A review on the potential of repurposing of metformin for colon cancer and breast cancer

By

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of
Bachelor of Pharmacy (Hons.)

School of Pharmacy
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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The thesis titled "A review on the potential of repurposing of metformin for colon cancer and breast cancer" submitted by Jannatun Noor Oishy (19146086) of Spring, 2019 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

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Ethics Statement

This study does not involve any kind of animal and human trial.

Abstract

Metformin, a hypoglycemic agent is currently being repurposed for the treatment of different types of cancer due to its pleiotropic functions, affordability, stability at room temperature with long shelf life, broadly favorable safety profile. Several potential mechanisms have been suggested for the ability of metformin to suppress cancer growth in vitro and vivo: activation of LKB1/AMPK pathway, inhibition of cancer cell growth by suppressing mTORC1, inhibition of Generation of ROS, reduction of IGF-1, and IGF-2, inhibition of chronic inflammation, activation of the immune system, modulation of ADORA1, and downregulation of gluconeogenesis in the mitochondria. However, by the in-dept summary and assessment of existing clinical data based on OS, PFS, HR, 95% CI in both CRC and BC, it can be said that metformin exhibits greater promise in CRC patients than the BC patients. The affirmation of the curative effect of metformin for the treatment in cancer will be greatly reinforced by comprehensive randomized clinical investigations on diverse participants. Of note, the data collected suggests that the daily dose of 1500–2000 mg of metformin is well tolerated and multiple clinical trials have reported promising results.

Keywords: Metformin; Drug repurposing; Cancer; Clinical trials; Dose.

Dedication

I want to dedicate this project to my parents for their endless support and encouragement.

Acknowledgement

I would like to proceed by thanking the Almighty who is the source of our strength and knowledge which have enabled me to complete this project with full diligence.

I would like to express my deepest gratitude and appreciation to my project supervisor, Namara Mariam Chowdhury (Senior Lecturer, School of Pharmacy, Brac University), whose expertise, ample time spent and consistent guidance in every step have helped me to accomplish this project efficiently. I would like to thank her for the great advice and patient behavior throughout this phase whenever I encountered difficulty.

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List of Acronyms

T2DM	Type 2 diabetes mellitus
AMPK	Adenosine monophosphate-activated protein kinase
ACC	Acetyl-CoA carboxylase
HIF-1a	Hypoxia-inducible factor-1 alpha
IGF	Insulin growth factor
IGF-1	Insulin-like growth factor-1
IGF-1R	Insulin-like growth factor-1 receptor
IR	Insulin receptor
IL-1	Interleukin 1
NF-B	Nuclear factor kappa
OCT1	Organic cation transporter 1
OCT2	Organic cation transporter 2
ROS	Reactive oxygen species
STAT	Signal transducer and activator of transcription
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
HR	Hazard ratio
95% CI	95% Confidence interval

5-FU	5-Flurouracil
ACF	Colorectal aberrant crypt foci
DDD	Defined daily dose
TRG	Tumor regression grade
SIR	Standardized incidence ratio
PFS	Progression free survival
HOMA	Homeostasis model assessment
BCS	Breast-conserving surgery

Chapter 1

Introduction

1.1 Metformin:

Metformin (dimethylbiguanide), was approved by FDA in 1994 and is the gold standard oral anti-hyperglycemic agent used worldwide also referred to as "Optimal Foundation Therapy" for those with initially diagnosed type 2 diabetes mellitus (T2DM) due to its potent glucose-lowering characteristics, pleiotropic functions, affordability, stability at room temperature with long shelf life, weight-neutrality, broadly favorable safety profile (particularly the absence of hypoglycemia as a detrimental consequence), and moderate cardio-protectivity (Sanchez-Rangel & Inzucchi, 2017). The chemical formula of Metformin is 3-(diaminomethylidene)-1,1-dimethylguanidine where it comprises of two imino and one amino group (primary, secondary, or tertiary) which serve as donor centers (Ismail et al., 2021).

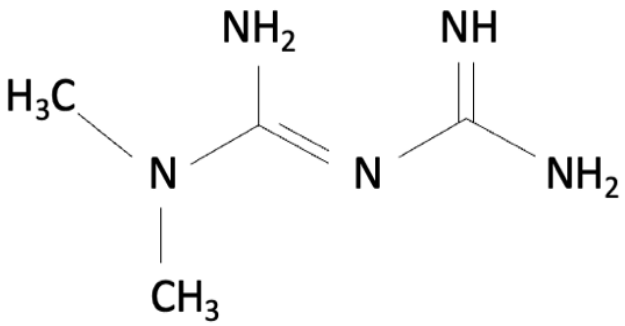

Figure 1: Metformin (CAS 1115-70-4)

1.2 Overall mechanism of action of metformin:

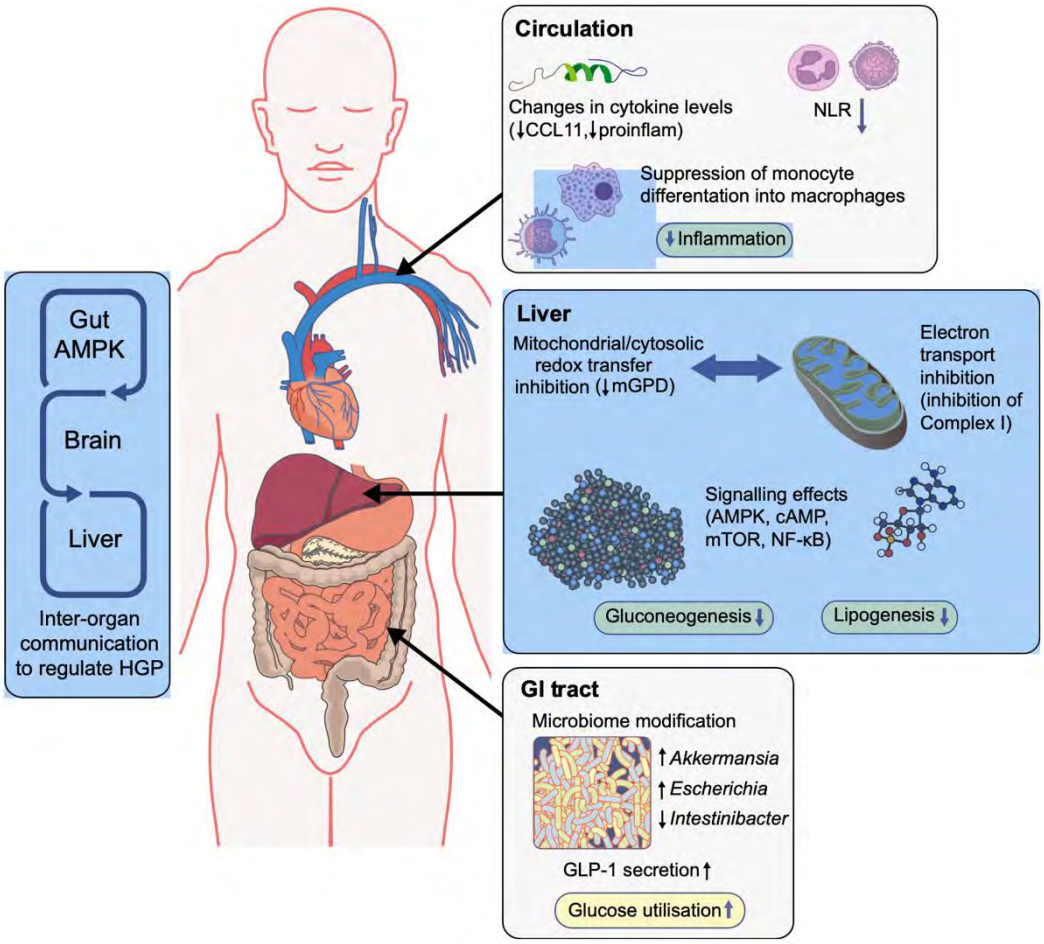

Figure 2: Overall mechanism of action (Rena et al., 2017a)

Schematic representations of metformin effects in the liver, intestines, and blood are presented. Neutrophil to lymphocyte ratio (NLR) is decreased in the blood of people with T2DM in observational studies, and metformin therapy has additionally been proven to inhibit other cytokines, such as C-C motif chemokine 11 (CCL11, also referred as eotaxin-1), in randomized placebo-controlled trials. Additional results demonstrate that this medication has effects on monocytes and macrophages, influencing the differentiation of monocytes into macrophages that secrete proinflammatory cytokines. It alters the microbiota, incretin (GLP-1) secretion, and gut metabolism in the intestines. Furthermore, there is proof that metformin acts through a gut-mediated mechanism that interacts with the brain and liver and indirectly controls hepatic

glucose production. Due to its effects on mitochondrial activity and molecular signaling, metformin lowers lipogenesis and gluconeogenesis in the liver (Rena et al., 2017b).

