UNIVERSIDADE D
COIMBRA

Ana Rita de Jesus Oliveira

Quality of Meta-Analysis Reporting the Effect of Metformin on Rectal Colon Cancer

Dissertação no âmbito do Mestrado em Farmacologia Aplicada orientada pelo do Professor Doutor Fernando Fernandez-Llimos e pela Professora Doutora Isabel Vitória Neves de Figueiredo Santos Pereira apresentada à Faculdade de Farmácia da Universidade de Coimbra.

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Julho 2023

À minha mãe.

"Valeu a pena? Tudo vale a pena
Se a alma não é pequena.
Quem quer passar além do Bojador
Tem que passar além da dor.
Deus ao mar o perigo e o abismo deu,
Mas nele é que espelhou o céu."

Fernando Pessoa

Agradecimentos

Este trabalho simboliza o culminar de dois anos repletos de trabalho, dedicação e valiosas amizades. Ao completar esta gratificante jornada, sinto-me compelida a expressar minha mais profunda gratidão pelo apoio, colaboração e amizade de todos aqueles que participaram ativamente ao longo desta longa jornada.

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Contents

List of Figure

List of Table

Abbreviations list

95% CI - 95% Confidence Interval

AMP - Adenosine Monophosphate

AMPK - Adenosine 5′-Monophosphate-Activated Protein Kinase

AMSTAR - Assessing the Methodological Quality of Systematic Reviews

ANGPTL6 - Angiopoietin Like 6

APC - Adenomatous Polyposis Coli

ATP - Adenosine Triphosphate

B-RAF - Rapidly Accelerated Fibrosarcoma B protein kinase

BAK - Bcl-2 Antagonist/Killer

BAX - Bcl-2-associated X Protein

Bcl-2 - B-cell Lymphoma 2

BRAF - Proto-oncogene B-Raf

CC - Colon Cancer

CDK - Cyclin-dependent Kinases

cGAS - Cyclic GMP-AMP Synthase

CIMP - CpG Island Methylator Phenotype

CIN - Chromosomal Instability

CMS - Consensus Molecular Subtypes

COX - Cyclooxygenase

CRC - Colonrectal Cancer

CREB - cAMP Response Element-binding Protein

CT - Computed Tomography

CTL - Cytotoxic T Lymphocytes

DCC - Deleted in Colorectal Carcinoma

DDR - DNA Damage Response

DGS - Portuguese General Health Direction

dMMR - Defective Mismatch Repair

DNA - Deoxyribonucleic Acid

EGFR - Epidermal Growth Factor Receptor

EMT - Epithelial-Mesenchymal Transition

ERK - Extracellular Signal-regulated Kinase

FAS - Fatty Acid Synthase

FGF19 - Fibroblast Growth Factor 19

FOXO - Forkhead Box Proteins Class O

FXR - Farnesoid X Receptor

Fz - Transmembrane Frizzled

GIP - Glucose-dependent Insulinotropic Polypeptide

GLP-1 - Glucagon-Like Peptide 1

GLUT - Glucose Transporter

GPBAR1 - G Protein-coupled Bile Acid Receptor 1

GTP - Guanosine Triphosphate

HGF - Hepatocyte Growth Factor

HMGB1 - High Mobility Group Box 1

HR - Hazard Ratio

IGF - Insulin Growth Factor

IL - Interleukin

iLC - Innate Lymphoid Cells

IR - Insulin Receptors

IRS - Insulin Receptor Substrate

KRAS - Kirsten Rat Sarcoma Viral Oncogene Homolog

LKB - Liver Kinase B

LOH - Loss of Heterozygosity

MAPK - Mitogen-activated Protein Kinase

Mcl - Myeloid Cell Leukemia

MDSC - Myeloid-derived Suppressor Cells

MEK - MAPK/ERK Kinase

MeSH - Medical Subject Heading

MMR - Mismatch Repair

MOOSE - Meta-analysis of Observational Studies in Epidemiology

MPC - Mitochondrial Pyruvate Carrier

MSI - Microsatellite Instability

MSI-H - Microsatellite Instability High

mTOR - Mammalian Target of Rapamycin

MYC - Family of Regulator Genes and Proto-oncogenes

NF-κB - Nuclear Factor Kappa-light-chain-enhancer of Activated B Cells

NK cells - Natural Killer Cells

OCT - Organic Cation Transporters

OS - Overall Survival

OXPHOS - Oxidative Phosphorylation

PAD4 - Peptidyl Arginine Deiminase 4

PAMP - Pathogen-Associated Molecular Patterns

PET - Positron Emission Tomography

PI3K - Phosphoinositide 3-Kinase

PKB - Protein Kinase B

PLKs - Polo-like Kinases

PMN MDSCs - Polymorphonuclear MDSCs

PRISMA - Preferred Reporting Items for Systematic Reviews and Meta-Analyses

PTEN - Phosphatase and Tensin Homolog

PUMA - p53-Upregulated Modulator of Apoptosis

RAF - Rapidly Accelerated Fibrosarcoma

RAS - Rat Sarcoma

RB - Retinoblastoma-Associated

RC - Rectal cancer

RCT - Randomized Controlled Trials

RNA - Ribonucleic Acid

RoB - Risk of Bias

ROS - Reactive Oxigen Specie

SCFAs - Short-chain Fatty Acids

SCNA - Somatic Copy Number Alteration

SGLT1 - Sodium Glucose Cotransporter 1

SMAD 4 - Small Mothers Against Decapentaplegic 4

SREBP - Sterol Regulatory Element-Binding Protein

ssDNA - Single-strand DNA Damage

T2DM - Type 2 Diabetes Mellitus

TAM - Tumor-associated Macrophages

TAN - Tumor-associated Neutrophils

TERT - Telomerase Reverse Transcriptase

TGF-β - Tumor Growth Factor β

TIMP - Tissue Inhibitor of Metalloproteinases

TME - Tumor Microenvironment

TNF-α - Tumor Necrosis Factor Alpha

TP53 - Tumor Suppressor 53

TSC - Tuber Sclerosis Complex

TSG - Tumor Suppressor Gene

TSP-1 - Thrombo-Spondin-1

VEGF - Vascular Endothelial Growth

VEGFR - Vascular Endothelial Growth Receptor

WHO - World Health Organization

WoS - Web of Science

Resumo

Introdução: A reutilização de medicamentos consiste em identificar novos usos para um medicamento existente, que vão além do uso médico originalmente aprovado. Para isso, os profissionais devem basear as suas escolhas numa síntese precisa e confiável de evidências. O objetivo desta pesquisa é investigar a qualidade metodológica e de *report* das meta-análises publicadas que associam os resultados de sobrevivência do cancro colorretal (CRC) ao tratamento com metformina em doentes diagnosticados com CRC.

Métodos: Foi realizada uma pesquisa de meta-análises na PubMed, Scopus e WoS até à data de inserção de 31 de março de 2023. Os critérios de inclusão adotados foram 1) meta-análise, 2) doentes diagnosticados com CRC, 3) tratamento com metformina e 4) qualquer resultado de sobrevivência para CRC. Foi realizada uma análise descritiva dos resultados obtidos.

Resultados: Foram identificados um total de 93 artigos, dos quais 8 publicações cumpriam os critérios de inclusão. Em relação à qualidade metodológica (AMSTAR2), as falhas críticas mais frequentes estão nos itens 2 (72,73%), 7 (100,00%) e 15 (54,55%), e as falhas não críticas mais frequentes estão nos itens 3 (100,00%), 10 (100,00%) e 12 (54,55%). Por outro lado, em relação à qualidade de *report*, as falhas mais frequentes estão principalmente nos itens 3 (100,00%), 5 (90,91%), 9 (90,91%), 11 (100,00%) e 12 (100,00%) da lista de verificação de Abstract PRISMA2020, e nos itens 13a (100,00%), 13b (100,00%), 13c (100,00%), 14 (90,91%), 15 (100,00%), 16b (90,91%), 21 (100,00%), 22 (100,00%), 24a (81,82%), 24b (90,91%) e 24c (100,00%) da lista de verificação PRISMA2020.

Discussão/Conclusão: A análise revelou que a qualidade metodológica das meta-análises incluídas foi geralmente "baixa" e "criticamente baixa", em consonância com estudos anteriores de YU H. *et al.* (2019) e NOWICKA Z. *et al.* (2023). Esses resultados enfatizam a necessidade de aprimorar o rigor metodológico em estudos futuros.

Palavras-chave: Reutilização, Meta-análises, Metformina, Cancro Colorretal.

Abstract

Background: Drug repurposing consist of identifying new uses for an existing drug that are outside the original medical approved use. Thus, decision-makers should base their decisions on accurate and credible synthesis of evidence. The purpose of this research is to investigate the methodological and reporting quality of published meta-analyses that associate the CRC survival outcomes with Metformin treatment in patient diagnosed with CRC.

Methods: A literature search of meta-analyses was performed on PubMed, Scopus and WoS from inception to 31 March 2023. Inclusion criteria adopted are meta-analysis, patient diagnosed with CRC, Metformin as treatment and any survival outcome for CRC. A descriptive analysis of the obtained results was conducted.

Results: A total of 93 articles were identified, of which 11 publications met our inclusion criteria. Regarding methodological quality (AMSTAR2), the most frequent critical flaws are in items 2 (72.73%), 7 (100.00%) e 15 (54.55%) and the most frequent non-critical flaws are in items 3 (100.00%), 10 (100.00%) e 12 (54.55%). On the other hand, with regard to the reporting quality, the most frequent flaws are mainly in items 3 (100.00%), 5 (90.91%), 9 (90.91%), 11 (100.00%) e 12 (100.00%) of the PRISMA2020 abstract checklist and in items 13a (100.00%), 13b (100.00%), 13c (100.00%), 14 (90.91%), 15 (100.00%), 16b (90.91%), 21 (100.00%), 22 (100.00%), 24a (81.82%), 24b (90.91%) e 24c (100.00%) of the PRISMA2020 checklist.

Discussion/Conclusion: Our analysis revealed that the methodological quality of the meta-analyses reporting this relationship was generally "low" and "critically low", aligning with previous findings by YU H. *et al.* (2019) and NOWICKA Z. *et al.* (2023). These results emphasize the need for improved methodological rigor in future studies.

Keywords: Repurposing, Meta-analyses, Metformin, Rectal Colon Cancer, Colorectal Cancer.

1. Introduction

Drug repurposing consist of identifying new uses for an existing drug that are outside the original medical approved use.[1,2,3,4] This strategy has been proposed as an alternative to develop new therapies and offers diverse advantages than developing new drugs.[1,2,3] An example of drug repurposing is the use of Metformin in colon rectal cancer.

According to WHO, cancer is the principal cause of death worldwide, accounting approximately 10 million deaths in 2020.[5,6] In 2020 colon and rectum cancer registered 1.93 million new cases and was responsible for 916 000 deaths.[6]

Recently, new effects of metformin have been discovered. In monotherapy or in combination with other drugs, Metformin has been shown to be effective in the treatment of several diseases, such as cancer.[7] The anticancer properties of Metformin are related to its effect on the modulation of signaling pathways involved in cellular proliferation, apoptosis and metabolism.[5] Metformin can inhibit growth, survival and metastasis of different types of tumor cells: breast, liver, bone, pancreas, endometrial, colorectal, kidney and lung cancers.[7]

Drug repurposing should be based on reliable studies and supported by robust scientific evidence. Improving the efficiency of scientific research and producing more credible and more useful research, can result in further development.[8] Meta-analyses statistically combine evidence between therapeutic alternatives, so they are the gold standard for synthetizing evidence in scientific literature.[9,10,11] Meta-analyses can enhance precision, provide robust estimates and answer questions for which single studies are underpowered,[10] which is extensively useful for clinical decision.[11,12] In this way, decision-makers can base their decisions on accurate, succinct, credible and comprehensible synthesis of evidence.[12,13,14,15]

To evaluate the methodological quality of meta-analyses the revised version of AMSTAR was created.[12,16] To evaluate the report quality of meta-analyses the PRISMA statement is used.[16] So, we established as a research question: Do the meta-analyses that promote repurposing of drugs such as metformin in rectal colon cancer have methodological quality and appropriate report?

