Kang MJ, Byun WS, Kim SA, Seo IH, Han JA, Moon JW, Kim JH, Kim SJ, Lee EJ, In Park S, Park SH, Kim HS. Metformin overcomes resistance to cisplatin in triple-negative breast cancer (TNBC) cells by targeting RAD51. Breast Cancer Res 2019;21. https://doi.org/10.1186/s13058-019-1204-2.

XXV. Li Y, Wang M, Zhi P, You J, Gao J. Metformin synergistically suppress tumor growth with doxorubicin and reverse drug resistance by inhibiting the expression and function of P-glycoprotein in MCF7/ADR cells and xenograft models. Oncotarget 2018;9:2158–74. https://doi.org/10.18632/oncotarget.23187.

XXVI. Kheraldine H, Hassan AF, Saeed S, Merhi M, Mateo JM, Ulamec M, Peric-Balja M, Vranic S, Al-Thawadi H, Moustafa A-EA. Neratinib and metformin: A novel therapeutic approach against HER2-Positive Breast Cancer. Biomed Pharmacother 2025;187. https://doi.org/10.1016/j.biopha.2025.118034.

XXVII. Shi Y, Niu P, Cheng Q, Chen L, Weng Y, Yang X. Metformin enhances alpelisib sensitivity in HER2+ breast cancer by suppressing cancer stemness and oncogenic signaling. Front Oncol 2025;15. https://doi.org/10.3389/fonc.2025.1631415.

XXVIII. Xue L, Chen F, Yue F, Camacho L, Kothapalli S, Wei G, Huang S, Mo Q, Ma F, Li Y, Jiralerspong S. Metformin and an insulin/IGF-1 receptor inhibitor are synergistic in blocking growth of triple-negative breast cancer. Breast Cancer Res Treat 2021;185:73–84. https://doi.org/10.1007/s10549-020-05927-5.

XXIX. Zhang Z, Zeng D, Zhang W, Chen A, Lei J, Liu F, Deng B, Zhuo J, He B, Yan M, Lei X, Wang S, Lam EW-F, Liu Q, Wang Z. Modulation of oxidative phosphorylation augments antineoplastic activity of mitotic aurora kinase inhibition. Cell Death Dis 2021;12. https://doi.org/10.1038/s41419-021-04190-w.

XXX. Gu Z, Ye F, Luo H, Li X, Gong Y, Mao S, Jia X, Han X, Han B, Fu Y, Cheng X, Li J, Shao Z, Wen P, Hu X, Zhuang Z. Metformin sensitizes triple-negative breast cancer to histone deacetylase inhibitors by targeting FGFR4. J Biomed Sci 2025;32. https://doi.org/10.1186/s12929-025-01129-7.

XXXI. Ariaans G, Jalving M, de Vries EGE, de Jong S. Anti-tumor effects of everolimus and metformin are complementary and glucose-dependent in breast cancer cells. BMC Cancer 2017;17. https://doi.org/10.1186/s12885-017-3230-8.

XXXII. Wang K, Huang J. Combination of oxabicycloheptene sulfonate (OBHS) and antidiabetic drugs synergistically inhibits proliferation in human breast cancer cells. Oncologie 2025;27:57–69. https://doi.org/10.1515/oncologie-2024-0471.

XXXIII. Liu J, Wang H, Zhang M, Li Y, Wang R, Chen H, Wang B, Gao X, Song S, Wang Y, Ren Y, Li J, Liu P. Metformin and simvastatin synergistically suppress endothelin 1-induced hypoxia and angiogenesis in multiple cancer types. Cancer Sci 2023;114:640–53. https://doi.org/10.1111/cas.15602.

XXXIV. Rico M, Baglioni M, Bondarenko M, Laluce NC, Rozados V, André N, Carré M, Scharovsky OG, Márquez MM. Metformin and propranolol combination prevents cancer progression and metastasis in different breast cancer models. Oncotarget 2017;8:2874–89. https://doi.org/10.18632/oncotarget.13760.