1.3 Pharmacokinetic profile:

Critiquing the pharmacokinetic characteristics of metformin is crucial to obtain a comprehensive grasp of its pharmacological mechanisms of action. The relative oral bioavailability of metformin ranged between 40 to 60% and is not adequately absorbed from the stomach (approximately 10% throughout the 4-hour interval) (Li & Scheen, 1996). After being absorbed in the upper small intestine's duodenum and jejunum within six hours of administration, it circulates mostly unbound within the body without interacting with plasma proteins (Song, 2016). The range of the mean apparent volume of distribution is 63 to 276L. Metformin is predominantly excreted from the body by active tubular secretion in the kidney and no metabolites are present in urine. For patients with adequate renal function consuming several doses of metformin, the elimination half-life ($t_{1/2}$) is around 5 hours (Robert et al., 2003). Patients who have moderate to severe chronic renal impairment should not be prescribed since it is not metabolized and is excreted unaltered in urine without significant biotransformation (Maideen et al., 2017). Metformin-associated lactic acidosis (MALA) is often induced by increased plasma levels (as observed in people with kidney dysfunction) and a subsequent condition or disease that further compromises lactate production or disposal (e.g., cirrhosis, sepsis, or hypo-perfusion). Despite the fact that it is a very exiguous condition (10 occurrences per 100,000 patient-years of exposure, according to enormous assessments) (Defronzo et al., 2016). To counteract the emergence of this adverse consequence, researchers recommended that the mean plasma concentrations of metformin along a dosing interval have remained below 2.5mg/L (Graham et al., n.d.). Particularly such individuals having an adequate renal function (serum creatinine 133 mol/L [1.5 mg/dL] in males and 124 mol/L [1.4 mg/ dL] in females) should be encouraged to take it. It is suggested that it should not be administered

to older patients with reduced muscle mass if their creatinine clearance is below than 1–1.17 mL/s (60–70 mL/min) (Hundal & Inzucchi, 2003). The remaining 50% of metformin, that is not absorbed, builds up in the distal small intestine's gut mucosa at concentrations 30 to 300 times higher than those detected in plasma before being excreted through feces (Song, 2016).

Metformin's oral absorption, hepatic uptake, and renal clearance are primarily controlled by organic cation transporters (OCTs) (Graham et al., n.d.). At physiological pH, it mostly exists as a protonated cation due to its strong base with a pKa of 12.4. Despite having hydrophilic nature, metformin can penetrate cell membranes with the support of OCT (Kinaan et al., 2015). OCTs are poly-specific transporters that are typically present in the liver and kidney, in which are necessary for extracting organic cations from the bloodstream (Motohashi & Inui, 2013). OCT1 and OCT2 both have a key role in transportation. OCT1 has been shown to be vital for metformin absorption in the liver, which is required for both the drug's therapeutic efficacy and feared adverse effects such as lactic acidosis. Conversely, OCT2, that is formed on the basolateral membrane of kidney tubular cells, has been associated with metformin excretion through the kidneys (Zhou et al., 2007).

1.4 Uses of metformin:

Over time, metformin has been proven to have an expanded role besides its effects on glucose level solely, including polycystic ovarian syndrome (PCOS), obesity, cancer (e.g., breast cancer, endometrial cancer, bone cancer, colorectal cancer, and squamous cell carcinoma), immunoregulatory, cardiovascular disease, renal disease, liver disease, HIV associated metabolic abnormalities, and nonalcoholic steatohepatitis (NASH) by targeting different site of action (Lv & Guo, 2020) (Hundal & Inzucchi, 2003).

1.5 Drug repurposing:

It takes an average of 13 years of research to establish a new drug. The effectiveness, tolerability, pharmacokinetic and pharmacodynamic characteristics of the drug in ex-vivo and in-vivo-based research must also be evaluated in addition to design and production. It costs expenses to move a single novel medicine from a bench to the bedside (Z. Zhang et al., 2020). Because of the prolonged discovery process, drug repurposing paved the way for a feasible strategy to accelerate the development process of a new drug (Parvathaneni et al., 2019).

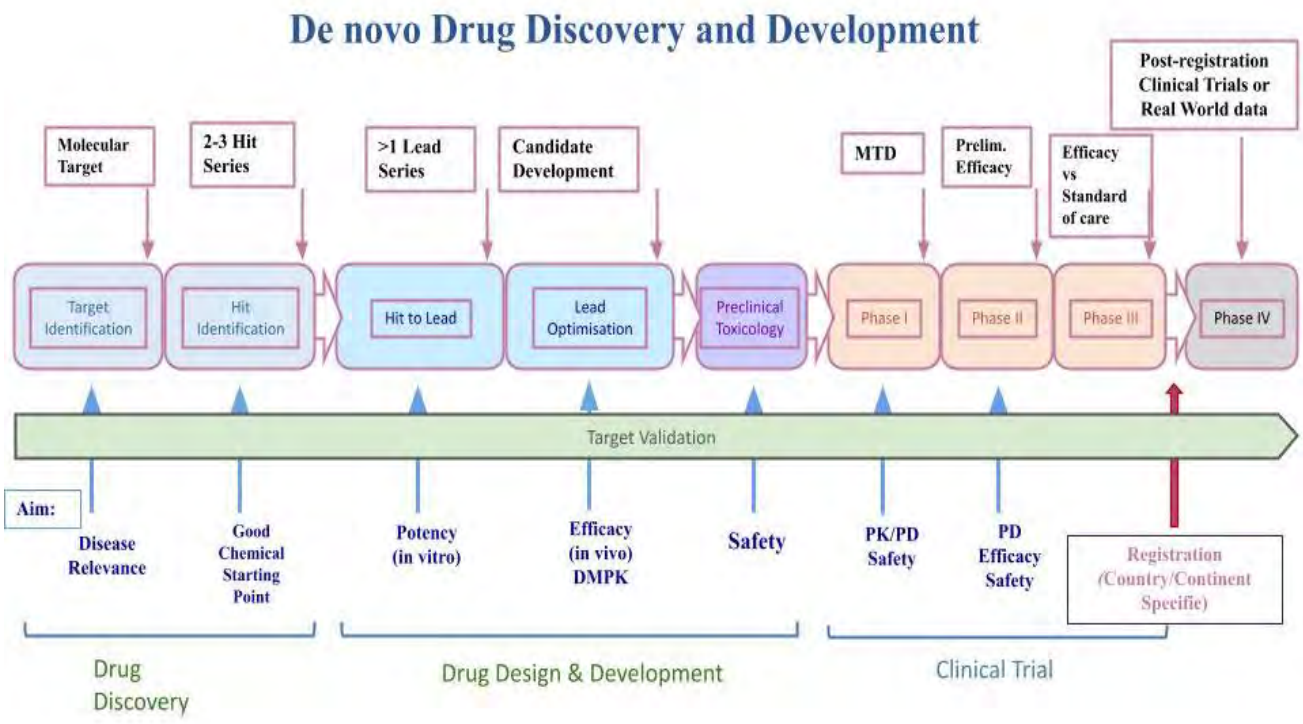

Figure 3: De novo Drug Discovery and Development (Z. Zhang et al., 2020)

The establishment of the alternative approach of drug repurposing—the development of current medications for brand-new therapeutic utilization necessitated due to the persistent impediments towards the discovery of novel medications for cancer therapy as it is cost-effective and permits speedy therapeutic translation (Z. Zhang et al., 2020). The inadequacy of

traditional therapies to effectively obliterate tumor cells is a factor in the poor prognosis of tumors due to their continued multiplication, susceptibility to metastasis, sensitivity to radiation and chemotherapy, and perhaps other physiological properties (Yu et al., 2019).