2. Diabetes and Metformin

2.1. Diabetes

According to WHO, diabetes is described as a "group of metabolic disorders characterized and identified by the presence of hyperglycemia in the absence of treatment" and results from defects in insulin secretion, insulin resistance or a combination of both.[17,18] The International Diabetes Federation estimated that, in 2013, 382 million adults worldwide aged between 20 and 70 years had Type 2 Diabetes Mellitus and it is expected that by 2035 this number will rise to 592 million.[17]

In 2019, WHO defined a new classification system for diabetes, that prioritizes clinical care and helps health professionals select the appropriate treatment. This new classification system divides diabetes into subgroups: type 1 diabetes, type 2 diabetes, hybrid forms of diabetes, other specific types, unclassified diabetes and hyperglycemia first detected during pregnancy (Table 1).[18]

Table 1 - WHO Classification system for diabetes

Type 1 diabetes
Type 2 diabetes
Hybrid forms of diabetes
Slowly evolving immune-mediated diabetes of adults
Ketosis prone type 2 diabetes
Other specific types
Monogenic diabetes
Monogenic defects of β-cell function
Monogenic defects in insulin action
Diseases of the exocrine pancreas
Endocrine disorders
Drug- or chemical-induced
Infections
Uncommon specific forms of immune-mediated diabetes
Other genetic syndromes sometimes associated with diabetes
Unclassified diabetes (used temporarily when there is not a clear diagnostic category)
Hyperglycemia first detected during pregnancy
Diabetes mellitus in pregnancy
Gestational diabetes mellitus

2.1.1. Type 2 Diabetes Mellitus

Type 2 Diabetes Mellitus is a multifactorial disease that is associated with genetic and environmental factors. Physiological changes in Type 2 Diabetes Mellitus are related to β cell and α cell dysfunction, insulin resistance and chronic inflammation.[17,19] These physiological alterations contribute to increased glycemia levels and to the development of micro and macrovascular complications.[17]

2.1.1.1. Pathogenesis

The pathogenesis of Type 2 Diabetes Mellitus is complex. It is characterized by a dysregulation of glucose homeostasis associated with a reduction in insulin secretion and action.[20] β cell and α cell dysfunction, changes in the microbiome, genetic component, environmental changes, inflammation and other factors play an important role in Type 2 Diabetes Mellitus pathogenesis.[17,19,21]

β Cell Dysfunction and Insulin Resistance

In Type 2 Diabetes Mellitus, the ability of β cells to respond to stimuli of intravenous secretagogues (for example glucose) is reduced. This reflects an insulin insensitivity, that results in increased postprandial glycemia, increased production of glucose by liver and decreased uptake of glucose in muscle and adipose tissue.[20,21]

In normal cells, insulin is release from β cells in response to stimulation. Insulin then mediates the uptake of glucose, aminoacids and fatty acids by insulin-sensitive tissues. In Type 2 Diabetes Mellitus, insulin resistance is present and β cells increase insulin secretion to maintain normal glycemia levels. However, plasma glucose concentrations increase.[21] Insulin resistance is related to a dysfunction in insulin receptor tyrosine kinase cascade and consequent alteration in the translocation of GLUT4 transporters.[20] This process is associated with increased phosphorylation of IRS proteins and insulin receptor in a serine instead of a tyrosine, which inhibits receptor activation and signaling.[17,20]

In addition to the alteration of β cell function, the mass of β cells is reduced in these patients, resulting in insufficient insulin secretion. This change possibly results from glucolipotoxicity and amyloid deposition leading to β-cell apoptosis.[20,21] β cells loss is not replaced, as the pancreas appears to be unable of renew these cells after the age of 30.[21]

Approximately 80% of Type 2 Diabetes Mellitus individuals are overweight or obese. Thus, lipid accumulation (especially in intra-abdominal cavity), inflammation, mitochondrial dysfunction and endoplasmic reticulum stress have been related to some of these abnormalities.[17,20,21]

α Cell Dysfunction

The intra-islet communication hypothesis describes a dynamic crosstalk of high hormone levels within the islet that controls the adequate secretion and regulation of insulin and glucagon. β and δ cells regulate α cells through the action of insulin and somatostatin as inhibitors of glucagon secretion. In Type 2 Diabetes Mellitus this communication is compromised, leading to dysregulated glucagon secretion and the development of hyperglycemia.[21,22] These patients have impaired of β cell function, elevated somatostatin secretion and somatostatin resistance in alpha cells.[22]

α cell dysfunction in Type 2 Diabetes Mellitus patients is not uniform. This dysregulation does not only affects glucose-induced inhibition of glucagon secretion, but also affects the stimulation of glucagon secretion at low glucose concentration.[22]

Gut and Gut Microbiome

Several peptides produced by gastrointestinal tract modulate the absorption of some nutrient. Incretins, for example GLP-1 and GIP, act on the pancreatic islet to modulate insulin and glucagon secretion. GLP-1 acts both on β cells to increase insulin secretion and on α cells to inhibit glucagon secretion.[21]

Bile acids also play an important role in glucose metabolism. Bile acids are endogenous ligands of the FXF that activate the receptor, resulting in the release FGF19. They also activate GPBAR1 in intestinal cells and lead to GLP-1 secretion.[21]

The gut microbiome is also important to the pathogenesis of Type 2 Diabetes Mellitus, although that is not clear which bacterial species can change human metabolism. Microbiome has about 100 times more genetic information than the human genome, and together they form the human metagenome.[21]

Genetic and Environmental Factors

Genes are strong determinants in Type 2 Diabetes Mellitus etiology, with a relative risk 4 times greater for people who have a Type 2 Diabetes Mellitus parent or sibling and increases to 6 times if both parents have this condition.[17,20,21] Type 2 Diabetes Mellitus is a complex multigenic condition and has multiple susceptibility loci that contribute to the final phenotype. In recent studies, more than 50 genetic loci with clear associations with Type 2 Diabetes Mellitus have been identified. Most genes associated with Type 2 Diabetes Mellitus are related to β cell function, insulin action and obesity.[20,21]

Environmental factors (for example intrauterine development, age and diet) also seem to have important role in Type 2 Diabetes Mellitus. The nutritional composition of our diet, particularly saturated fat, is important for the development of obesity and consequent insulin resistance, β-cell dysfunction and glucose intolerance. Furthermore, there is a decrease in the responsiveness of β cells to carbohydrate with ageing, reducing the glucose tolerance. The intrauterine environment, associated with the size of the mother's body, can induced epigenetic and gene-expression changes that affect the risk of obesity and Type 2 Diabetes Mellitus development.[21]

Environmental factors and hyperglycemia contribute to epigenetic changes in DNA and histones. This leads to gene expression changes in organs implicated in Type 2 Diabetes Mellitus pathogenesis and progression (including β cells).[21]

Chronic Inflammation

Type 2 Diabetes Mellitus is associated with obesity and systemic inflammation is a characteristic condition. β-cell dysfunction is related to systemic inflammation through activation of the intra-islet immune response. In Type 2 Diabetes Mellitus patients, interleukin 1β production in islets is increased by glucose and fatty acids.[21]

An increased adipose tissue is associated with the accumulation of activated macrophages. These cells express numerous pro-inflammatory genes (cytokines, for example Tumor Necrosis Factor α) that locally impair insulin signaling. Produced cytokines are released into the circulation where they can act at distant organs (for example, liver or skeletal muscle) to increase insulin resistance.[21]

2.2. Metformin

Metformin (1,1-dimethylbiguanide hydrochloride) is an oral biguanide, derived from guanidine, and a hypoglycemic agent. Metformin is the first-line treatment for glucose control in Type 2 diabetic patients and its use has expanded to gestational diabetes, T2DM non-alcoholic fatty liver disease, diabetic nephropathy, T2DM-associated cardiovascular complications, premature puberty and polycystic ovarian syndrome, due to its safety, efficacy and tolerability profile.[5,23,24]

2.2.1. History

Metformin, Phenformin and Buformin are derived from Galegine, a natural product which was extracted from the plant *Galega officinalis* in the 1920s.[24,25] Chemically, Galegine is an isoprenyl derivative of Guanidine, while Metformin and Phenformin containing two Guanidine molecules (biguanides) with additional substitutions.[25]

In 1918, Guanidine was found to have anti-diabetic properties, however it had toxic properties. So, this led scientists to research safer substitutes and, in the 1920s, Metformin and Phenformin were synthesized. However, they were not introduced for clinical use until the 1950s.[7,25] In the 1970s, Phenformin and Buformin have been withdrawn due to their risk profile of causing cardiac mortality and lactic acidosis.[24] Since then, Metformin became the first-line to treat Type 2 Diabetes Mellitus due to its capability to decrease plasma glucose levels.[7]

2.2.2. Mechanism of action

Multiple mechanisms are responsible for the antihyperglycemic effect of metformin. In general, Metformin promotes the uptake of glucose, increases insulin sensitivity, reduces hepatic glucose production and act on the intestinal glucose absorption and gut microbiota, which leads to a reduction in glucose levels.[23,26,27] The consumption of metformin reduces the risk of cardiovascular disease by reducing cholesterol levels while controlling the blood glucose level.[5,23,26]

Improvement in insulin sensitivity

Metformin improves insulin sensitivity through increased insulin receptor expression, increased tyrosine kinase activity and via the incretin pathway. Metformin stimulates GLP-1 secretion and increases beta-cell GLP-1 receptor expression, thereby increasing insulin secretion and lowering plasma glucose levels.[7,23]

Decreased hepatic gluconeogenesis

Another mechanism for the antihyperglycemic effect of metformin is the decrease in hepatic gluconeogenesis through AMPK-dependent or independent pathways, by reducing the uptake of gluconeogenic substrates or inhibiting enzymes involved in the process.[7,23]

AMPK is a master regulator of different metabolic pathways. Metformin can activate it by increasing its phosphorylation at Thr-172.[24] The AMPK-dependent pathway leads to activation of Small Heterodimer Partner and inhibition of phosphorylation of CREB binding protein. In this way, it suppresses the expression of gluconeogenic genes, such as Glucose 6 Phosphatase, Phosphoenolpyruvate Carboxykinase and Pyruvate Carboxylase. In addition, low doses of metformin leads to inhibition of mTOR Complex I through AMPK activation, which also results in the suppression of gluconeogenesis.[7,24]

On the other hand, AMPK-independent pathway leads to attenuation of the glucagon ability or inhibits mitochondrial Glycerol-3-Phosphate Dehydrogenase. This leads to impaired utilization of lactate for gluconeogenesis. Metformin also inhibits hepatic glucose production by directly targets Fructose-1,6-Bisphosphatase-1, a controlling enzyme in gluconeogenesis. In addition, metformin activates IRS 2, improving GLUT1-mediated glucose transport into hepatocytes and decreasing plasma glucose levels.[7]

Mitochondrial membrane

Mitochondria is the predominant site of action of Metformin. Metformin is a positively charged molecule while the mitochondrial membrane is negatively charge.[23] When interacting with mitochondria, metformin inhibits complex I of the mitochondrial electron transport system, decreasing the production of ATP and increasing the intracellular concentration of AMP.[7,23,24] Increased cellular levels of AMP, activates AMPK which is an important regulator of several metabolic pathways, including glucose and lipid metabolism and energy homeostasis.[7,23] Activated AMPK converts the cell from an anabolic state to a catabolic state, leading to inhibition of protein, lipid and glucose synthesis and increased uptake of glucose and fatty acid into the cell, contributing to cellular energy balance.[23]

In addition, metformin suppresses the mitochondrial Glycerophosphate Dehydrogenase, reducing the conversion of lactate and glycerol to glucose.[24]

Gastrointestinal tract

Recent studies suggest that the gastrointestinal tract and gut microbiota may be involved in the antidiabetic effect of Metformin.[7,24] Metformin may affect the composition and function of the gut microbiota, decreasing Bacteroides fragilis count. The result is an increase in Glycoursodexoycholic Acid levels, which suppress intestinal FXR, improving glucose tolerance.[7] Additionally, Metformin upregulates SGLT1 in upper small intestine, through increasing Lactobacillus.[24]

2.2.3. Pharmacokinetics and Pharmacodynamic

Metformin is mainly absorbed in the small intestine and oral bioavailability is approximately 50% to 60%. Absorption of metformin is saturable and incomplete, considering that it is non-linear pharmacokinetics. Metformin maximum plasma concentration is reached in about 2.5 hours.[20,26,28]

Metformin doesn't bind to plasma proteins and is distributed to erythrocytes. Red blood cells probably represent a secondary compartment of distribution, with the mean volume of distribution being between 63-276L.[20,26,28]

The presence of two methyl substitutes is responsible for the reduced lipophilicity of metformin that help in hepatic lactate clearance and unchanged metformin excretion in the urine. Metformin is not metabolized and is eliminated by renal tubular secretion and glomerular filtration. Elimination half-life of Metformin is about 6.5 hours and hypoglycemic effects last more than 24 hours.[5,20,26,28]

Metformin transport into cells is mediated by OCT. OCT 1 is responsible for transporting Metformin to hepatocytes and myocytes and OCT 2 to the renal tubules for excretion. Genetic mutations in OCT 1 may affect the response to metformin.[20]

2.2.4. Posology

Usually, the initial dose of Metformin is 500mg or 850mg, 2 or 3 times a day and taken with or after meals. After 10 to 15 days, the dose should be adjusted based on blood glucose values. The maximum recommended dose of metformin is 3g/day, taken in 3 doses.[26]

Metformin and insulin can be used in combination to achieve better glycemic control. In this case, Metformin should be administered at the starting dose of 500mg or 850mg, 2 to 3 times a day, while the insulin dose should be adjusted based on blood glucose values.[26]

Metformin dose should be adjusted based on renal function, in elderly patients, due to a potential decrease in renal function.[26] Elderly patients have reduced muscle mass, so their GFR should be estimated by a 24 hour urine creatinine collection. If the estimated GFR is less than 60mL/min, Metformin should not be given.[28]

Glomerular Filtration Rate should be assessed before starting the treatment and then monitor function at least annually.[20,26] In patients with higher risk of renal failure, renal function should be assessed more frequently.[26]