XXXV. Cheng G, Zielonka J, Hardy M, Ouari O, Chitambar CR, Dwinell MB, Kalyanaraman B. Synergistic inhibition of tumor cell proliferation by metformin and mito-metformin in the presence of iron chelators. Oncotarget 2019;10:3518–32.

https://doi.org/10.18632/oncotarget.26943.

XXXVI. Wu Y, Lyu H, Tan C, Larsen ME, Tang S-C, Huang S, Liu B. Synergistic anti-tumor activity of ciclopirox olamine and metformin in triple-negative breast cancer. Genes Dis 2026;13. https://doi.org/10.1016/j.gendis.2025.101538.

XXXVII. Hosseini SS, Askari N, Yaghoobi MM. Combined anti-tumor effects of metformin and azacitidine in breast cancer cells. BMC Cancer 2025;25. https://doi.org/10.1186/s12885-025-14908-0.

XXXVIII. Long L, Hu X, Li X, Zhou D, Shi Y, Wang L, Zeng H, Yu X, Zhou W. The anti-breast cancer effect and mechanism of Glimepiride-Metformin adduct. Onco Targets Ther 2020;13:3777–88. https://doi.org/10.2147/OTT.S240252.

XXXIX. Wawszczyk J, Wolan R, Kapral M, Orchel A. Anticancer Activity Of Melatonin and Metformin Alone and In Combination Against Melanoma and Breast Cancer Cells. Acta Pol Pharm - Drug Res 2024;81:767–83. https://doi.org/10.32383/appdr/197350.

XL. Zhao M, Wang Y, Du C, Liu Y, Zhang N, Luo F. Aspirin and metformin exhibit antitumor activity in murine breast cancer. Oncol Rep 2018;39:1414–22. https://doi.org/10.3892/or.2018.6190.

XLI. Xin M, Wang Y, Ren Q, Guo Y. Formononetin and metformin act synergistically to inhibit growth of MCF-7 breast cancer cells in vitro. Biomed Pharmacother 2019;109:2084–9. https://doi.org/10.1016/j.biopha.2018.09.033.

XLII. Chatran M, Pilehvar-Soltanahmadi Y, Dadashpour M, Faramarzi L, Rasouli S, Jafari-Gharabaghlou D, Asbaghi N, Zarghami N. Synergistic anti-proliferative effects of metformin and silibinin combination on T47D breast cancer cells via hTERT and cyclin D1 inhibition. Drug Res (Stuttg) 2018;68:710–6. https://doi.org/10.1055/a-0631-8046.

XLIII. Falah RR, Talib WH, Shbailat SJ. Combination of metformin and curcumin targets breast cancer in mice by angiogenesis inhibition, immune system modulation and induction of p53 independent apoptosis. Ther Adv Med Oncol 2017;9:235–52. https://doi.org/10.1177/1758834016687482.

XLIV. Salim EI, Alabasy MM, Nashar EME, Al-Zahrani NS, Alzahrani MA, Guo Z, Beltagy DM, Shahen M. Molecular interactions between metformin and D-limonene inhibit proliferation and promote apoptosis in breast and liver cancer cells. BMC Complement Med Ther 2024;24. https://doi.org/10.1186/s12906-024-04453-x.

XLV. Wokoun U, Hellriegel M, Emons G, Gröndker C. Co-Treatment of breast cancer cells with pharmacologic doses of 2-deoxy-D-glucose and metformin: Starving tumors. Oncol Rep 2017;37:2418–24. https://doi.org/10.3892/or.2017.5491.

XLVI. Mahmoudi G, Ehteshaminia Y, Kokhaei P, Jalali SF, Jadidi-Niaragh F, Pagheh AS, Enderami SE, Kenari SA, Hassannia H. Enhancement of targeted therapy in combination with metformin on human breast cancer cell lines. Cell Commun Signal 2024;22. https://doi.org/10.1186/s12964-023-01446-0.