1.6 Metformin repurposing:

Multiple properties of metformin are considered suitable for repurposing it as an anti-cancer treatment. A case study with 923 T2DM patients belonging to the United Kingdom was the first epidemiological research to relate metformin towards the prophylaxis of cancer, where it was observed that taking metformin was attributed to a 23% lesser probability of establishing cancer. Since then, it has resulted in a notable upsurge in the number of retrospective researches inquiring into the relationship between metformin consumption and the likelihood of developing cancer. On a corollary, a series of meta-analyses have been conducted to consolidate the current statistics. The major aim of cancer treatment is to surgical resection, radiotherapy, ease symptoms, boost the effectiveness of adjuvant therapy, or inhibit relapse. In various in-vitro and in-vivo animal trials, metformin has been confirmed to possess a strong antagonistic effect on metabolism-related tumors, growth-inhibiting properties in breast, endometrial, lung, liver, gastric, and medullary thyroid cancer cell lines moreover augment the effectiveness of chemotherapy (Morales & Morris, 2015).

1.7 Mechanism of action of Metformin as a promising anti-cancer agent:

The following are some of the methods through which metformin exerts its anti-neoplastic effects:

1.7.1 Metformin and Mammalian Target of Rapamycin Complex 1:

In order to reduce the development of cancer cells, suppression of the mammalian target of rapamycin complex 1 (mTORC1) is vital. A multiprotein complex called mTORC1 is mostly

made up of the protein kinase mTOR and the scaffolding protein raptor. Tuberous sclerosis Complex (TSC2) can be directly phosphorylated by Adenosine Monophosphate Protein Kinase (AMPK), which promotes TSC2's suppression of mTORC1. The fact that protein synthesis is stimulated by mTOR highlights this protein's function in the growth and metabolic activity of cancer cells. An assortment of malignancies with activated RTKs or IR may benefit from mTOR inactivation (Andrzejewski et al., 2018).

1.7.2 Initiation of Adenosine Monophosphate Protein Kinase (AMPK):

The elevated AMP/ATP proportion can trigger the cellular energy sensor AMPK. The activity of respiratory complex I may be inhibited by metformin, causing in decreased oxidative phosphorylation and ATP generation, which lowers cellular ATP and activates AMPK (Zi et al., 2018). By the phosphorylation of S722 and S792 on the mTOR binding raptor, mTORC1 is directly blocked. This is comparable to how metformin acts to control diabetes (Kahn et al., 2005).

1.7.3 Inhibition of Generation of Reactive Oxygen Species (ROS):

Numerous cancer forms exhibit a dramatic elevation in the ROS signaling systems, which culminate in aberrant growth and division. Peroxides, super-oxides, hydroxyl radicals, singlet oxygen, and alpha oxygen are various reactive oxygen species (Ugwueze et al., 2020). The reversible oxidation of tyrosine phosphatases, tyrosine kinases, and transcription factors is considered to be mediated by hydrogen peroxide, a prominent form of ROS (Lennicke et al., 2015). Metformin's effect on Complex 1 of the respiratory chain, that decreases electron access to the chain and consequently ROS formation, mediates the reduction of ROS formation (Algire et al., 2012). The AMPK system has no impact on lowering endogenous ROS production. The DNA, which is among the crucial targets of ROS-induced cellular damage, is

distorted structurally due to mutation. According to flow cytometry, cells primed with metformin can minimize ROS levels upon paraquat exposure (Aljofan & Riethmacher, 2019).

1.7.4 Reduction of Serum Levels of Insulin, IGF-1, and IGF-2:

IGFs are peptides with highly identical insulin sequences that promote cell growth, motility, and act as both endocrine hormones and paracrine growth factor. The frequency of triggers that induce the multiplication of cancer cells is decreased by metformin. In cancer sufferers, excessive levels of IGF-1 and IGF-2 are associated with cancer progression or relapse. IGF-IR, a transmembrane tyrosine kinase that is structurally similar to the insulin receptor, mediates the activities of IGF proteins. IGF-1 and IGF-2 binding to IGF-receptors ultimately activates mTOR, which increases tumorigenesis and inhibits mortality (Ugwueze et al., 2020).

1.7.5 Inhibition of Chronic Inflammation:

A crucial factor in the initiation and promotion of carcinogenesis is chronic inflammation. The very first stage of the inflammatory response related to cellular transformation and the development of cancer stem cells is inhibited by metformin. Inactivation of NFKB induced by metformin consequences in decreased secretion of pro-inflammatory cytokines (Podhorecka et al., 2017)

1.7.6 Modulation of Adenosine A1 Receptor (ADORA1) Expression:

ADORA1 is notably significant for its immunoregulatory mechanism in malignancies. Four members of the ADORA family includes ADORA1, ADORA2a, ADORA2b and ADORA3. According to studies, adenosine has been observed to disproportionately store in the tumor microenvironment and attach to adenosine receptors to retain the immune system homeostasis

in malignancies. Particularly, ADORA1 promote the tumor progression in CRC, BC, RCC, and leukemia (Lin et al., 2021).

1.7.7 Downregulation of Gluconeogenesis in the Mitochondria:

Metformin's anticancer action is associated with its ability to reduce gluconeogenesis in the mitochondria. Multiple signaling pathway that regulate tumor growth, motility, and penetration are modified by hyperglycemia. As a response, hyperglycemia produces the adenosine triphosphate (ATP) that cancer cells need in order to replicate efficiently (Bridges et al., 2014)

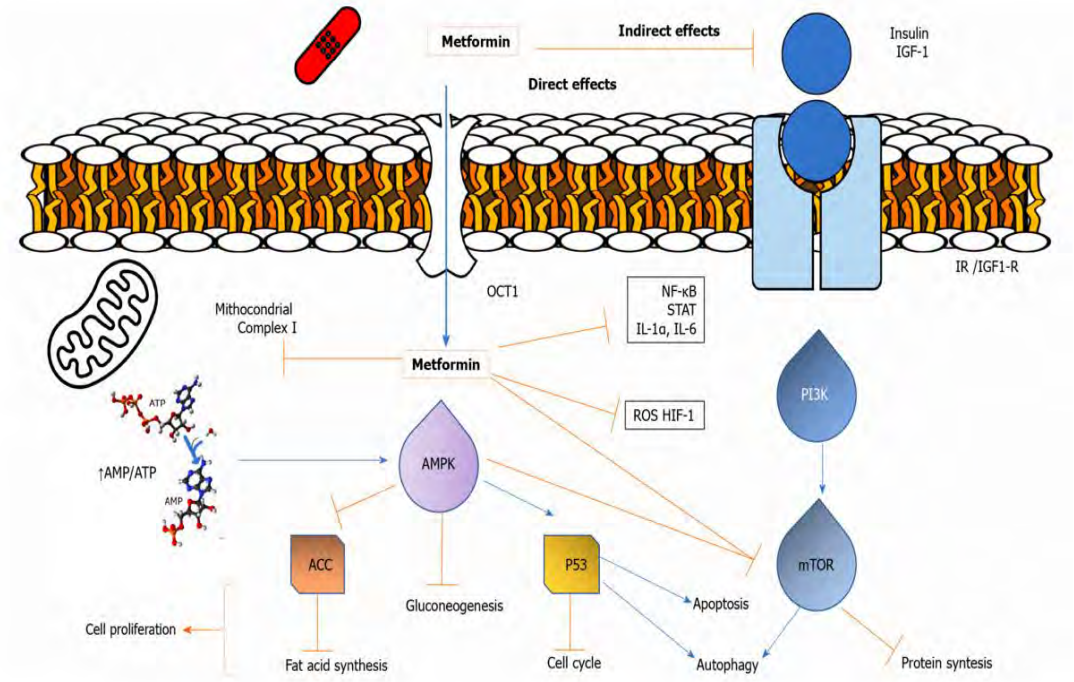

Figure 4: MOA of metformin in cancer (Júnior & Jbc, n.d.)

Despite the fact that metformin has a variety of therapeutic advantages in the treatment of various types of cancer, there are not enough studies to conclusively demonstrate which cancer types metformin is more successful in curing as well as the role of metformin dose that play a significant role in overall survival of cancer patient. Therefore, considering these gaps, the present review aimed to identify which type of cancer metformin shows better performance in a way to promotes overall survival rate and hence what should be its dosage requirements. The current study also discusses different types of the potential mechanism of action in the inhibition of cancer relating to metformin. The purpose of this research work is to deliver an exceptional insight into metformin usage with respect to cancer treatment as well as to give vast access of gaining current information in terms of encouraging to conduct more studies of this drug.

Chapter 2

Research Methodology

For conducting this review study, relevant data were gathered by a comprehensive literature search. A number of reliable sources, including peer-reviewed journals and an online scholarly database, were used to compile the information. Here is a list of a few of the several databases that have been searched for this study.