In pediatric patients, Metformin can be used in children from 10 years of age. The initial dose is 500mg or 850mg of Metformin once a day, taken with or after meals. After 10 to 15 days, the dose should be adjusted based on blood glucose levels. The maximum recommended dose of Metformin is 2g/day, taken in 2 or 3 doses.[26]

2.2.5. Safety profile

The safety profile of Metformin is now well established due to its use for over 50 years. The most common adverse effects of metformin are gastrointestinal, such as nausea, indigestion, anorexia and loss of weight, abdominal discomfort and diarrhea. These side effects can be minimized by having patients take it with or right after meals and by starting the treatment with low doses and gradually titrating to a target dose over several weeks. Gastrointestinal side effects tend to be transitory and decreasing in severity.[20,26,28]

Metformin has been associated with lactic acidosis, however it is a rare occurrence. Lactic acidosis risk may be increased with tissue hypoperfusion, for example in acute kidney injury, acute cardiac or hepatic disease, severe lung disease, sepsis, hypoxic states, shock or chronic alcohol abuse. This is relevant in cancer patients, in whom these conditions may occur.[5,20,26,28]

Another comorbidity reported is renal failure therefore it is important to assess renal function periodically. Metformin is not approved for use in stage 4 chronic kidney disease.[5,20,26]

Metformin is associated with lower blood levels of vitamin B12, possibly due to interference with vitamin B12 absorption, but no neurological or hematological consequences have been reported.[20,26,28] Has been documented a metallic taste and hypoglycemia during intense exercise.[26,28]

2.2.6. Drug Interactions

Cationic drugs (such as Cimetidine, Furosemide and Nifedipine) that are secreted by renal tubular secretion have the potential to interact with metformin by competing for common renal tubular transport systems.[20,26,28]

Some medications (Nonsteroidal Anti-Inflammatory Drugs, Selective Cyclooxygenase 2 Inhibitors, Angiotensin Converting Enzyme Inhibitors, Angiotensin II Receptor Inhibitors and Diuretics) can negatively affect renal function, which can increase the risk of lactic acidosis. When starting or using these drugs in combination with Metformin, monitoring of renal function is recommended.[26]

In patients with these medications is recommended monitoring blood glucose values and dose adjustment of Metformin.[20,26]

3. Rectal Colon Cancer and Metformin

3.1. Cancer

In 2018, the incidence of cancer worldwide was nearly 18 million cases and in Europe was almost 5 million cases.[29] WHO reports cancer as the second cause of death worldwide, accounting approximately 10 million deaths in 2018.[5, 6]

According to WHO (Figure 1), in 2020 breast cancer was the most common, with 2.26 million new cases, followed by lung cancer with 2.21 million new cases, colon and rectum cancer with 1.93 million new cases and prostate cancer with 1.41 million new cases. The most common causes of cancer death in 2020 were lung cancer, with 1.80 million deaths, followed by colon and rectum cancer with 916 000 deaths, liver cancer with 830 000 deaths and stomach cancer with 769 000 deaths.[6,30]

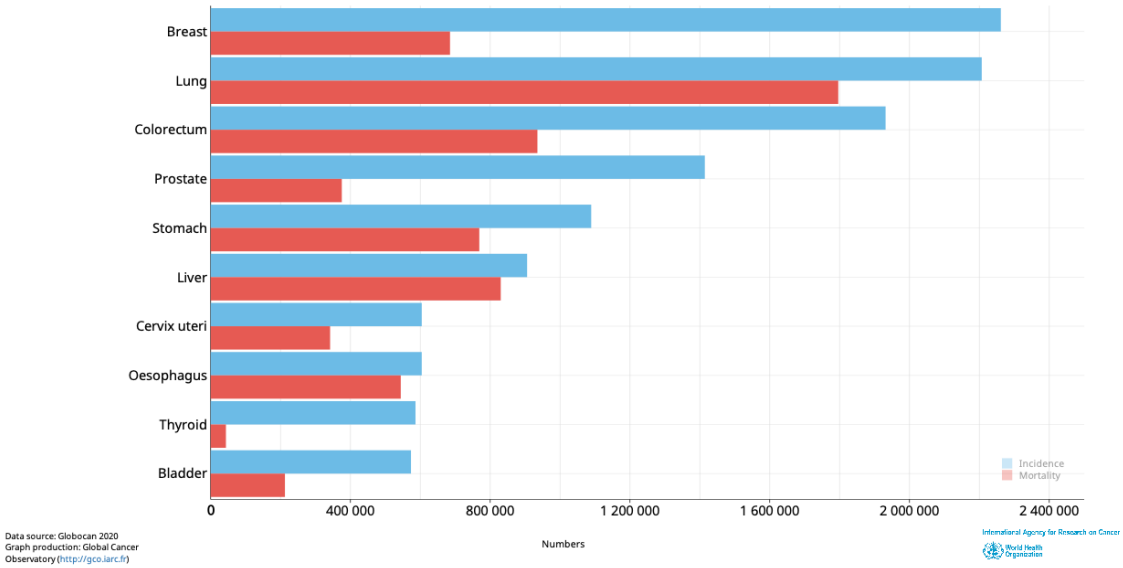

Figure 1 - Estimated number of incident cases and deaths worldwide, both sexes, all ages
International Agency for Research on Cancer, Global Cancer Observatory official site: https://gco.iarc.fr consulted on 25, July 2022.

Cancer is characterized by the rapid proliferation of abnormal cells that grow beyond their usual boundaries. These cells can then invade other tissues and spread to other organs. This process is called as metastasis and is the primary cause of death from cancer.[6] The tumor process is the result of the interaction between genetic factors and external agents. This external agents fall into in three categories:[6]

- ◊ physical carcinogens, such as ultraviolet radiation;
- ◊ chemical carcinogens, such as components of tobacco smoke and alcohol;

biological carcinogens, such as infections from viruses, bacteria or parasites.

3.1.1. TNM stage

One of the most used system to cancer staging is the TNM system. This system is used in most cancers, with the exception of brain and spinal cord tumors and blood cancers, which use a different system. In the TNM system, the "T" refers to the invasion or adherence of the primary tumor to adjacent organs or structures, the "N" refers to the number of regional lymph nodes that have cancer and the "M" refers to the existence or absence of metastasis.[31,32,33]

The following staging system is applied to all carcinomas appearing in the colon or rectum:[31,32,33]

◊ Primary tumor (T) - size and/or extent of the main tumor

TX: Main tumor cannot be measured.

T0: Main tumor cannot be found.

Tis: Carcinoma in situ.

T1: Main tumor invades submucosa.

T2: Main tumor invades muscularis propria.

T3: Main tumor invades through the muscularis propria into pericolorectal tissues.

T4a: Main tumor penetrates to the surface of the visceral peritoneum.

T4b: Main tumor directly invades or is adherent to other organs or structures.

◊ Regional lymph nodes (N)

NX: Cancer in nearby lymph nodes cannot be measured.

N0: There is no cancer in regional lymph nodes.

N1: There is metastasis in one to three regional lymph nodes.

N1a: There is metastasis in one regional lymph node.

N1b: There is metastasis in two to three regional lymph nodes.

N1c: There is metastasis in in the subserosa, mesentery, or nonperitonealized pericolic or perirectal tissues without regional nodal metastasis.

N2: There is metastasis in four or more regional lymph nodes.

N2a: There is metastasis in four to six regional lymph nodes.

N2b: There is metastasis in seven or more regional lymph nodes.

◊ Distant metastasis (M) - cancer has spread to other parts of the body

MX: Metastasis cannot be measured.

M0: Cancer has not distant metastasis.

M1a: Cancer has metastasis confined to one organ or site.

M1b: Cancer has metastasis in more than one organ/site or the peritoneum.

The TNM combinations are grouped into five stages (Table 2):[31,32,33]

◊ Stage 0: Abnormal cells are present but have not spread to nearby tissue.

◊ Stage I, Stage II, and Stage III: Cancer is present. The higher the number, the larger the cancer tumor and the more it has spread into nearby tissues.

◊ Stage IV: The cancer has spread to distant parts of the body.

Table 2 - Colon Rectal Cancer Stages

Staging System	T Stage	N Stage	M Stage
0	Tis	N0	M0
I	T1 or T2	N0	M0
IIa	T3	N0	M0
IIb	T4a	N0	M0
IIc	T4b	N0	M0
IIIa	T1 or T2	N1 or N1c	M0
	T1	N2a	M0
IIIb	T3 or T4a	N1 or N1c	M0
	T2 or T3	N2a	M0
	T1 or T2	N2b	M0
IIIc	T4a	N2a	M0
	T3 or T4a	N2b	M0
	T4b	N1 or N2	M0
IVa	Any T	Any N	M1a
IVb	Any T	Any N	M1b

EDGE S. B. *et al.* - AJCC Cancer Staging Manual. 7th Edition. Springer New York Dordrecht Heidelberg London, 2015.

The y prefix should be added to the stage of patients with high-risk rectal cancers, who received adjuvant treatment prior to surgical resection and pathological stage annotation. The r prefix should be used for the recurrent tumor stage.[33]

3.1.2. Hallmarks of Cancer

Tumors are a complex tissue that are composed of a diversity of cell types. Stromal cells, contribute to the development and expression of certain hallmark capabilities. Figure 2 shows all the hallmarks of cancer described by Douglas Hanahan and Robert A. Weinberg[34] in 2011. These hallmarks are cell capabilities that allow tumor growth and metastatic dissemination and represents a therapeutic target in cancer.[34]

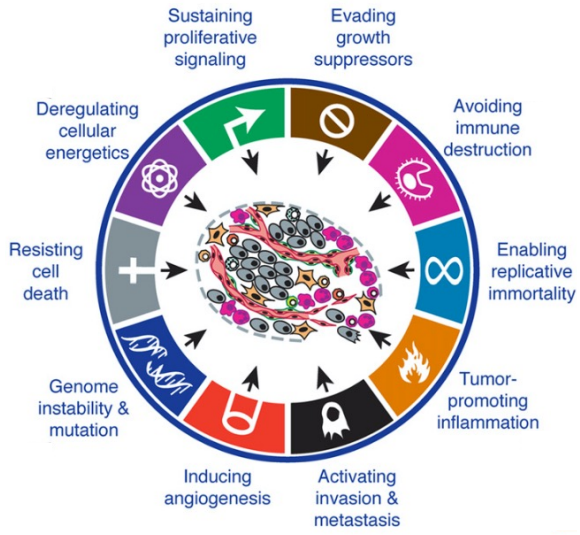

Figure 2 - Hallmarks of Cancer
HANAHAN D., WEINBERG R. A. - Hallmarks of Cancer: The Next Generation. Cell (2011) 144(5):646-74.

3.1.2.1. Sustaining Proliferative Signaling

Cancer cells are capable of sustaining chronic proliferation. While normal tissues maintain homeostasis of cell number and thus preserve the normal tissue architecture and function, cancer cells become masters of their own destinies, by deregulating this process. To maintain cell number homeostasis, normal tissues control the production and release of growth-promoting signals that promote cell growth and proliferation. This signals are transmitted by growth factors that bind to receptors on the cell surface and contain intracellular tyrosine kinase domains. The receptors emit intracellular signals that regulate progression through the cell cycle as well as cell growth.[34]

Cancer cells can acquire the capability to sustain proliferative signaling in a number of alternative ways: produce growth factor ligands themselves; send signals to stimulate normal cells within the supporting tumor-associated stroma; deregulate receptor signaling by elevating the levels of receptor proteins displayed at the cancer cell surface; structural alterations in the receptor molecules that facilitate ligand-independent firing.[34]

Some somatic mutations can promote a constitutive activation of signaling pathways, for example:[34]

- ◊ mutations in the structure of the B-RAF protein, resulting in constitutive signaling through the RAF to Mitogen-activated Protein Kinase (MAPK) pathway;
- ◊ mutations in the catalytic subunit of Phosphoinositide 3-Kinase (PI3K), which hyperactivate the PI3K signaling pathway, including Akt/PKB signal transducer.

Also defects in negative-feedback mechanisms can promote an increase of proliferative signaling. These feedback mechanisms are important to reduce some signals and to ensure homeostatic regulation of the flux of signals, for example:[34]

- ◊ Ras GTPase operate as an intrinsic negative-feedback mechanism that normally ensures that active signal transmission is transitory. The mutations in RAS genes affect Ras GTPase activity;
- ◊ PTEN phosphatase neutralize PI3K by degrading its product. The mutations in PTEN increase PI3K signaling and promote tumorigenesis;
- ◊ Through negative feedback, mTOR activation promote the inhibition of PI3K signaling.

Lastly, excessively elevated signaling by oncoproteins such as RAS, MYC, and RAF can induce cell senescence and/or apoptosis. For example, high levels of RAS oncoprotein promote cell senescence and lower levels may avoid senescence and proliferate.[34]

3.1.2.2. Evading Growth Suppressors

Tumor suppressor downregulate cell proliferation. The two most important tumor suppressors are RB and TP53 proteins, which control the decision of cells to proliferate or activate senescence and apoptotic programs:[34]

- ◊ The RB protein incorporate signals from intra and extracellular sources and choose whether or not a cell should progress its growth and division cycle. With a defects in RB function, the skills of a gatekeeper of cell-cycle progression are lost, which allows a persistent cell proliferation.
- ◊ TP53 receives signals from stress and abnormality sensors of intracellular systems: damage genome, difference in the levels of nucleotide pools and suboptimal growth-promoting signals, glucose or oxygenation. In these situations, TP53 can halt cell-cycle progression until conditions normalize or can trigger apoptosis in the case of overwhelming or irreparable damage to cellular subsystems.