- Journal Database
- Newspaper Database
- Professional website
- Library Catalogue

In order to assemble as much essential information as possible regarding the use of metformin in cancer treatment, and associated clinical trial data, a thorough search of several journals, review articles and research papers from official websites and research databases was performed. Utilizing well-known and reliable sources including PubMed, SCOPUS, Google Scholar, and ScienceDirect, clinicaltrials.gov the data for this review study was collected. Relevant papers were gathered using appropriate important keywords, such as metformin, drug repurposing, cancer, clinical trials data. Around 135 articles have been assessed based on the title and keyword content. Then, 52 papers were reduced after reading the abstracts. The 83 papers that made up this review research were carefully selected and examined. Mendeley software was used for accurate and fair referencing in order to show respect for the writer's original works.

Chapter 3

Colon Cancer

Unregulated cell division is a hallmark of the condition recognized as cancer. It is referred to as colorectal cancer (CRC) when this type of malignancy arises in the colon or rectum. With over 500,000 cases reported and 600,000 deaths annually, it is the second most prevalent cancer in women as well as the third highly widespread cancer in men and the fourth most commonly diagnosed cancer leading to death. Younger than 50 years old, the rate is minimal; however, it rises dramatically with age (Brenner et al., 2014). Throughout various nations over the recent decades, the prognosis of individuals suffering from bowel carcinoma has modestly but consistently progressed. Men experience 25% more morbidity and fatality than women, and the 5-year and 10-year survival rates are 65% and 58%, respectively (Li et al., 2021). This accelerated surge in CRC rates in developed nations may be driven by unhealthy eating patterns, sedentary lifestyles, diabetes mellitus, overweight, alcohol and tobacco consumption. Alongside environmental conditions, hereditary and epigenetic alterations can also trigger the transformation of healthy gut epithelial cells into malignant cells (Dalal et al., 2020). The large intestine, the last portion of the gastrointestinal (GI) tract, is comprised of the colon and rectum (colorectum), and anus. Since the large intestine is also alluded to as the large bowel, CRC has often termed bowel cancer.

3.1 Colorectal Polyp:

A precancerous polyp, a non-malignant development which forms in the mucosal layer (interior layer) of the colon or rectum, is typically where CRC originates. These polyps' proliferating cells may eventually aggregate substantial genetic modifications to offer them the potential to penetrate the gut wall and ultimately, they might mutate further, migrate to adjacent lymph nodes, and thereafter to distal metastatic sites. Adenomas and sessile serrated polyps (SSPs)

are indeed the two primary forms of polyps with carcinogenic capacity, and both have been correlated to a particular chance for progressing into a CRC (Simon, 2016). It is thought that less than 10% of polyps develop into metastatic tumors.

3.2 Stages of colon cancer:

- Stage 0 (carcinoma in situ): At this stage the mucosa, the colon wall's innermost layer, contains aberrant cells. These aberrant cells might transform into carcinoma and metastasize to surrounding healthy tissue.
- Stage I: In this stage, the mucosa has developed cancer, which has subsequently migrated to the submucosa, the layer of tissue right beneath the mucosa, or the muscle layer.
- Stage II: During this stage, the serosa (exterior part) of the colon wall has been penetrated by cancer, which has then reached surrounding organs.
- Stage III: In this phase, colon cancer has progressed to one or even more lymph nodes beyond the colon.
- Stage IV: In the terminal stage, different body organs such as the lung, liver, abdominal wall, or ovary have been compromised by the cancer's dissemination via the plasma and lymphatic nodes (in females).

It is highly acknowledged that colorectal cancers developed from the stimulation of oncogenes (KRAS) as well as the inactivation of tumor suppressor genes (APC, p16, p53, and DCC). One of most widely changed gene is KRAS. As aberrant KRAS proteins are tiny, scarcity of binding sites, they are perceived as an inaccessible target (Ruiz-Bañobre & Goel, 2021). Notably, KRAS mutation is detected in 30%–40% of incidences of colon cancer. KRAS alterations in colon cancer have been linked to a worse prognosis and more vigorous tumor progression.

KRAS mutations in colorectal cancer also cause inhibition to several types of therapy (Daniela, n.d.). On the other side, the Adenomatous Polyposis Coli (APC) gene prevents the formation of tumors. If the APC gene is mutated, it becomes stagnant and thus more liable to further mutations that might contribute to rectal and colon cancers. Another tumor suppressor gene known DCC (deleted in colorectal cancer), produces a protein with receptor interactions involved in cell motility and death. Approximately 70% of colorectal tumors lost the DCC allele (Mehlen & Fearon, 2004). Moreover, under conditions of cellular stress, the tumor suppressor p53 protein functions as a transcription mediator to trigger cell cycle arrest, senescence, and apoptosis. The adenocarcinomas transformation throughout the metastatic tumor pathogenesis and progression is hypothesized to be significantly impacted by p53 alterations. The p53 mutation is observed in 34% of proximal colon tumors and 45% of distal colorectal tumors in CRC (X. L. Li et al., 2015).

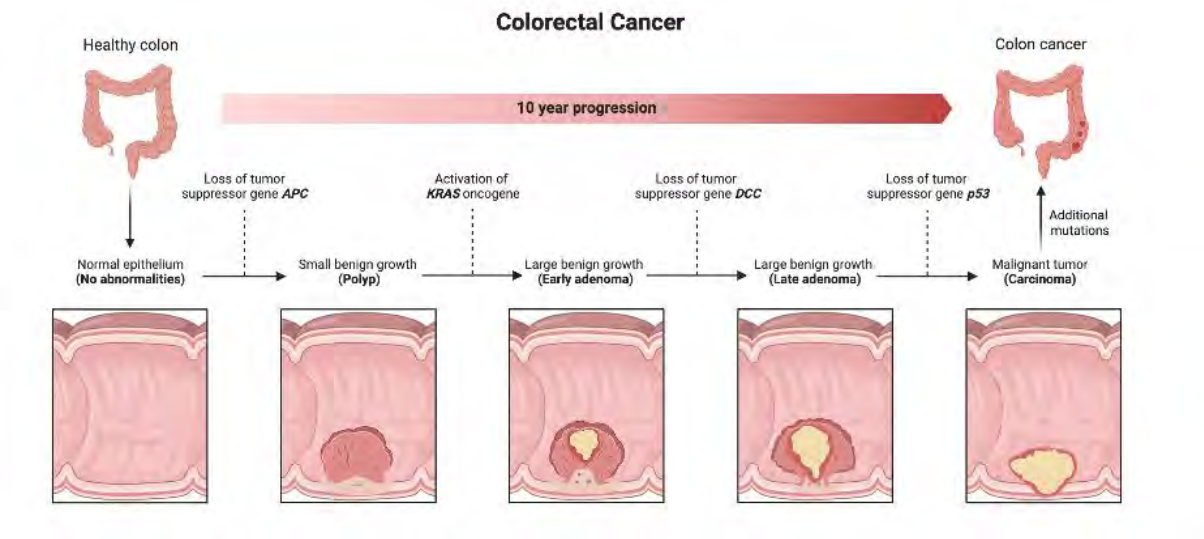

Figure 5: Stages of colorectal cancer (Hossain et al., 2022)

3.3 Currently available therapies for treating CRC:

At present, the major therapeutic possibilities are chemotherapy and surgery, including a right colectomy, sigmoid colectomy, and complete abdominal colectomy with ileorectal anastomosis. Moreover, 5-fluorouracil (5- FU) alone or in addition with additives including oxaliplatin and avastin is frequently prescribed to cure individuals with severe levels of CRC. The patients are generally beset by serious adverse effects like severe nausea, vomiting, weight loss, and the likelihood of viral comorbidities owing to immunocompromised, despite the fact that these treatment approaches are efficacious at relieving symptoms and overall mortality. Nowadays, the recommended treatment strategy for locally progressed CRC is neoadjuvant concurrent chemoradiotherapy (CCRT) accompanied by total mesorectal excision (TME). This may promote sphincter retention, increase resectability, and minimize local recurring (Oh et al., 2016). Among the most widely performed chemotherapeutic treatments for progressive malignancy is FOLFOX, which combines oxaliplatin and 5-fluorouracil (Field et al., 2008; Oweira et al., 2018). According to the plethora of scientific findings, Metformin has been shown to be effective against CRC by inhibiting the processes of invasiveness and hyperproliferation and suppressing carcinogenesis with its anti-angiogenesis, radio-chemosensitizer, and antimetabolic properties (Kamarudin et al., 2019).

Table 1: The summarization of metformin clinical use for CRC

Number of patients	Disease Condition	Interventions	Dose	Life quality/ side effects	Summary of findings	Reference
595 patients (Metformin user: 258 patients and Nonuser: 337 patients	Both CRC and diabetes	(i) Metformin (ii) Aspirin	Not mentioned	No serious side effects	Metformin use showed a lower risk of CRC-specific mortality (HR = 0.66; 95%CI: 0.45-0.975) in patients with diabetes.	(Lee et al., 2012)
23 patients (Metformin user: 9 patients and Control group: 14 patients)	CRC	Metformin	250mg/day	No side effects	Directly suppressed both colorectal epithelial proliferation and ACF formation.	(Hosono et al., 2010)
151 patients (Metformin user: 79 patients and Control group: 72 patients)	CRC Everyone had previous resections experience.	Metformin	250mg/day for one year	No serious adverse effects	Following polypectomy, reduced dose of metformin lowered metachronous adenomas or polyps.	(Higurashi et al., 2016)
Diabetic and non-diabetic 50 patients. Older than 18 years (mean – 57 years old)	Refractory metastatic CRC. Everyone received chemotherapy and radiation treatment.	(i) Metformin (ii) 5-FU (ii) Leucovorin	(i) Metformin: 850 mg orally 2 times/day (ii) 5-FU: 425 mg/m2 (iii) Leucovorin: 50 mg by I.V.	Diarrhea, nausea, vomiting, and myelotoxicity	(i) Treatment - median PFS of 1.8 months and OS of 7.9 months (ii) 22% met primary end-point – median PFS of 5.6 months and OS of 16.2 months.	(Miranda et al., 2016)

Number of patients	Disease Condition	Interventions	Dose	Life quality/ side effects	Summary of findings	Reference
2088 cases (66-80 years); and 9060 control (61-77 years)	CRC Patients had Chemotherapy and radiation treatment.	(i) Metformin (ii) Aspirin (iii) NSAIDs (iii) Sulfonylurea	2000 g (DDD) cumulatively in 5 years	Not mentioned	(i) Lower incidence of CRC OR 0.83, 95% confidence interval 0.68-1.00 (ii) Metformin dose and interval response: significantly lessened incidence of CRC > 250 DDD and > 1 year	(Cardel et al., 2014)
1304 patients (18 years old and above) Diabetic patient (Metformin user): 133 Diabetic patient (Metformin non-user): 144 Nondiabetic patient: 1027	CRC	(i) Metformin (ii) Diabetes controlling other oral agents except metformin (iii) Insulin	Not mentioned	No side effects	Extended OS (91% at 1 year, 80.5% at 2 years, and 72.2% at 3 years).	(Ramjeesingh et al., 2016)
543 patients Diabetic patients: (Metformin user): 42 patients; (Metformin non-user): 29 patients Non-diabetic patients: 472 patients	CRC Patient received neoadjuvant chemoradiotherapy, radical surgery.	(i) Metformin (ii) 5-FU (iii) Leucovorin (iv) Capecitabine	(i) Metformin (500mg; 2 times/day). (ii) Chemotherapy (concurrently with radiotherapy) using either - 5-FU (425mg/m^2/day) and Leucovorin (20mg/m^2/day) for 5 days. Other patients received Capecitabine (825mg/m^2/day) bid during radiotherapy.	No side effects	Metformin using patients exhibited greater rates of T downstaging, N downstaging, TRG, and pCR.	(Oh et al., 2016)

Number of patients	Disease Condition	Interventions	Dose	Life quality/ side effects	Summary of findings	Reference
424 patients	CRC Patients received chemotherapy, radiation.	(i) Metformin (ii) Insulin (iii) Aspirin (iv) ADDs (v) Anti-cholesterol	Not mentioned	Not mentioned	Metformin ensures 30% improvement in OS as compared to other ADDs.	(Kamarudin et al., 2019)
202 patients Diabetic patients: (metformin user): 104 patients and (metformin non-user): 98 patients	CRC and T2DM	(i) Metformin (ii) Diabetes controlling other oral agents except metformin	Not mentioned	No side effects	Improved overall survival, fewer recurrences, and reduced metastases.	(Henderson et al., 2017)
111109 Patients	CRC and diabetes	Metformin	Increasing cumulative dose	24718 (22.2%) have died during study period.	CRC risk significantly decreased with increasing cumulative dose of metformin ($P < 0.001$)	(Dulskas et al., 2020)

Numerous researches investigated metformin's effectiveness in preventing CRC. (Lee et al., 2012) assess the relationship between the intake of metformin and CRC fatality in diabetes patients. For this 595 people with CRC and diabetes mellitus were detected. Patients were divided into two groups; 258 diabetic individuals consuming metformin and 337 diabetic patients not consuming metformin. Patient characteristics, disease manifestations, overall survival, and CRC-specific fatalities were examined. Following a median follow-up of 41 months, 258 patients who administered metformin reported 71 total deaths (27.5%) and 55 CRC-specific deaths (21.3%), in comparison to 337 individuals who did not take metformin, experienced 136 total deaths (40.4%) and 104 CRC-specific deaths (30.9%). According to a univariate study, using metformin was related to lower rates of overall mortality (p=0.018) and CRC-specific mortality (p=0.042). After adjusting for clinically significant variables, metformin use in CRC patients with diabetes suggested a decreased risk of overall death (HR, 0.66; 95% CI 0.476-0.923; p=0.015) and CRC-specific mortality (HR, 0.66; 95% CI 0.45-0.975; p=0.037). Diabetes and CRC patients have a lesser incidence of both CRC-specific and overall death while using metformin. Furthermore, to count the number of aberrant crypt foci (ACF; a potential precursor to CRC), participants were randomly assigned to receive either extremely low dose metformin (250 mg/day) or no treatment for one month. A substantial drop in ACF (mean ± SD) was seen in the metformin arm but not in the control group, going from 8.78 ± 6.45 to 5.11 ± 4.99 (p=0.007). This study ensures the safety and direct suppression of both colorectal epithelial proliferation and ACF formation (Hosono et al., 2010). In multi-centre, double-blind, placebo-controlled**,** randomized phase 3 trials, adult non-diabetic patients who had previously undergone endoscopic removal of one or more colorectal polyps or adenomas were included in the study. Implementing a stratified machine randomization procedure, patients who fulfilled the criteria were arbitrarily selected (1:1) to receive either oral metformin (250 mg daily) or identical placebo tablets. The patients were classified by the

institute, age, gender, and BMI. The randomization process involved 151 patients, of whom 79 received metformin and 72 received a placebo. A 1-year follow-up colonoscopy was conducted on 71 metformin-treated individuals and 62 placebo-treated participants. Total polyp predominance (hyperplastic polyps plus adenomas) and adenomas were considerably less in the metformin group than in the placebo group (total polyps: metformin group 27 [38·0%; 95% CI 26·7–49·3] of 71 patients, placebo group 35 [56·5%; 95% CI 44·1–68·8] of 62; p=0·034, risk ratio [RR] 0·67 [95% CI 0·47–0·97]; adenomas: metformin group 22 [30·6%; 95% CI 19·9–41·2] of 71 patients, placebo group 32 [51·6%; 95% CI 39·2–64·1] of 62; p=0·016, RR 0·60 [95% CI 0·39–0·92]). This study suggests that, upon polypectomy, a moderate dose of metformin improved the occurrence and frequency of metachronous adenomas or polyps as well as it was risk-free for the patient who were taking lose dose of metformin without having diabetes (Higurashi et al., 2016). Then in a single-center, single-arm phase 2 clinical trial who had previously received treatment with 5-fluorouracil (5-FU), irinotecan, oxaliplatin, and an anti-epidermal growth factor receptor (if the tumor was RAS dominant allele) were enlisted to begin receiving metformin 850 mg orally twice daily and including 5-FU 425 mg/m2 and leucovorin 50 mg intravenously weekly till the advancement of cancer. 11 (22%) patients out of the 50 total patients attained the main endpoint. The median overall survival was 7.9 months, whereas the median progression-free survival was 1.8 months. When solely the 11 patients who already had clinical prevention at 8 weeks were reviewed, their median overall survival was 16.2 months and their median progression-free survival was 5.6 months (Miranda et al., 2016). Additionally, (Cardel et al., 2014) did a nested case–control experiment where 2088 cases and 9060 controls were selected based on exclusion criteria. Metformin was demonstrated to exert a prophylactic effect in this investigation, with an adjusted OR of 0.83 (95% CI 0.68-1.00) on the probability of obtaining CRC. Concerning the correlation between dose and response, there was a declining chance of CRC with cumulative