3.1.2.3. Resisting Cell Death

Apoptosis consist of programmed cell death and serves as a barrier to cancer development. This process consists of both regulators and effector components. Regulators and effectors are the trigger for apoptotic process and are controlled by members of the Bcl-2 family. Bcl-2 inhibits of apoptosis by binding to and suppressing two proapoptotic triggering proteins (BAX and BAK). Then, BAX and BAK disrupt the outer mitochondrial membrane, which leads to the release of proapoptotic signaling proteins (such as Cytochrome C). The Cytochrome C that was released activates a cascade of Caspases that induce several cellular modifications associated with the apoptotic program, with their proteolytic activities.[34]

The conditions that trigger apoptosis are not yet fully enumerated:

- ◊ TP53 protein, that function as tumor suppressor and detect DNA- damage;
- ◊ Signaling hyperactivation by certain oncoproteins, such as MYC.

There are a variety of strategies that tumor cells use to limit apoptosis. The most common are the loss of function of TP53 and downregulation of proapoptotic factors (BAX), which increases the expression of antiapoptotic regulators (Bcl-2) or survival signals (Igf1/2).[34]

Autophagy is an important process that operates at basal levels in cells and can be induced in cellular stress. This program permits cells to break down cellular organelles, allowing the resulting catabolites to be recycled and thus used for biosynthesis and energy metabolism. The resulting low-molecular-weight metabolites support survival in the stressed and nutrient-limited environments. The autophagy machinery has also both regulatory and effector components. Recent research has shown intersections between the regulatory circuits of autophagy, apoptosis and cellular homeostasis. For example, the signaling pathway involving the PI3K, AKT and mTOR kinases, which when activated block apoptosis and equally inhibit autophagy. Stressed cancer cells have been shown to contract through autophagy to a state of reversible latency.[34]

3.1.2.4. Enabling Replicative Immortality

The normal cells are able to pass through only a restricted number of successive cell growth-and-division cycles. This limitation is associated with senescence and crisis, that are two barriers to proliferation. Senescence is an irreversible access into a non-proliferative but viable state and crisis involves cell death. On rare occasion, is immortalization. This term refers to cells that emerge from a state of crisis and show unlimited replicative potential.[34]

In contrast, cancer cells demonstrate unlimited replicative potential, in order to generate macroscopic tumors. Some evidence shows that telomeres are involved in the ability for unlimited proliferation. Immortalization has been attributed to the ability of the cells to maintain telomeric DNA at lengths sufficient to avoid senescence or apoptosis. This process is mostly achieved by upregulating expression of telomerase.[34]

3.1.2.5. Inducing Angiogenesis

As normal tissues, cancer cells need nutrients and oxygen as well as eliminate metabolic wastes and carbon dioxide. The tumor-associated neovasculature responds to these needs. For that, angiogenesis is frequently activated and remains on, leading to the formation of new vessels. Angiogenesis is induced at an early stage in the development of invasive cancers and is characterized by a chronically activated angiogenesis and an aberrant unbalanced mix of proangiogenic signals.[34]

Angiogenesis result from the combination of factors that induce or oppose angiogenesis. One example of angiogenesis inducers is VEGF-A and of angiogenesis inhibition is TSP-1:[34]

- ◊ VEGF-A is involved in orchestrating new blood vessel development and its expression can be upregulated both by hypoxia and oncogene signaling.
- ◊ TSP-1 counterbalance in the angiogenic switch by induction of suppressive signals that can reduce proangiogenic stimuli.

3.1.2.6. Activating Invasion and Metastasis

The progressed of tumor to higher pathological grades of malignancy is associated with local invasion and distant metastasis. This process involved the loss of E-cadherin by cancer cells, an essential cell-to-cell adhesion molecule, and is often observed downregulation and mutational inactivation of E-cadherin in human carcinomas. Also the expression of genes encoding other adhesion molecules is altered in many highly aggressive tumors.[34]

Invasion and metastasis process consists of a sequence of biologic changes in cancer cells, starting with local invasion, followed by entrance in adjacent blood and lymphatic vessels (intravasation) and transport through them, then escape into the parenchyma of distant tissues (extravasation), the formation of new nodules of cancer cells (micrometastasis) and, at last, the growth of micrometastatic lesions into macroscopic tumors (colonization).[34]

3.1.2.7. Genome Instability and Mutation

The genome modifications of cancer cells are responsible for the acquisition of several hallmarks. These mutations can be acquired by chance or through epigenetic mechanisms (for example DNA methylation or histone modifications) and can affect the regulation of gene expression.[34]

In the course of the disease, cancer cells frequently increase the number of mutation and this is reached through increased sensitivity to mutagenic agents, a failure in one or more mechanisms of the genomic maintenance, or both. Also the surveillance systems of the genome can be compromised and lead to an accumulation of genome mutations. Another source of genomic instability are the loss of telomeric DNA (that lead to karyotypic instability) and amplification and deletion of chromosomal segments.[34]

Theses failures in DNA maintenance and repair machineries are advantageous and crucial for tumor progression, because they increase the rate at which premalignant cells can accumulate favorable genotypes.[34]

3.1.2.8. Tumor-Promoting Inflammation

Many tumors are densely infiltrated by cells of both innate and adaptive immune system that play an important role in inflammatory conditions. This inflammatory response contributes to the increase of tumorigenesis and tumor progression by providing molecules to the tumor microenvironment that facilitate angiogenesis, invasion, and metastasis. Some example of these molecules are growth factors, survival factors, proangiogenic factors, extracellular matrix-modifying enzymes. Inflammatory cells can also release chemicals, such as Reactive Oxygen Species, that are mutagenic for adjacent cancer cells and that increase their malignancy.[34]

In some tumors, inflammation is evident at the earliest stages of neoplastic progression.[34]

3.1.2.9. Reprogramming Energy Metabolism

Beyond deregulated cell proliferation, in order to fuel this growth and division, cancer cells have adjustments of their energy metabolism. Cancer cells, even in the presence of oxygen, process glucose mostly by glycolysis ("aerobic glycolysis"). They do this through upregulating glucose transporters, particularly GLUT1, which increases glucose transport into the cytoplasm. Some tumors contain two subpopulations of cancer cells that differ in their energy metabolism pathways. One subpopulation (hypoxic cancer cells) is the glucose-

dependent cells. These cells use glycolysis to process glucose and secrete lactate. The other subpopulation cells (oxygenated cancer cells) import and use the lactate produced as their main energy source.[34]

Glycolytic process has been shown to be related with activated oncogenes, such as RAS and MYC and also related with mutant tumor suppressors, such as TP53. Many tumors experience states of hypoxia that increased glycolysis by upregulate glucose transporters and multiple enzymes of the glycolytic pathway. With the increase of glycolysis, the glycolytic intermediates are diverted to others biosynthetic pathways, such as those generating nucleosides and amino acids. This facilitates the biosynthesis of macromolecules and organelles required for cell proliferation.[34]

3.1.2.10. Evading Immune Destruction

The theory of immune surveillance suggests that cells and tissues are constantly monitored by an immune system that is responsible for recognize and eliminate cancer cells and thus emerging tumors. In this way, solid tumors that appear have to avoid detection by this immune surveillance system or have been able to limit the immunological response.[34]

In truth, cancer cells that are immunogenic can escape immune destruction by disabling components of the immune system that have been dispatched to eliminate them. For example:[34]

- ◊ Cancer cells may paralyze infiltrating CTLs and NK cells, by secreting an immunosuppressive factor (such as TGF-β),
- ◊ Recruitment of inflammatory cells that have an immunosuppressive activity, such as regulatory T cells and myeloid-derived suppressor cells.

In both cases, cancer cells can suppress the actions of cytotoxic T cells.

3.2. Colon Rectal Cancer

Colorectal cancers comprehend two types of cancers: colon and rectal. Both are common and highly aggressive.[35] According to WHO (Figure 3), in 2020 in Portugal, colon and rectum cancer was the most common, with 10 501 new cases and the secund causes of cancer death with 4 320 deaths. Colon and rectum cancer was also the most prevalent cancer in 2020 in Portugal (Figure 4), followed by breast and prostate cancers.[6,30]

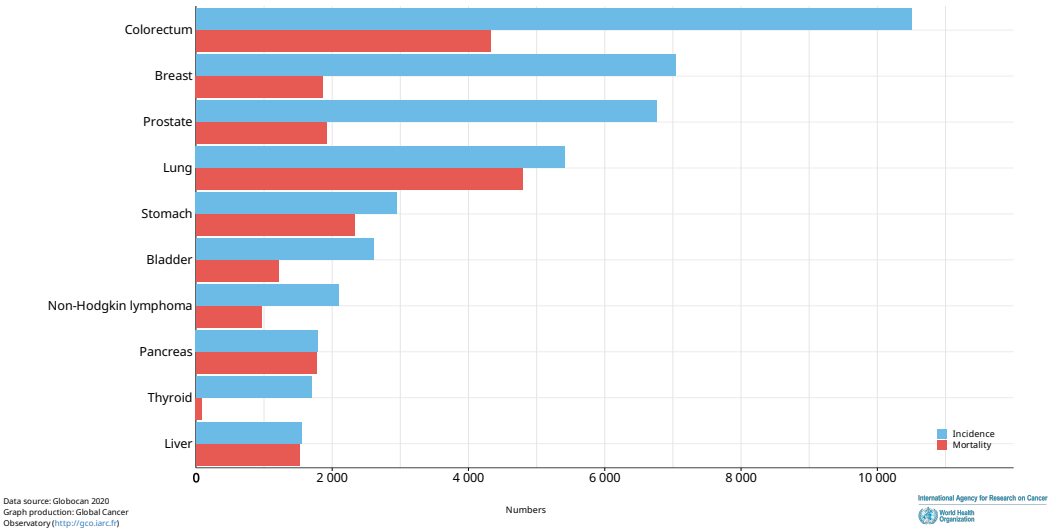

Figure 3 - Estimated number of incident cases and deaths, Portugal, both sexes, all ages
International Agency for Research on Cancer, Global Cancer Observatory official site: https://gco.iarc.fr consulted on 25, July 2022.

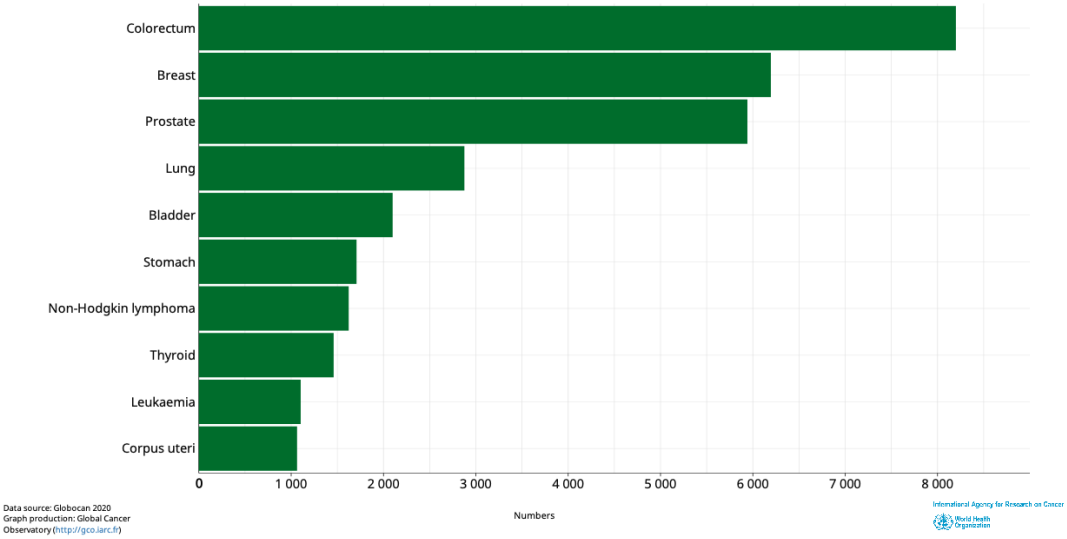

Figure 4 - Estimated number of prevalent cases (1 year) Portugal, both sexes, all ages (IA
International Agency for Research on Cancer, Global Cancer Observatory official site: https://gco.iarc.fr consulted on 25, July 2022.