doses of metformin > 250 DDD and with administration > 1 year. Gender seemed to have a massive effect on the relationship. Long-term metformin use had a protective impact against CRC in women (OR = 0.66, 95% CI = 0.49-0.90), but not among males (OR = 0.96, 95% CI = 0.75-1.23). Metformin use has been connected to obesity, a risk factor for CRC. In a retrospective study, they included 1304 CRC patients. Patients with diabetes who were taking metformin lived significantly longer than those who were taking other diabetes medications except metformin, according to a subgroup analysis (OS for the metformin group: 91% at 1 year; 80.5% at 2 years; 72.2% at 3 years and OS for the group taking other treatments, including diet control: 80.6% at 1 year; 67.4% at 2 years; 53.5% at 3 years). Metformin treatment was found to be positively correlated with prognosis (Ramjeesingh et al., 2016). In a different study, (Oh et al., 2016) investigated metformin improves the tumor responsiveness to preoperative CCRT in T2DM patients who had locally established CRC. Altogether 543 individuals had examined: 42 patients received metformin, 29 patients received no metformin, and 472 patients had no diabetes history. Compared with the non-diabetic category, participants in the metformin and non-metformin groups were elderly and had increased BMI ($p < 0.001$ and $p = 0.012$, correspondingly). Simultaneous radiation and chemotherapy were provided by either a capecitabine- or 5-fluorouracil (FU)-based regimen. Throughout the initial and fifth weeks of radiation, 356 of the 543 patients got 5-FU (425 mg/m2/day) by IV route and leucovorin (20 mg/m2/day) for 5 days. All underwent TME surgery between six and ten weeks when neoadjuvant CCRT was finished. Six to ten weeks following the completion of neoadjuvant CCRT, each had surgery with TME. To measure the clinical tumor response, analysis of T downstaging, N downstaging, TRG, and pCR was carried out. Regarding T downstaging and pathologic complete response, there were no appreciable variations amongst the subgroups. Nevertheless, the metformin group experienced notably increased N downstaging (85.7%) in comparison to the non-metformin (51.7%) or non-diabetic (73.1%) groups ($p = 0.006$). Also,

compared to the non-metformin (34.5%) and non-diabetic (42.2%) groups, the metformin group had a considerably increased rate of TRG 3-4 (61.9%) (p = 0.029). In the multivariate analysis for N downstaging, metformin use (OR = 6.132; 95 % CI = 1.864–20.169; p= 0.003) and an interval to surgery of more than 8 weeks (p = 0.031) were key indicators. Metformin use (OR = 3.988; 95 % CI =1.261–12.610; p = 0.019) and young age (p = 0.019) were related to increase rates of TRG 3–4. In another cohort study conducted by (Henderson et al., 2017), A total of 202 CC patients were selected for the cohort for the project's first arm. There were 104 patients in the metformin group and 98 patients in the non-metformin group. CEA levels, relapses, metastases, subsequent malignancy, and survival were monitored. Metformin use reduced tumors and relapses in CC patients. Reduced mortality was detected for CC in the metformin group 48% vs 76%, p<0.001), recurrences (4% vs 19%, p= 0.002), metastases (23% vs 46%, p=0.001), better 5-year survival rates (57% vs 37%, p=0.004), overall survival years (5.7 vs 4.1, p=0.007), and grater CEA decrease (72% vs 47%, p=0.015). In another retrospective cohort research, they estimated SIRs for colorectal malignancies as a proportion of the actual occurrence of carcinoma diagnoses among individuals who had diabetes to the anticipated number of cancer diagnoses in the underlying normal community. In total, 70 038 females and 41 071 males formed the last group. At the completion of the following period, 86 391 diabetes patients (77.8%) were surviving, while 24 718 (22.2%) had passed away during the trial timespan. (Dulskas et al., 2020) analyzed that CRC risk significantly decreased with an increasing cumulative dose of metformin ($P < 0.001$). In a comprehensive trial from China, researchers discovered that a higher dose of metformin at greater than 0.25 average DDD (equivalent to more than 500 mg/day) was related to an 80% decreased risk of developing cancer in comparison to a decreased dosage (Chen et al., 2015). In Ireland, a robust correlation was found between high-intensity intermittent metformin therapy and a decline in CRC specific

mortality as compared to more than 300 diabetes individuals and 3500 non-diabetic patients (Spillane et al., 2013).

The above analysis depicts the beneficial use of metformin from up-to-date clinical reports, as potential chemotherapeutic and adjuvant therapy for CRC. The studies suggested that for ensuring better efficacy metformin dose should be higher than the usual dose suggested for diabetes. A higher dose of metformin at greater than 0.25 average DDD (equivalent to more than 500 mg/day) was related to an 80% decreased risk of developing cancer.

Chapter 4

Breast cancer

Breast cancer (BC) is a heterogeneous devastating condition that is comprised of several entities with diverse histological and physiological features, therapeutic indications and behaviors, and pharmacological responses. It is the second most common cancer reported globally, irrespective of gender, having an occurrence of 11.9%. Aging, heredity, family background, nutrition, alcoholism, overweight, lack of physical activity, and endocrine variables (both endogenous and exogenous) are some of the possible causes of BC. These variables potentially function independently or in combination to develop BC. It is predicted to affect one in eight women worldwide, and only 5-10% among all instances are likely to be carried forward by hereditary issues, with the other 90-95% being attributed to environmental exposures and way of living (Kolak et al., 2017).

4.1 Gene related to BC:

A substantial specific chance for acquiring genetic BC is conferred by germ-line mutations in high-penetrance BC susceptibility genes including BRCA1, BRCA2, p53, and PTEN, that account for 5-10% of all BC (Abdulkareem, 2013). The two most prominent anti-oncogenes, BRCA1 and BRCA2, are present in chromosomes 13q12 and 17q21, correspondingly, are responsible for encoding tumor suppressor proteins. Lack of BRCA1 causes aberrant centrosome proliferation, genetic mutations, disruption of the cell cycle checkpoint, and ultimately cell death (Sun et al., 2017). BRCA1 deleterious mutation carriers have a 60–85% likelihood of developing cancer, with an increased risk at younger ages (Romagnolo et al., 2015). Mammary tumors with a luminal phenotype that are related to BRCA2 are more likely to be advanced invasive ductal cancer (Bane et al., 2007). Women who are BRCA1 carriers are moderately more prone than BRCA2 carriers to acquire BC by the age of 70. Additionally,

triple negative BC, which is more aggressive and challenging to cure than some other subtypes of BC, is generally associated with BRCA1 mutations. In contrast, males with the BRCA1 gene mutation have a 1% lifelong threat and those with the BRCA2 gene mutation get a 6% lifetime chance of acquiring male BC. Particularly in the pre-menopausal age range, gene-positive patients' risk of developing BC is 80% (Abdulkareem, 2013). Then, human epidermal growth factor receptor 2 (HER2), located on the long arm of human chromosome 17 (17q12) and accelerates rapid cell growth in BC. HER2-positive BC cells are defined as having higher than-average levels of HER2. Although these tumors have a tendency to grow and spread more quickly than HER2-negative BC, they also have a significantly higher propensity to respond to HER2 protein-targeting medications. Genetic multiplication and re-arrangement are the major ways that trigger the HER2 gene's expression. PTEN/Akt/mTORC1 signaling enhances the amount of cancer stem cells and HER2 is found to be overexpressed in 20% of initial breast tumors, that is a marker of poor treatment prognosis (Davis et al., 2014; Elizalde et al., 2016). Furthermore, the EGFR protein, a part of the tyrosine kinase group of cell membrane glycoproteins, is initiated by interacting with substances like EGF, TGF-α, amphiregulin, betacellulin, and others. It is positioned on the short arm of chromosome 7 (7p12) (Sun et al., 2017). The EGFR family is comprises of EGFR1 (EGFR, HER1, c-ErbB1), EGFR2 (HER2, c-ErbB2), EGFR3 (c-ErbB3, HER3) and EGFR4 (c-ErbB4, HER4) proteins (Butti et al., 2018; Macdonald-Obermann & Pike, 2014; Wilson et al., 2009). As a result of gene amplification, EGFR is frequently overexpressed in various human malignancies (Gan et al., 2013). Elevated EGFR1 activation was noticed in 16.4% of the tumors, enhanced HER2 activation was showed in 22.8% of the tumors, increased EGFR3 activity was observed in 17.5% of the tumors, and increased EGFR4 activity was seen in 11.9% of tumors. Patients with EGFR1, HER2, or EGFR3 overexpression in their BC malignancies had lower chances of survival. Patients with BC who had tumors that exhibited increased amounts of EGFR4 had a favorable prognosis

compared to patients whose tumors expressed EGFR1, HER2, or EGFR3 (Davis et al., 2014). With over 30% of incidences of inflammatory breast cancer (IBC), an extremely malignant form of BC indicates overexpression of EGFR. In comparison to patients with EGFR-positive IBC have a worse prognosis than those with EGFR-negative IBC (Alanazi & Khan, 2016; D. Zhang et al., 2009). In order to eradicate these cancerous growths, it may be advantageous to target the EGFR pathway. Additionally, p53 is a tumor suppressor gene but it behaves as an oncogene in its muted form and involved in the development of both sporadic as well as some heredity breast tumors (Coles et al., 1992).