Some factors contribute to an increased risk of developing CRC, such as diet high in fat and low in fiber, obesity, smoking, alcohol consumption, genetic predisposition, sedentary lifestyle, diabetes mellitus, long-standing inflammatory bowel disease, Crohn disease and ulcerative colitis.[5,35]

Environmental and dietary factors are responsible for sporadic CRC, which accounts 70% of cases. Familial CRC cases accounts for 25% of the cases and is related to individuals who have family history of CRC. Genetic or inherited cases account 5–10% of the cases.[35]

3.2.1. Morphology

The large intestine corresponds to the extent from the terminal ileum to the anal canal, including the vermiform appendix, cecum, colon, rectum and anal canal. The colon is divided into: right/ascending colon, middle/transverse colon, left/descending colon and sigmoid colon.[33] Proximal colon tumors show CpG Island Methylator Phenotype, Microsatellite Instability, deficient DNA Mismatch Repair Mechanisms, mutation in KRAS and BRAF and have a worse prognosis in terms of survival.[36] On the other hand, distal colorectal tumors are characterized by Chromosomal Instability and show a more favorable prognosis.[36]

The regional nodes location are along the vascular arcades of the marginal artery, along the course of the major vessels supplying the colon and rectum and adjacent to the colon. These lymph nodes include the pericolic and perirectal nodes and those along the ileocolic, right colic, middle colic, left colic, inferior mesenteric artery, superior rectal and internal iliac arteries.[33] The metastasis can affect any organ, although carcinomas of the colon and rectum commonly metastasize to liver and lungs.[33] The pathological staging of CRC is usually after surgical exploration of the abdomen, surgical tumor resection and pathologic examination of the resected specimen.[33]

Depending on the location of cancer along the colon and rectum, differences in clinical outcomes and drug responsiveness are seen. Factors that contribute to this include the distinct physiological functions and gut microbiome, the regionally resident immune cell types, the carcinogens from diet, timing of disease detection and regional differences in gene expression.[36]

Clinical assessment of CRC is based on medical history, physical examination, sigmoidoscopy, and colonoscopy with biopsy. Examination of the presence of metastasis include chest radiographic films, Computed Tomography to abdomen, pelvis and chest, Magnetic Resonance Imaging and Positron Emission Tomography or fused PET/CT scans.[33] Some molecular markers using in CRC diagnosis are Deleted in Colorectal Cancer, 18q Loss of Heterozygosity, p27Kip1, DNA Microsatellite Instability, KRAS mutation or thymidylate synthase.[33]

3.2.2. Classification for Colon Rectal cancer

Two molecular pathological classifications for CRC are described. One by The Cancer Genome Atlas and the other by GUINNEY *et al.* (2016).[35]

The Cancer Genome Atlas classified CRC into two groups, using integrated molecular analysis. The first group represent approximately 16% of cases and consists of hypermutated or ultra-mutated tumors. Hypermutated tumors represent approximately MSI and dMMR and ultra-mutated tumors are characterized by the presence of DNA Polymerase Epsilon or Delta 1 exonuclease domain mutations.[35]

The second group consisted of non-hypermutated tumors and represent approximately 84%. The tumors of these group are characterized by the presence of microsatellite stability (MSS) with a high frequency of DNA SCNAs. Is also present a deregulation of Wnt pathway with frequent mutations in genes involving APC, KRAS, Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha, Small Mothers Against Decapentaplegic 4 and TP53.[35,37]

Guinney *et al.* (2016) combined gene expression datasets and analytical approaches and described the four Consensus Molecular Subtypes of CRC: CMS1 (MSI-immune), CMS2 (canonical), CMS3 (metabolic), and CMS4 (mesenchymal).[35,38]

CMS1 subtype is characterized by the presence of deregulated DNA mismatch repair, MSI, low prevalence of SCNAs, high CIMP, occurrence of BRAF mutations and immune infiltration and activation. CMS1 has a very poor survival rate after relapse.[35,38] CMS2 subtype is characterized by the presence of higher CIN translated into a high prevalence of SCNAs, expression of epithelial signatures with Wnt and MYC signaling activation, loss of TSGs and overexpression of oncogenes.[35,38] CMS3 subtype is characterized by the presence of low CIMP and SCNAs, MSI, metabolic deregulation and KRAS mutations. CMS2 and CMS3 have better survival rate after relapse compared to other subtypes.[35,38] Lastly, CSM4 subtype is characterized by the presence of high prevalence of SCNAs stromal infiltration, angiogenesis, TGF-β activation and upregulation of expression of EMT genes. CSM4 has the worse overall and relapse-free survival compared to other subtypes.[35,38]

3.2.3. Molecular Mechanisms

According to the multistep genetic model, CRC formation is the result of the accumulation of genetic and epigenetic mutations in key genes, involving silencing of TSG and activation of oncogenes.[35]

Adenoma-Carcinoma Model (Vogelstein model)

The conventional adenoma-carcinoma model occurs in 70-90% of CRC cases.[35,36] Fearon and Vogelstein, in 1990, described the genetic model of CRC formation.[35,36] According to this model, an accumulation of genetic and epigenetic alterations is at the origin of CRC formation. These mutations lead to a dysplastic crypt that grows into a benign adenomatous polyp, which evolves into CRC over approximately 10–15 years.[35,36] The accumulation of these genetic mutation in "APC-KRAS-TP53" genes is a specific genetic signature of CRC. As showed in Figure 5, adenoma appearance overlaps with inactivating mutation or deletion of APC gene, adenocarcinoma sustains inactivating mutations or deletion of TP53 gene, telomere dysfunction and CIN, and metastatic disease shows activation of oncogene KRAS.[36]

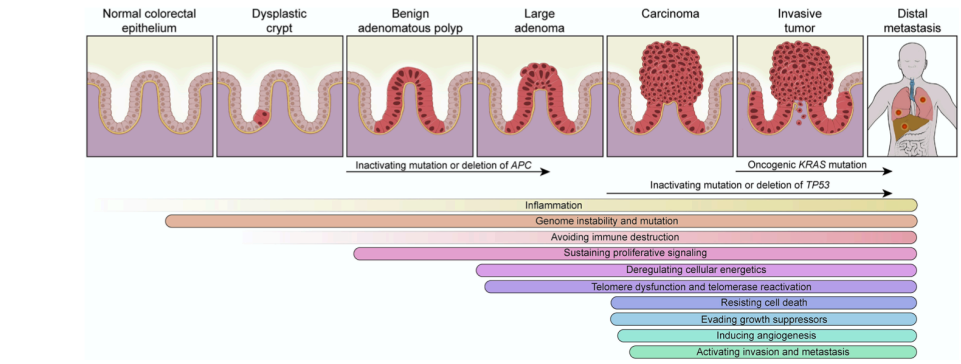

Figure 5 - Colorectal cancer "conventional adenoma–carcinoma–metastasis" model and corresponding cancer hallmarks
JIEXI LI *et al.* - Genetic and biological hallmarks of colorectal cancer. Genes & Development (2021) 35(11-12):787-820.

Serrated pathway

Otherwise, 10-20% of CRCs can evolve along a serrated pathway.[36,39]

While the majority of serrated polyps (80–90%) can be characterized as benign hyperplastic polyps, a subset of serrated lesions can progress to colorectal carcinoma. There are two presentations for colorectal carcinoma progress by serrated pathway, according to premalignant precursor lesions:[36,39]

- ◊ A Sessile Serrated Pathway, resulting from sessile serrated adenomas/polyps (SSA/P) and which is characterized by a microvesicular hyperplastic polyp progresses to a sessile serrated adenoma and then to either Microsatellite Instability or Microsatellite Stable carcinoma.
- ◊ A traditional serrated pathway, resulting from Traditional Serrated Adenomas and which is characterized by a goblet cell-rich hyperplastic polyp progresses to a traditional serrated adenoma and then to Microsatellite Stable carcinoma. These

serrated carcinoma show an activating mutations in BRAF and KRAS, increased CIMP, and rarely APC mutations.

Cancers result from these two serrated pathways are heterogeneous in terms of molecular patterns. Usually are observed mutations in KRAS or BRAF, hyperactivation of the MAPKinase pathway and downregulation of EphB2. Theses cancers are associated with a poor prognosis and therapy resistance.[39]

3.2.3.1. Genome Instability and Mutation

The accumulation of genetic mutations and chromosomal aberrations result in tumor heterogeneity. The genomic instability in CRC appears as Chromosome Instability, Microsatellite Instability and CpG Island Methylator Phenotype pathway.[35,36] When CIN or MSI are absent, CRC are classified as Genome Stable.[36]

Chromosome Instability

CIN is present in approximately 85% of adenocarcinoma.[35] CIN tumors is characterized by inactivation of Tumor Suppressors Genes (APC and TP53), activation of oncogenes (KRAS and BRAF) and a Loss of Heterozygosity for the long arm of chromosome 18 (18q LOH) .[35,39] These tumors show chromosomal numerical and structural alterations, such as aneuploidy and somatic copy number alterations, deletions, insertions, amplifications or loss of heterozygosity, respectively.[36]

APC gene mutations underlie WNT pathway activation and are characteristic of approximately 80% of adenomas and CRC.[36,39] Also β-catenin mutations or epigenetic changes can be present and leads to hyperactivation of the WNT pathway.[39] APC is a negative regulator of the WNT pathway by promoting the degradation of β-catenin.[36,39] WNT pathway mutations leads to a dysregulation of cell proliferation and differentiation. β-catenin accumulation, as a consequence of APC inactivation, translocate to the nucleus leading to MYC and other genes activation.[39]

Mutation and/or loss of the TP53 is related with the transition from adenoma to invasive carcinoma.[39] These inactivation/deletion of TP53 provides a permissive context for genome instability mechanisms. Combined telomere dysfunction and p53 deficiency are an important mechanism of genome instability.[36]

The allelic loss or gain of material allows development of mutated cells, leading to transformation into cancer cells. In CRC are identified allele losses that include losses at chromosomal arms 1p, 5q, 8p, 17p, 18p, 18q, 20p, and 22q and are identified chromosomal

gains at chromosome 7 and chromosomal arms 1q, 8q, 12q, 13q, and 20q.[35] LOH refers to the absence of one copy or alleles of a gene and the remaining allele is often mutated. In advanced CRC, LOH in the 18q region is the most commonly affected region and is associated with poor prognosis. LOH at 18q is associated with the presence of DCC, SMAD2 and SMAD4.[35] LOH in the DCC gene region is located on chromosome 18q21.2 and is present in approximately 70% of CRCs. Mutation in DCC gene lead to abnormal cell survival. On the other hand, SMAD2 and SMAD4 are present on chromosome 18q21.1 and are related with adenoma development and adenocarcinoma progression in mice models.[35]

Microsatellite Instability

MSI is another type of genomic instability in CRC and result from mutational inactivation of MMR genes. Microsatellites are DNA sequences that contain regions that tend to accumulate mutation.[35,36] Tumors with unstable loci in ≥30% markers are classified as "Microsatellite High" (MSI-H), with 10–29% unstable loci are classified as "Microsatellite Low" and with no unstable markers are classified as "Microsatellite Stable".[35]

In sporadic colorectal cancers with defective mismatch repair, the mechanism is mostly by promoting hypermethylation of both alleles of the MLH1 gene.[39] In these MSI-H cancers, small alterations in the coding regions of TSGs or oncogenes result in mutations that contribute to tumorigenesis. A genomic screening of the microsatellites coding region found mutations in nine loci: TGF-βR2, Bax, MSH3, ActRIIB, SEC63, AIM2, NADH-ubiquinone oxidoreductase, COBLL1 and EBP1. The most commonly mutated loci are TGF-βR2 and Bax, reducing susceptibility to apoptosis by mismatch-related DNA damage.[36,39]

Sporadic CRCs with MSI are related with the serrated pathway and usually carry BRAFV600E mutations.[35]

CpG Island Methylator Phenotype

DNA methylation consists of the addition of a methyl group to cytosine in the 5′-position that is catalyzed by DNA methyltransferases. This process is named as CpG transcription.[35]

In normal cells, the CpG sites are methylated while CpG islands are unmethylated. However, genome instability in CRC is related with hypermethylation of CpG islands and global DNA hypomethylation. These alterations in methylation pattern can affect some signaling pathways including TP53, TGFβ/SMAD, Wnt and tyrosine kinases receptor involved in process such as cell cycle regulation, transcription regulation, DNA stability, apoptosis, angiogenesis and metastasis.[35]

In CRC, some genes are known to be methylated and silenced: APC, MLH1, MGMT, SFRP1, SFRP2, CDKN2A, TIMP3, VIM, SEPT, CDH1 and HLTF. There is a subgroup of CRCs, known as the CIMP and represent approximately 20% of CRCs. These tumors are subclassified into CIMP2, CIMP-low and CIMP-high.[35]

3.2.3.2. Telomere Dysfunction and Telomerase Reactivation

Telomeres functions are to protect and maintain chromosomal integrity. With aging, telomeres become dysfunctional and leads to CIN in the early stages of human CRC. In approximately 85%–90% of all cancer types, cancer progression is related with telomerase reactivation.[36]

Telomere dysfunction

In addition to CIN, telomere dysfunction leads to inflammation, which contribute to colon cancer development. The dysfunction in telomere biology results in up-regulation of pro-IL-18, which is cleaved by Caspase-1 into mature IL-18. This increase in IL-18 produces inflammation. Also extrachromosomal telomere fragments can interact with cGAS/STING and induce chronic inflammation. Thus, this telomere–cGAS/STING connection may be a contributing factor in the development of cancers associated with inflammation. As a result of the inflammatory state, the ROS produced accelerate telomere damage and attrition, leading to genomic instability.[36]

Telomere dysfunction induces the production of ROS and reactive nitrogen species, which promote damage to lipids, proteins and DNA. Polyunsaturated fatty acids are oxidized to free radicals and other substances such as lipid oxidation products, which increase membrane permeability and lead to signaling defects and inflammation. Protein oxidation leads to loss of protein function, which can affect DNA repair enzymes and proteasome system. Lastly, DNA nucleotides oxidization is present in CRC and is mutagenic by itself.[36]

Telomerase reactivation

During adenoma-carcinoma progression, an increase in TERT levels and telomerase activity are observed. This increase is correlated with a poor prognosis, which may be associated with TERT procarcinogenic activities or with upregulation of TERT gene transcription by MYC or WNT.[36]

Telomere dysfunction and telomerase reactivation can promote carcinogenesis through various processes, such as CIN, inflammation and increased ROS production.[36]

3.2.3.3. Sustaining Proliferative Signaling

A cellular proproliferative state is conferred through somatic mutations in key signal transduction pathways. In CRC, the mutated signaling pathways are EGFR/RAS and WNT/β-catenin.[36] Figure 6 shows proliferative signal transduction pathways in CRS.