4.2 Stages of BC development:

Stage 0: The epithelium of ducts or portions of the breast may have abnormal cells, but they do not disseminate to neighboring tissue. increased chance of BC. In this stage, the survival rate is 100%.

Stage I (Early stage): Very limited region of tissue has been affected by the cancerous growth. The diameter of the tumor is less than two centimeters. The survival rate is 98% throughout this stage.

Stage II (Localized stage): Despite being confined to a small portion of the breast, cancer has spread. The size of the tumor is two-five centimeters. Auxiliary lymph nodes may become affected by cancer and the survival rate is 88% approximately.

Stage III (Regional spread): Tumor is more than five centimeters in diameter, and secondary lymph nodes have been affected by malignancy. In certain instances, there may be no tumor at all. Skin or the chest wall may have undergone cancerous spread. Skin color variations or potential irritability start to appear. There is a 52% survival rate.

Stage IV (Distant Spread): Far beyond the breast, cancer has migrated toward other organs. The survival rate drops to 16% at this stage, which is the lowest of all.

It is exceedingly challenging to develop a BC medication agent which is beneficial due to the enormous heterogeneity in oncogene and tumor suppressor genes and abnormalities among diverse subtypes of BC (Cai et al., 2019).

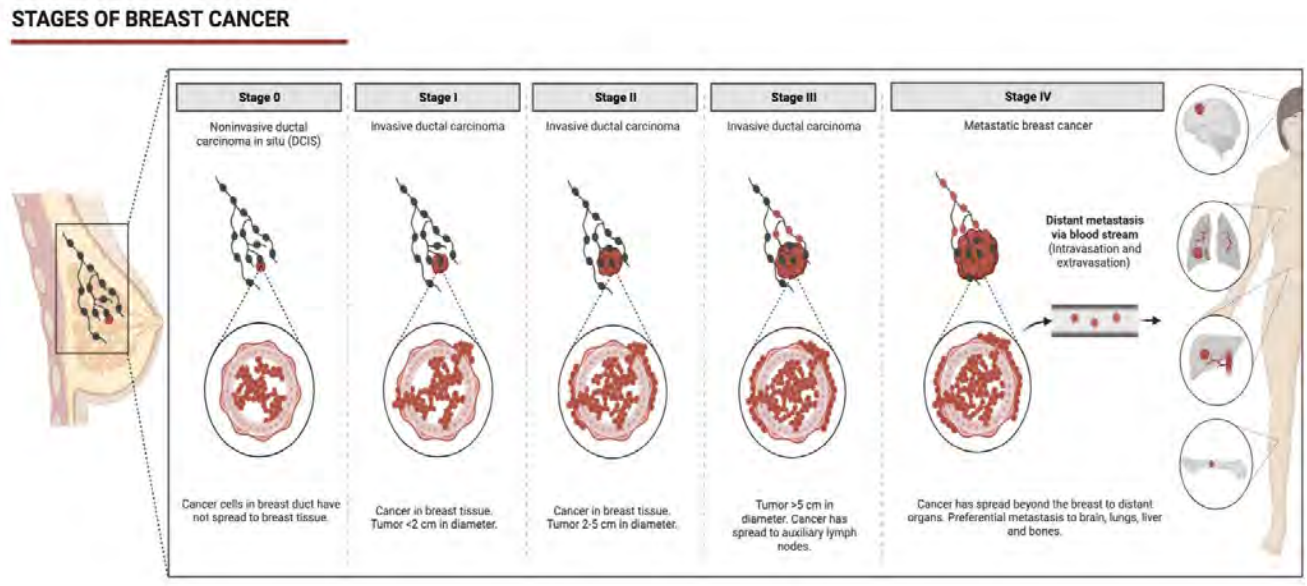

Figure 6: Stages of breast cancer(Hammer et al., 2008)

4.3 Currently available therapies for treating BC:

Surgery of operable tumors, radiation, neoadjuvant therapy, adjuvant therapy, and targeted therapy are all included in the holistic strategy to treat BC. Based on the metastasis stage and histology, the treatment options are varying. Stage 0 does not include any initial treatment. However, prophylaxis with tamoxifen can be considered. Breast-conserving surgery (BCS) and radiation therapy are commonly performed to treat stage I and stage II BC since they reduce recurrence and mortality. Neoadjuvant chemotherapy with anthracyclines followed by taxane chemotherapy is ubiquitously used in early-stage breast cancer (EBC) and locally advanced BC because it tends to increase the likelihood of BCS by downstaging the condition and

assessing the tumor reaction to chemotherapy (Fisusi & Akala, 2019; Ishigami et al., 2017). Docetaxel or paclitaxel (PTX) have been utilized as taxane regimens, while 5 fluorouracil, epirubicin, and cyclophosphamide (FEC), epirubicin and cyclophosphamide (EC), or doxorubicin and cyclophosphamide (AC) have been preferred as anthracycline drug therapies. Usually, stage III BC necessitates induction chemotherapy to shrink the tumor. Even though it is classified under stage III, severe IBC demands induction therapy accompanied by mastectomy. In stage IV, radiation therapy or bisphosphonates, along with endocrine therapy, or chemotherapy could be employed depending on the patient's ability to tolerate adverse effects and the prognosis of the condition (Maughan et al., 2010). Recent times, the spotlight has been drawn to the application of nanoparticle albumin-bound PTX (nab-PTX) as a therapy for advanced or recurring BC (Blum et al., 2007; Gradishar et al., 2005). The advancement of molecular diagnostics, targeted medicines, and gene sequencing offers the possibility for personalized BC treatment based on the distinctive malignant properties of each individual.

Even with the leaps and bounds in attaining success in the prevention and therapy of BC by surgery, chemotherapy, and radiotherapy, BC appears the most commonly occurring cancer in women and the most common cause of cancer-related deaths among women. Multiple studies have suggested that metformin has promising possibilities to cure BC as it decreased cancer risk, increased the time to develop malignancies, and lowered the mortality rate (Thompson, 2014).

Table 2: The summarization of metformin clinical use for BC

Number of patients	Disease condition	Interventions	Dose	Life quality/ side effects	Summary findings	Reference
5464 patients Metformin user: 2760 patients; Metformin non-user: 2704 patients.	BC and Diabetes	Metformin	Not mentioned	No side effects	Prolonged survival and lowered all-cause mortality in BC patients (HR: 0.53; 95% CI: 0.39-0.71)	(Xu et al., 2015)
39 patients	BC	Metformin	500mg tid	No side effects	Reduced cell proliferation and increased apoptosis	(Niraula et al., 2012)
68019 patients (Postmenopausal)	BC Diabetes	i) Metformin (ii) Anti-diabetic drug except Metformin	Not mentioned	No side effects	Reduced incidence of invasive BC (HR, 0.75; 95% CI, 0.57 to 0.99)	(Chlebowski et al., 2012)
24 patients (Postmenopausal)	Obesity ER+ and PR+ BC	(i) Metformin (ii) Exemestane (iii) Rosiglitazone	(i) Metformin: 2000mg (ii) Exemestane: 25mg (iii) Rosiglitazone: 8mg	Nausea Diarrhea	Combination dose responded well.	(Esteva et al., 2013)

Number of patients	Disease condition	Interventions	Dose	Life quality/ side effects	Summary findings	Reference
2529 patients Diabetic patient:155 (Metformin user: 68 patients Metformin non-user: 87 patients) Non-diabetic: 2374 patients	BC and Diabetes	Metformin	Not mentioned	No side effects	pCR rate was higher (24%) than other groups.	(Jiralerspong et al., 2009)
112 patients	HER2 BC	(i) Metformin (ii) Myocet (iii) Cyclophosphamide	(i) Metformin: 1000mg (ii) Myocet: 60mg/m2 (iii) Cyclophosphasmide: 600mg/m2	No side effects	Combination therapy increases PFS	NCT01885013
460 patients	BC	Metformin	2500mg	No side effects	Reduced BC related mortality	(El-Benhawy & El-Sheredy, 2014)
24 patients (postmenopausal)	(i) BC (ii) Obesity	Metformin	850mg bid	No side effects	Lowered risk for BC in obese patients	NCT01793948