EGFR pathway

ErbB/HER family include EGFR and contains four members: ErbB1 (EGFR/HER1), ErbB2 (Neu/HER2), ErbB3 (HER3) and ErbB4 (HER4).[35] EGFR activation triggers RAS/RAF/MEK/ERK, PI3K/AKT and JAK/STAT3 signaling cascades to regulate cell growth, survival, and migration.[35,36] In CRS, EGFR is mutated in only 1% of cases and is overexpressed in approximately 80% of cases. Methylation of R198 and R200 by Protein Arginine Methyltransferase 1, can improved EGFR signaling activation. Furthermore, the MET receptor could override EGFR signaling through HGF activity, bypassing EGFR inhibition in CRC. HGF is secreted by cancer-associated fibroblasts and is the MET receptor ligand. Also HER2/ERBB2 activation can trigger EGFR signaling cascades.[36]

EGFR activation leads to RAS-RAF activation and, in turn, phosphorylation of Mitogen-Activated Protein Kinase (MAPK or MEK) and activation of ERK, which are involved in regulation of cell proliferation, differentiation, apoptosis and senescence.[35]

RAS, RAF, MEK or ERK can be mutated in CRC, also contributing to cancer cell proliferation and survival.[36] Mutations in BRAF and RAS are related with a poor prognosis for CRC. These mutations can activate MEK/ERK, which triggers MYC and NF-κB transcription. Mutant KRAS can interact with PI3K and activate it and the activation of mTOR promote cell growth and proliferation.[36]

Wnt/β-catenin signaling

Chronic Wnt activation promotes CRC. The binding of β-catenin to the destruction complex (AXIN, APC, CK1, GSK3) is related to its levels in the cytoplasm. β-catenin dissociate from the destruction complex, with the accumulation of Wnt ligands, and migrate to the nucleus. There it couples to TCF or LEF and activates tumor growth genes, including TERT, increasing cell growth, differentiation, migration and adhesion.[35,36]

Wnt gain-of-function mutations can be divided into ligand-independent and ligand-dependent alterations. Ligand-independent alterations are mutations in intracellular signal transduction proteins, such as APC and β-catenin (occurring in 80% and 5% of cases, respectively). Ligand-dependent alterations are mutations which amplify endogenous Wnt

signal transduction, such as R-spondin (RSPO) fusions, and need Wnt binding to the seven-pass transmembrane frizzled (Fz) receptor and its coreceptors, LRP5 and LRP6.[36]

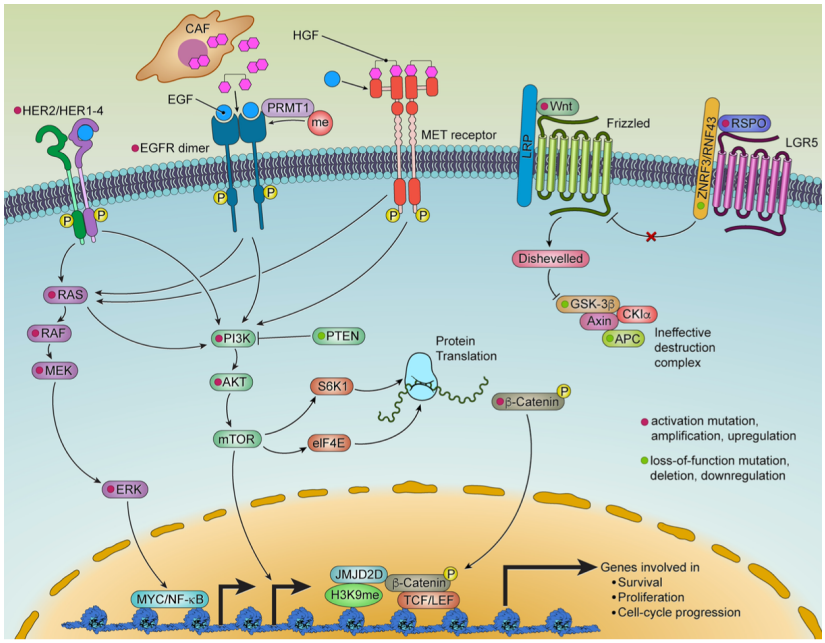

Figure 6 - Growth signaling pathways in CRC
JIEXI LI *et al.* - Genetic and biological hallmarks of colorectal cancer. Genes & Development (2021) 35(11-12):787-820.

3.2.3.4. Evading Growth Suppressors

Cancer cells bypass growth restrictions by deactivating cell cycle checkpoints, tolerating of DNA damage and overcoming of senescence.[36]

Avoiding cell cycle checkpoints

Cyclin-dependent Kinases, checkpoint kinases, aurora kinases and Polo-like Kinases are responsible for controlling the cell cycle phases (G0/G1, S, G2, and M). The transition from G1 to S phases are regulated by cyclin/CDK complex and the progression through S phase and the transition from G2 to M phases are regulated by cyclin– CDK complex, PLK1 and aurora A/B.[36]

CDKs receive activating signals from mitogenic pathways (such as RAS) and inhibitory signaling from DNA damage checkpoints (such as p53). In CRC, up-regulation of aurora A kinase causes transient aberrations in mitotic spindle, which promote the formation of lagging chromosomes and aneuploidy.[36]

Tolerating DNA damage

Mutagenic mechanisms and unrepaired DNA damage lead to genetic mutations in cancer cells. The first step from DNA Damage Response is "damage sensing" by sensors specific to DNA Double-strand Breaks and by sensors specific to Single-strand DNA damage (ssDNA). The generated signals are then transduced and amplified. p53 plays an important role in determining cell cycle arrest, senescence or apoptosis. In CRC, its mutation is frequent. Both p53 loss and increased p53 degradation contribute to unrepaired DNA damage and promotion of tumorigenesis.[36]

Overcoming senescence

Two mechanisms can induce cell senescence: "replicative senescence" and "premature senescence". "Replicative senescence" result from a cell division mechanism and "premature senescence" result from DNA damage, oncogenic activation or oxidative stress and protects cells from tumorigenesis.[36]

In CRC, p53/p21 senescence signaling pathway is compromised by p53 degradation, inactivation or mutation and by inhibition of p21 expression. Cancer cells senescence is also related with inflammation, which promote tumorigenesis through loss of growth control capacity and acceleration of cancer cell growth and invasiveness.[36]

3.2.3.5. Resting Cell Death

Apoptosis resistance can also involve resistance mechanisms to nonapoptotic forms of cell death such as necrosis, ferroptosis and autophagy. To avoid cell death programs, cancer cells promote changes in key regulators (p53, MYC) and effectors (Bcl-2 family, FAS) of the apoptosis process. This strategies are essential for survival under various stresses.[36]

Resisting intrinsic and extrinsic apoptosis

Apoptosis pathways are classified as "intrinsic" or "extrinsic". Bcl-2 family proteins regulate the "intrinsic pathway", which are apoptosis promoters (BAX, BAK) or inhibitors (Bcl-2, Bcl-XL). Activation of the intrinsic apoptotic cascade initiates with Bcl-2 proteins inhibition and activation of BAX and BAK by PUMA and NOXA. Then, the outer mitochondrial membrane permeability increases and Cytochrome C is released. The caspase-9/caspase-3 cascade is activated by Cytochrome C, resulting in cell death. In CRC, procarcinogenic signals and hypoxia state lead to Bcl-2 and Bcl-2L1 induction, protecting cancer cells from apoptosis and promoting tumorigenesis.

The "extrinsic pathway" is also present in CRC and bypasses the mitochondrial step. In its place, FAS and TNF receptors activate caspase-8, which in turn activates caspase-3 and leads to cell death. In CRC cells, FAS mutation decrease activation of this pathway, promoting cell survival.[36]

p53 and cell death

The p53 gene is known as the "guardian of the genome" as it encodes proteins that regulate cell cycle, DNA repair, senescence and apoptosis.[35] p53 are related with intrinsic and extrinsic apoptosis pathways through activation of PUMA, NOXA, FAS and other death receptors transcription. Furthermore, p53 can induce itself apoptosis via its translocation to mitochondria and MDM2 is responsible for its degradation.[36] p53 mutations or loss of function are reported in 50 to 75% of CRC cases. The p53 loss of function increases cell proliferation and uncontrolled cell cycle, promoting tumorigenesis.[35]

Moreover, p53 is also involved in autophagy. Autophagy is the process of removing damaged organelles, which prevents necrosis and reduce inflammation. In CRC, the balance between apoptosis and autophagy is maintained through HMGB1/p53 complex. The loss of p53 leads to cytosolic accumulation of HMGB1, which increases autophagy and decreases apoptosis. The loss of HMGB1 increases cytosolic p53, resulting in increased apoptosis and decreased autophagy.[36]

Other p53-mediated apoptosis regulator is MYC. In normal cells, MYC inhibits Bcl-XL and Bcl-2 and activate BAK and BAX. In addition, MYC sensitizes cells to TNF and FAS ligands. In CRC, the loss of APC increases MYC, promoting cell proliferation and survival and tumorigenesis.[36]

Resisting nonapoptotic cell death

In CRC, other forms of cell death are also deactivated, for example necrosis and ferroptosis. Necrosis is related with higher tumor stages and inflammation. To resist to necrosis, cancer cells elevate HGF-MET signaling, reducing the level of necroptosis mediator RIPK1. Ferroptosis, is the process of cell death through iron oxidation and can be suppressed by phospholipid glutathione peroxidase GPX4.[36]

3.2.3.6. Deregulating Cellular Energetics

To meet energy needs, normal cells principally use oxidative phosphorylation. Under low-oxygen conditions, anaerobic glycolysis is the preferred pathway, producing abundant lactate and limited ATP.[36]

Cancer cells have elevated energy requirements and available nutrients regulate cell proliferation and differentiation. High metabolic plasticity allows cancer cells adaption to nutritional conditions. For example, "aerobic glycolysis" or "the Warburg effect" is a metabolic reprogramming of cancer cells that generate lactate even in the presence of oxygen. In addition, glutamine utilization, lipid metabolism, one-carbon metabolism and short-chain fatty acids metabolism supported cancer cell growth. Figure 7 shows the mechanisms of aerobic glycolysis and metabolic remodeling in CRC. A feature of CMS3 CRCs is the activation of multiple metabolism signatures.[36]

Together, these metabolic mechanisms allow cancer cells to adapt to limited nutrient availability and serve to suppress immune surveillance via several mechanisms.[36]

Aerobic glycolysis

Metabolic reprogramming of cancer cell is the result of genetic alterations in the regulators of metabolic enzymes and mechanisms. Some examples are Wnt signaling that leads to glycolysis, p53 loss of function that leads to oxidative phosphorylation and high LDHA levels that increase glycolysis.[36]

In CRC, the aerobic glycolysis can be enhanced by deletion or underexpression of mitochondrial pyruvate carrier. This decrease of MPC results from mitochondrial DNA mutations. CRC cells exhibit metabolic heterogeneity in the tumor microenvironment, thus, to generate ATP some cancer cells show anaerobic glycolysis in the presence of low MPC levels and others use OXPHOS with high MPC levels.[36]

Nutrient deprivation decrease antitumor activity by reducing CTL mTOR activity, glycolytic capacity and IFN-γ production. In CRC cells, checkpoint blockade inhibits glycolysis, increasing glucose availability in the TME. Immunosuppressive regulatory T cells depend on folic acid oxidation and OXPHOS, thriving in TME low glucose conditions. The abundant lactate in the TME is profoundly immunosuppressive and can stimulate immune suppressor cells and inhibit antitumor immune cells (natural killer cells [NKs] and CTLs) .[36]

Glutamine, lipid and one-carbon metabolism

In glutamine metabolism, the entry of glutamine into the TCA cycle is supported by GLUD1 and enhanced by up-regulated GPT2.[36]

In lipid metabolism, CRC cells show increased uptake of extracellular lipids and upregulation of enzymes that enable lipid biogenesis. These mechanism support critical processes such as membrane formation, signal transduction, protein post-translational modifications and energy storage. Furthermore, CRC cells have lipid droplets that function as reservoirs for COX2 and for PGE2 synthesis, which increase inflammation.[36]