The preventative impact of metformin in BC prone women is constantly being investigated in a variety of trials. 5464 BC patients with a history of diabetes were included in a meta-analysis of large cohorts by (Xu et al., 2015), whereas 2760 patients were metformin user and 2704 patients were metformin nonuser. Metformin was related to prolonged overall survival times (HR: 0.53; 95% CI: 0.39-0.71) and cancer-specific survival times (HR: 0.89; 95% CI: 0.79-1.00), according to the meta-analysis. Following adjustment for hormone receptor expression, subgroup findings indicates that metformin increased overall survival by 65% (HR: 0.35; 95% CI: 0.15-0.84). After diagnosis with BC, using metformin referred to a longer overall survival. From a different study, 39 early diagnosed, untreated, non-diabetic BC patients were treated with 500mg tid (Three times in a day) metformin. (Niraula et al., 2012) concluded that metformin use for a brief period of time led to improved clinical and cellular alterations (lower BMI, weight, HOMA, and reduced Ki67 staining in aggressive tumor), which are compatible with a favorable anti-cancer impact. This was the inaugural analysis to demonstrate a correlation between metformin use in the neoadjuvant stage and an elevation in apoptosis in BC. In a prospective cohort, 68019 postmenopausal women were assigned and after observation it was found that metformin treated diabetic women had a decreased risk of metastatic BC (HR, 0.75; 95% CI, 0.57 to 0.99) than non-diabetic women who used other anti-diabetic drugs (HR, 1.16; 95% CI, 0.93 to 1.45). (Chlebowski et al., 2012) also demonstrated that metformin therapy was linked to a decreased incidence of breast tumors in women with diabetes that were negative for HER2 overexpression and positive for both estrogen receptor ER and progesterone receptors. A phase I open-label, dose-escalation trial examined the effectiveness of exemestane in combination with metformin and rosiglitazone in postmenopausal obese women who had HR+ metastatic BC. The dosage of oral exemestane prescribed to patients by the FDA for the treatment of metastatic BC was 25 mg/day. Exemestane (25 mg) and metformin (2000 mg) were administered orally on a daily basis, with

or without rosiglitazone (8 mg), and the combination was well tolerated and it increased stability. Rosiglitazone and metformin had no impact on the pharmacokinetic parameters of exemestane (Esteva et al., 2013). For this particular study, neoadjuvant chemotherapy was administered to 2429 patients with early-stage BC. The following groups of patients were compared: 2,374 non-diabetic patients, 87 diabetic patients not taking metformin, and 68 diabetic patients receiving metformin. The proportion of pCR was significantly higher in the metformin group (24%; 95% CI, 13% to 34%) than it was in the nonmetformin group (8.0%; 95% CI, 2.3% to 14%) and the nondiabetic group (16%;95% CI, 15% to 18%) (Jiralerspong et al., 2009). Additional support for metformin as a therapeutic agent comes from the review, (El-Benhawy & El-Sheredy, 2014) investigated at 460 diabetic patients with BC undergoing adjuvant chemotherapy to assess if metformin treatment was related to improved overall results. Metformin daily dose was 2500mg. Diabetes patients' disease-free survival and overall survival were both considerably enhanced by using metformin. Additionally, the findings indicated that metformin therapy reduced the incidence of BC morbidity. Moreover, the effectiveness of metformin as a single or in combination with chemotherapy and/or radiotherapy for the treatment of BC has been examined in more than 30 ongoing and 23 successfully completed clinical trials. Metformin is being tested in a randomized phase II clinical study (ClinicalTrial.gov registration no. NCT01885013), where the fundamental goal was to compare the clinical effectiveness of Myocet/Cyclophosphamide + Metformin treatment to that of Myocet/Cyclophosphamide monotherapy in terms of PFS. The treatment regimen was maintained till the condition progresses. According to the study's findings, combined therapy improves both the characterization of insulin levels' sensitivity and PFS. Furthermore, a different randomized clinical trial (ClinicalTrial.gov registration no. NCT01793948) was conducted on 24 postmenopausal obese people who have a higher risk of developing BC due to genetic background or previous atypical hypertrophy of the breast. Patients were treated with

an 850mg oral dose of metformin twice a day for 12 cycles and the study result concluded that metformin use was associated with lower risk for BC patients.

As per the above discussion, metformin has been suggested as an adjuvant medication choice for the management of BC as it improved BC-related mortality, lowered the risk for developing, prolonged survival, and increased apoptosis at a higher dose (>2000mg). On the other hand, contradictory clinical results on the effectiveness and anti-tumor effects of metformin have been described in the literature (Col et al., 2012; DeCensi et al., 2010; Hong et al., 2017; Lega et al., 2013; Tang et al., 2018), which has emphasized the desire for additional analysis. The affirmation of the curative effect of metformin for the treatment of BC will be greatly reinforced by comprehensive randomized clinical investigations on diverse participants.

Chapter 5

5.1 Conclusion

Cancer is one of the most devastating diseases of the 20th century and spreading further with continuance and increasing incidence in the 21st century. Every fourth person has a lifelong risk of developing cancer, which is a shocking state of facts. Despite significant advancements in treatment and diagnosis techniques, there is still a dearth of information regarding the pathophysiology of the disease, which has a significant impact on the treatment's efficacy to reduce morbidity and mortality associated with cancer. Essentially, metformin, a useful metabolic agent for diabetes with pleiotropic biological targets, holds considerable therapeutic relevance not solely in the management of metabolic homeostasis but more critically, as a potential anti-neoplastic agent for cancer. Several potential mechanisms have been suggested for the ability of metformin to suppress cancer growth in vitro and vivo: (1) activation of LKB1/AMPK pathway, (2) inhibition of cancer cell growth by suppressing mTORC1, (3) inhibition of Generation of ROS, (4) reduction of IGF-1, and IGF-2, (5) inhibition of chronic inflammation, (6) activation of the immune system, (7) modulation of ADORA1, and (8) downregulation of gluconeogenesis in the mitochondria.

There is also growing evidence, mostly in the form of retrospective clinical studies, that suggest that metformin may be associated with a decreased risk of developing cancer and with a better response to chemotherapy. Additional longitudinal epidemiological data from both current and formerly researched cohorts, coupled with evidence on the putative signaling pathway of metformin for the prophylaxis and management of cancer, are continuing to be gathered. Regarding metformin usage in CRC patients exhibited greater rates of T downstaging, N downstaging, TRG, and pCR as well as lower rates of CRC-specific mortality, ACF formation.

Metformin medication is associated with improved overall survival, fewer recurrences and reduced metastases.

To conclude, it is undeniable that metformin has a tremendous amount of potential as a possible anti-tumor agent. However, by the in-dept summary and assessment of existing clinical data based on OS, PFS, HOMA, HR, 95% CI in both CRC and BC, it can be said that metformin exhibits greater promise in CRC patients than the BC patients. Of note, our findings suggest that the daily dose of 1500–2000 mg of metformin is well tolerated.

5.2 Future perspectives

Although great progress has been achieved over the past few years, there are still many challenges regarding metformin therapy. So, before initiating a therapy, it is necessary to evaluate the disease progression. To mitigate such issues further comprehensive study is necessary. Moreover, CRC cell proliferation has been found to be halted by the use of metformin, either alone or in combination with conventional chemotherapeutic agents (FOLFOX). Collectively, metformin is likely to decrease the EMT and CSCs, each of that are anticipated to be critical facets in tumor metastasis, suggesting as a promising therapy treatment to combat metastasis for patients with CRC. In terms of BC prophylaxis by metformin therapy shows lower mortality rate, lower cell growth and increase apoptosis. Furthermore, intriguing in vivo results reveal that metformin and natural anti-substances like curcumin may function synergistically to benefit patients. The combined therapy showed the best impact against tumor growth and proliferation. It dramatically decreased VEGF expression, activated Th2 immune reaction, incited Trp53 independent mortality, and exhibited negligible toxic effects (Falah et al., 2017). The affirmation of the curative effect of metformin for the treatment of cancer will be greatly reinforced by comprehensive randomized clinical investigations on diverse participants.

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