Finally, one-carbon metabolism is used by CRC cells to provides nucleotides and fatty acids for cell proliferation and chromatin remodeling.[36]

Short-chain fatty acids

SCFAs are products of fiber fermentation in the gastrointestinal tract. The production of SCFAs are through anaerobic bacterial fermentation and it seems to be protective of colon carcinogenesis by decreasing inflammation. SCFAs increase immune tolerance and mucus production, promoting gut homeostasis.[36]

Furthermore, SCFAs are tryptophan metabolites and contributes to an immunosuppressive TME in CRC. The expression of indoleamine 2,3-dioxygenase in CRC will metabolize tryptophan to kynurenine and facilitate tumor immune escape and support tumor growth.[36]

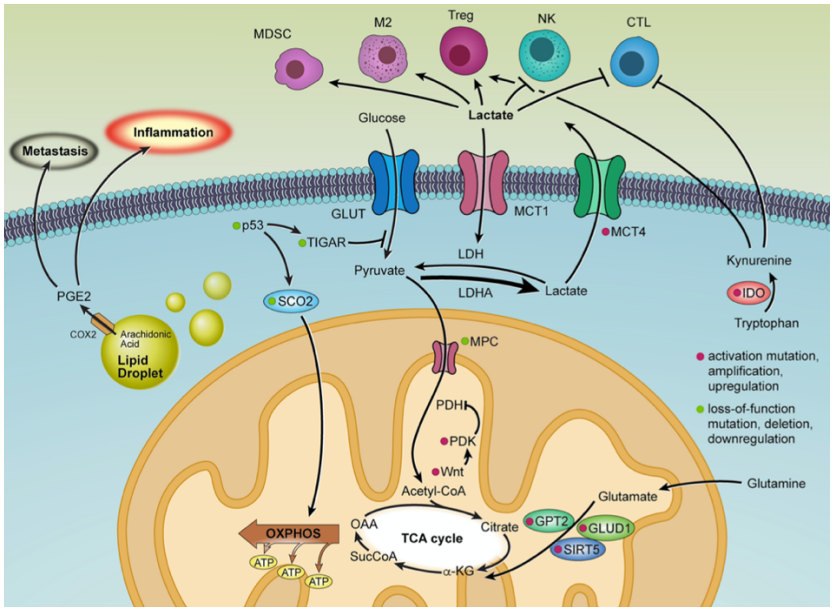

Figure 7 - Aerobic glycolysis and metabolic remodeling in CRC
JIEXI LI *et al.* - Genetic and biological hallmarks of colorectal cancer. Genes & Development (2021) 35(11-12):787-820.

3.2.3.7. Tumor-promoting Inflammation

Inflammation is an immune response to exogenous or endogenous signals. In intestine, inflammation have an important role in the resolution of pathogenic infection, maintenance of intestinal function and promotion of tumorigenesis.[36]

CRC inflammatory cytokine environment

Among other factors, cancer-driving mutations contribute to the formation of a network of local and systemic inflammatory cytokines. An important regulator of inflammation is NF-κB. NF-κB promotes the production of ROS and promotes TNF activation and IL-6 production, inducing DNA damage and enabling cellular neoplasia and antiapoptotic capabilities, respectively. Furthermore, NF-κB regulates the expression of cytokines in cancer and immune cells.[36]

More than 50% of CRC show abnormal activation of NF-κB. The classical activation of NF-κB pathway occurs through receptors activation (PAMPs) or cytokines (IL-1β, TNF, IL-17). An alternative pathway, activate NF-κB by RANKL and lymphotoxin-β.[36]

Loss of p53 increases intestinal permeability, also initiating inflammatory response by NF-κB activation. Furthermore, loss of p53 induces epithelial-mesenchymal transition.[36]

Microbiota and inflammation in CRC

"Dysbiosis" is a change in the functional composition and metabolic activities of microbiota. In CRC, dysbiosis induces the inflammatory response and contributes to tumor progression. Equally, inflammation can increase the ratio of genotoxic microorganisms in intestine and contributes to tumorigenesis.[36]

Innate lymphoid cells

iLC is an immune cell population that, in the intestine, regulate some processes that influence immunity, inflammation and bacterial homeostasis. They are located on mucosal surfaces and their functions are to enhance immune responses, sustain mucosal integrity and maintain tissue homeostasis. The ILC2 subset secretes IL-22 that contributes to CRC progression. IL-22 operates in epithelial cells and supports intestinal cell regeneration and inhibition of bacterial translocation. Furthermore, the IL-22 pathway together with the KRAS mutant increases the MYC pathway, leading to increased proliferation of cancer cells. The microbiome regulates iLCs to produce cytokines, which can be antitumorigenic or promote tumor progression.[36]

3.2.3.8. CRC Immunity/Avoiding Immune Destruction

There is a correlation between specific immune cells and relapse and metastasis that demonstrates the role of antitumor immunity in the development and progression of CRC.[36]

Immunosuppressive tumor microenvironment

Antitumor immune cells populations, including CTLs, NKs and activated dendritic cells are less abundance in CRC compared with normal mucosa.[36]

A hallmark of the immunosuppressive TME in CRC is a presence of polymorphonuclear MDSCs, including tumor-associated macrophages and tumor-associated neutrophils. TAMs are responsible for secrete anti-inflammatory cytokines and growth factors that contribute to tumor growth and proteolytic enzymes that allow for tissue remodeling and tumor expansion. Additionally, TANs and TAMs inhibit CTL activity through elevated production of ARG1 and ROS and promote CRC metastasis.[36]

Immune hallmarks of MSI-H and MSS subtypes

Of the consensus molecular subtypes, CMS 1 includes most MSI-H CRCs including 15% of cases and exhibits dense immune infiltrates. In contrast, CMS 2 to 4 are mostly MSS CRCs representing 85% of cases and are characterized as "immune-cold" tumors.[36]

MSI-H tumors show an increase in T-cell infiltration and CD8+ / CD45RO+ memory T-cell populations comparative to MSS CRCs. MSI-H CRC also have an increase in proliferative Th1-like cells, enhancing the activity of macrophages, B cells and CD8+ T cells.[36] In contrast, MSS tumors show an increase in proliferative Th17 cells, which antagonize Th1 cells.[36]

CMS 2 to 4 have distinct genetic characteristics. CMS2 CRC is characterized by APC inactivation and β-catenin activation that are related to decrease in T-cell infiltration. CMS3 tumors have high KRAS mutation that are related to downregulation of MHC-I, leading to low immunogenicity. KRAS hyperactivation also leads to an increase in GM-CSF production, which recruits immunosuppressive PMN MDSCs. Finally, CMS4 tumors have extensive stromal infiltration and abundance of TGF-β, which is an immunosuppressive factor. TGF-β can block the recruitment of CD8+ and CD4+ T cells, antagonize Th1 cell and polarizes TANs to secrete extracellular matrix remodeling enzymes and proangiogenic factors, promoting metastasis and angiogenesis.[36]

3.2.3.9. Activating Invasion and Metastasis

The accumulation of genetic mutations in CRC cells, particularly KRAS mutations and TGF-β signaling activation, enable cell metastatic potential by contributing to epithelial-mesenchymal transition, increasing cell intra and extravasation and secondary organs colonizing. Cells can disseminate and seed metastatic sites at early stages of CRC development where the primary carcinoma is still clinically undetectable or at later stages. Metastasis involves cell adaptation through metabolic and transcriptional reprogramming.[36]

The major cause of death in CRC is liver metastasis.[36]

TGF-β signaling activation

TGF-β and oncogenic KRAS signaling play an important role in CRC metastasis. The activation of TGF-β receptor starts complex formation and nuclear translocation of SMAD2/3/4 to trigger its target genes. In CRC, SMAD4 tumor suppressor is the most common mutated genes in metastasis, being mutated in 12% of patients with metastatic or unresectable CRC. Loss of SMAD4 activates a signaling pathway that facilitates cancer cell invasion and metastasis.[36]

Metastasis colonization

CRC cells create an inflammatory premetastatic site through secretion of tissue inhibitor of metalloproteinases and integrin subunit beta like 1. In the liver, a supportive stromal matrix for CRC metastases is produced by profibrotic hepatic stellate cells. In addition, hepatic ANGPTL6 complexes with E-cadherin and α(6) integrin on CRC cells to improve their targeting to the liver.[36]

After colonization, PAD4 is expressed by metastatic cancer cells, promoting greater adhesion and increasing expression of characteristic epithelial markers. Metastatic cancer cells adapt to a new TME through metabolic reprogramming and change to a specific liver transcription profile.[36]

3.2.3.10. Inducing Angiogenesis

Angiogenesis is the development of new blood vessels and provides nutrients and oxygen suppresses cell needs, playing an important role in tumor initiation, growth and metastasis. This process is regulated by the equilibrium between proangiogenic ligand (VEGF) and antiangiogenic ligands (TSP-1).[35,36]

In CRC, the intermediaries of VEGF pathway are usually hyperactivated. The VEGF upregulation and VEGFR hyperactivation leads to increased density and permeability of lymphatic vessel and increased metastases in the lymph nodes, lungs and livers. Upregulation of KRAS and TP53, increased COX-2 expression, tumor-infiltrating immune cells (for example neutrophils, CD11b+ myeloid cells and macrophages) and proangiogenic mRNA and microRNAs secreted from CRC cells regulate VEGF-VEGFR activity, promoting vascular permeability, angiogenesis and cancer growth and migration.[35,36]

3.2.4. Colon Rectal Cancer treatment

Surgery

Surgery is the key of treatment with curative intent and the quality of cancer resection is decisive.[40] Surgeries that are available are right colectomy, sigmoid colectomy and total abdominal colectomy with ileorectal anastomosis.[5]

Radiotherapy

Preoperative radiotherapy can reduce the risk of local recurrence in CRC. Chemoradiotherapy is the most used therapy, using a fluoropyrimidine (fluorouracil or capecitabine) as radiation sensitizer and a time interval for surgery usually 8 to 10 weeks.[40]

Systemic treatment

Fluoropyrimidine-based chemotherapy increases survival in a subset of stage II and III colon cancers. Several studies have established the addition of oxaliplatin to a fluoropyrimidine as the new standard. However, the addition of oxaliplatin can lead to the development of cumulative sensory neuropathy. The addition of other agents such as, irinotecan and biologics, do not work as an adjunct to the treatment of rectal cancer. For many years, the standard of care was 6 months of adjuvant chemotherapy, but new studies have showed that 3 months of adjuvant chemotherapy can reduce toxicity without impairing treatment efficacy, at least for low- risk stage III colon cancers.[40]

For metastatic colorectal cancer, the systemic therapy usually includes chemotherapy combined with a biologic. The chemotherapy used is with Fluoropyrimidines, Oxaliplatin and Irinotecan in regimens of two-drug or three-drug and the added biologic, such as anti-VEGF or anti-EGFR antibody, depends on tumour-specific and patient-specific factors.[40]

These treatment programs are effective at improve overall survival, however they have severe side-effects such as nausea, vomiting, weight loss and risk of infectious complications.[5]

In Portugal, according to the DGS norm 025/2012, for CRC the available treatment options are surgery, chemotherapy, radiotherapy and targeted therapy. To patients with advanced stage of CRC (stage II or III) are recommended chemoradiotherapy or radiotherapy complementary to surgery. The chemotherapy drugs used are fluoropyrimidines (5-Fluorouracil or Capecitabine), Irinotecan and Oxaliplatin. These agents can be used alone or in combination, with the exception of oxaliplatin, which is only used in combination with fluoropyrimidines. Concomitant chemotherapy with radiotherapy is done with fluoropyrimidines. For stage IV patients with unresectable metastatic disease, palliative chemotherapy protocols include fluoropyrimidines or irinotecan alone, or combinations of fluoropyrimidines with irinotecan or oxaliplatin with or without biologics. The biological agents used are VEGFR inhibitor (Bevacizumab) and EGFR inhibitors (Cetuximab and Panitumumab). Bevacizumab should only be used in combination with chemotherapy.[41]

3.3. Metformin in Colon Rectal Cancer

Recently, additional roles for metformin have been discovered. In addition to T2DM, Metformin also has an effect on cancers, cardiovascular disease, liver diseases, obesity, neurodegenerative diseases and renal diseases. Metformin in monotherapy or in combination with other drugs has been shown to be effective in the treatment of several diseases.[7]

3.3.1. Anticancer property

Metformin's anticancer properties are related to its ability to modulate signaling pathways involved in cellular proliferation, apoptosis and metabolism.[5] Metformin can inhibit growth, survival and metastasis of different types of tumor cells: breast, liver, bone, pancreas, endometrial, colorectal, kidney and lung cancers.[7]

Recent studies suggest that metformin can improve the effects of other anticancer drug, such as Cisplatin, Vincristine, 5-Fluorouracil and Doxorubicin. This suggests that metformin can act as part of combination therapy to reduce the chemotherapy dose and increase sensitivity to radio- and chemotherapy in cancer patients, minimizing gastrointestinal side effects and reducing toxicity.[5,24] Furthermore, Metformin is cheaper than other chemotherapy drugs and adjuvants, so it serve as a cost-effective treatment option for CRC intervention.[5]

3.3.2. Mechanism of action

Metformin has an effect on the growth, proliferation and angiogenesis of the CRC cell through diverse signaling pathways. The mainly anticancer effect pathways result from the mediation of the AMPK/mTOR and insulin/insulin-like growth factor pathways (Figure 8).[5]

The anticancer effect of Metformin results from two main mechanisms: Direct mechanism related to inhibition of mitochondrial complex I, leading to suppression of ATP production; Indirect mechanism associated with its endocrine effects that may suppress tumor development.[5,23]

AMPK activation

In the direct mechanism, the inhibition of mitochondrial complex I electron transport chain by Metformin reduces oxidative respiration, resulting in ATP/AMP ratio imbalance, which in turn also activates Liver Kinase B1. Activated LKB1 activates AMPK, the principal metabolic sensor involved in the regulation of cellular energy homeostasis.[5,23,24] Following AMPK activation, it induces the activation and inhibition of several molecular signaling pathways that promote cell death.[5]

Activated AMPK induces the activation of Tuber Sclerosis Complex/Tuberin-2, an inhibitor of mTOR pathway which is essential in protein synthesis and cell proliferation.[5, 7,24,42]

AMPK activation also modifies the cell cycle by phosphorylation of TP53 on Ser15. TP53 regulates cell cycle arrestment, DNA repair, programmed cell death and senescence. The suppression of cyclin D1 and expression of cyclin-dependent kinase inhibitors p27Kip1 and p21Cip1 are responsible for regulating TP53 activation. The activation of TP53 induces the transcription of TP21, increasing the expression of apoptotic genes that lead to DNA-damage and fragmentation as well as cell cycle arrest at G0/G1 phase.[5,7,23,24] TP53 activation also regulates microRNAs expression (such as miR-21, miR-26a, miR-33a, miR-140-5p, miR-142-3p, miR-181a, miR-192, miR-193b, R-20mi0, miR-205, miR-222, let-7a, and let- 7c), that modulate different signaling pathways, inhibiting CRC cells proliferation.[5, 7,24]

Furthermore, the activation of AMPK modulates several transcription factors, for example inactivating NF-κB and FOXO, which regulate processes such as cellular apoptosis, oxidative stress, inflammation and neoplastic malignancy.[5,7,23,24] AMPK activation have an anti-inflammatory effect, reducing pro-inflammatory cytokines such as TNF-α, IL-6, IL-8 and VEGF.[5,7,23]

Lastly, AMPK activation suppresses the expression of lipogenic transcription factor Sterol Regulatory Element-Binding Protein 1, reducing its targets such as FAS and 3-hydroxy-3-methyl glutaryl-CoA reductase. Plasma concentrations of insulin, glucose, triglycerides and cholesterol are modulated by this process, disturbing cellular homeostasis.[5]

mTOR inhibition

mTOR is composed of a catalytic subunit of two multiprotein complexes, mTORC1 and mTORC2, and act as an important regulator of protein synthesis and cell growth.[7,24] The activation of LKB1/AMPK/TSC2 pathway by metformin result in mTOR pathway inhibition that is vital for the suppression the CRC cells proliferation. Even in the absence of TSC2, activated AMPK can suppress mTOR pathway through phosphorylation of the raptor component of the mTORC1 complex.[5,24]

mTOR inhibition induces the activation of PTEN, which inactivates PI3K-Akt. This pathway promotes cells apoptosis by increasing the expression of caspase-3 and apoptosis inducing factor.[5]

Oncogenic Myc protein promotes mitotic progression during the cell cycle, exhibiting deregulated pro-proliferative activity. mTOR inhibition suppresses Myc protein, reducing cancer cells proliferation.[43]

Insulin and Insulin-like growth factors

In normal cells, Insulin binds to Insulin Receptors and IGF-1 binds to its receptors (IGF-1R). These receptors are expressed and upregulated in cancer cells. The binding of receptors to their ligand phosphorylates them, leading to the activation of downstream pathways such as PI3K/Akt/mTOR and RAS/RAF/MAPK. Activation of these pathways promotes the initial tumor proliferation, invasion and metastasis. In the indirect mechanism, the metformin anticancer effect occurs through these insulin/IGF-1 pathways. Metformin reduces insulin and IGF-1 levels, decreasing the phosphorylation of IRS-1. This leads to reduced activation of the mTOR cascade.[5,23] In this way, cancer growth is reduced.

It was observed that Insulin or IGF-1 promotes phosphorylation of β-catenin, causing the accumulation of β-catenin in the cytosol and nucleus, which increases its transcriptional activity and enhances the invasiveness of tumor cells. However, treatment with metformin in human CRC cell lines promoted the translocation of β-catenin to the plasma membrane, decreasing transcriptional activity and invasiveness of tumor cells. This occurs as a result of AMPK activation by metformin, followed by inhibition of PI3K/Akt signaling and phosphorylation of β-catenin.[43]

Other mechanism

Suppression of mitochondrial complex I by Metformin prevents the generation of ROS and decreases DNA damage. This contributes to suppressing cancer growth.[7]

Metformin in combination with TNF-related apoptosis-inducing ligand induce the dissociation of Noxa from Myeloid Cell Leukemia 1, leading to an increase of E3 ligase Mule activity. This process promotes polyubiquitination of Mcl-1, leading to cancer cells apoptosis.[5,43]

Other effects of Metformin contribute to the inhibition of the development and progression of CRC, such as: the increase in the pool of bile acids in the gut, which affects the secretion of GLP-1 and cholesterol levels; and also the alteration of the gut microbiome, affecting the regulation of glucose homeostasis, lipid metabolism and energy metabolism.[7]

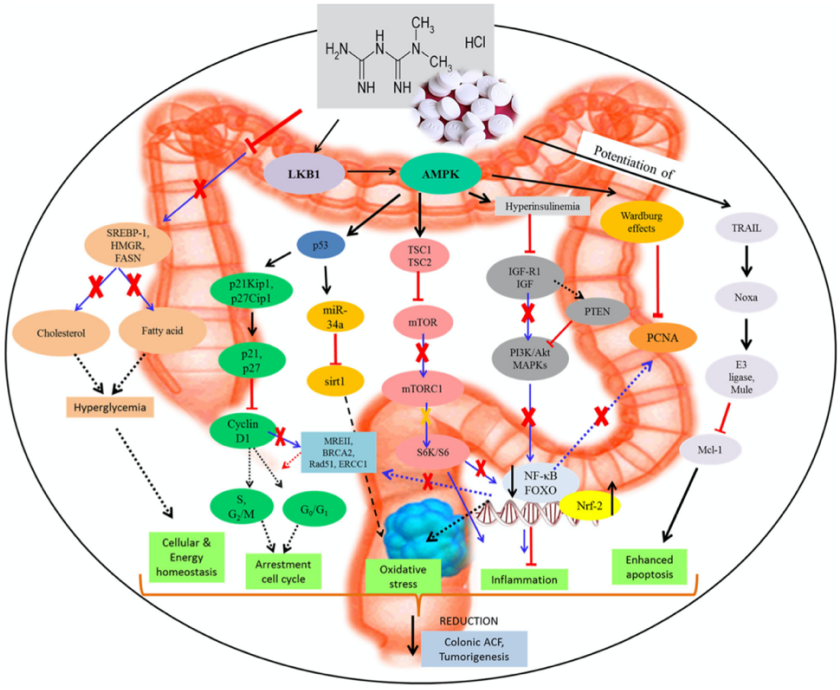

Figure 8 - Antitumor molecular mechanisms of Metformin
KAMARUDIN *et al.* - Metformin in colorectal cancer: molecular mechanism, preclinical and clinical aspects. Journal of Experimental & Clinical Cancer Research (2019) 38(1):491.

4. Drug Repurposing

Research and development of new drugs is an expensive, time-consuming and risky process.[1, 2, 3, 4] Increasing regulatory barriers can make this process even more expensive and time-consuming. Furthermore, de novo drug discovery and development takes about 10 to 17 years and approximately 90% of drugs that make it to clinical trials end up failing.[4,44] In this way, it is essential to have new strategies that bring drugs to market in a safer and cost-effective way and with less time-consuming in order to close the gap between our knowledge and the treatment of human disease.[4] An alternative strategy is drug repurposing, which has fewer risks, lower costs and shorter timelines (3-12 years) than developing new drugs.[2,44,45]

Drug repurposing is also called drug repositioning, reprofiling, re-tasking, redirection, rediscovery, or rescue.[1,2,3,44] All these terms describe the same process that is a strategy for identifying new applications for approved drug/compound that are outside the scope of the original medical indication and not currently prescribed or investigated.[1,2,3,4,44,45] Some authors distinguish between drug repurposing, drug repositioning and drug rescue. They refer to drug repurposing as the use of an approved drug for new indications and refer to drug repositioning to the development of an existing drug, previously evaluated but unapproved, for the treatment of a different disease. Drug rescue refer to the use of a drug that has failed for its primary indication.[2]

The use of Metformin in colon rectal cancer is one example of drug repurposing. Metformin, a common diabetes medication and now repurposed as cancer therapeutic. However, there are others examples, such as:[3,4,44,45]

- ◊ Sildenafil, a phosphodiesterase inhibitor, was originally developed as an antihypertensive drug and was repurposed for the treatment of erectile dysfunction;
- ◊ Thalidomide was originally developed as a sedative and was found to be effective in the treatment of Erythema Nodosum Leprosum (1964) and in multiple myeloma (1999);
- ◊ Bupropion was originally developed as an antidepressant and was repurposed for smoking cessation;
- ◊ Botox (onabotulinumtoxinA) was originally used for eye muscle disorders was found to be cosmetic effects;
- ◊ Minoxidil was originally developed to treat hypertension and was found to be effects in hair growth.
- ◊ Duloxetine, an antidepressant that blocks the reuptake of both serotonin and noradrenaline in the synaptic cleft, was originally developed to treat depression and was repurposed for the treatment of Stress Urinary Incontinence.

Approval rates for repurposed drugs are close to 30%. This drugs are generally approved between 3 to12 years, with reduced cost (about 50 to 60% of the cost) and with a lower risk.[2] With this strategy, the starting point is a drug for which its safety profile and pharmacokinetic properties are already well known. So, it may be possible to go straight to clinical trials for a new indication, avoiding the costly and lengthy research and development processes that is required for new drugs. The regulatory phase costs may remain similar to a new drug in the same indication. [2]

Drug repurposing offers some advantages over developing a new drug:[3,44]

- ◊ First, the risk of failure is lower: it is less probable to fail at least from a safety point because its safety and pharmacokinetic profiles are well-known.
- ◊ Second, the time frame for drug development can be reduced, because most of the studies will already have been completed and can therefore be bypassed: studies such as in vitro and in vivo screening, chemical optimization, toxicology, bulk manufacturing, formulation development and preclinical tests.
- ◊ Third, less investment is needed: the cost of drug repurposing will differ depending on the stage and process of development of the drug candidate. The regulatory phase costs may remain similar to a new drug in the same indication, but there may reduce the cost in preclinical and phase I and II.

New ideas for repositioning can come from different ways: serendipitous observations; from novel, informed insights; or from technology platforms.[44] Drug repurposing should be based on reliable studies and supported by robust scientific evidence. In this way, meta-analysis plays an important role in this process.

5. Meta-analysis

"Science remains the key driver of human progress" (Ioannidis JPA, 2018).[8] Improving the efficiency and producing more credible and useful results can create major benefits for research. Meta-research is the study of research itself and its practices and covers an extensive variety of theoretical, observational and experimental investigations.[8] Systematic reviews and meta-analyses constitute the highest level of evidence and are the gold standard for synthetizing evidence in scientific literature.[9,10,46]

Meta-analyses summarize, synthesize and integrate evidence from the literature on a given topic and can improve precision and provide robust outcomes.[10,13,14,15] However the validity of the conclusions of these reviews depends on the quality of the individual studies and the methodological quality of the evidence synthesis process.[10] As some studies have shown, a significant number of sub-optimal and conflicted meta-analyses are published, in health sciences area. It is determined that one-in-three meta-analyses in this area are redundant or unnecessary. A small minority of these reviews represent decent and reliable meta-analyses and these deficiencies impair the credibility of the results.[10]

Decision-makers can base their decisions on accurate, succinct, credible and comprehensible synthesis of evidence, such as meta-analysis.[12,13,14] In this way, it is important that these reviews can enhance precision and provide robust results.

5.1. Meta-analysis Quality

The number of published studies of healthcare interventions has increased rapidly and this produces benefits and risks. The benefits are the opportunities that decision-makers can base their decisions on accurate, succinct, credible and comprehensible synthesis of evidence minimizing error and bias. The risks involve disparity in quality.[12,47] The studies, especially meta-analysis, are expansively used by decision-makers as an important tool for achieving evidence-based healthcare.[12,13,14,15] So, it is important to evaluate the quality of published studies.

According to Ioannidis *et al.* (2016), only 3% of all meta-analyses published represent a good and truly informative study and the remaining are redundant, unnecessary, unpublished, not useful or have serious methodological flaws. This is due to their poor conduct and report.[14,46] Figure 9 illustrates a summarized overview of the meta-analyses produced.

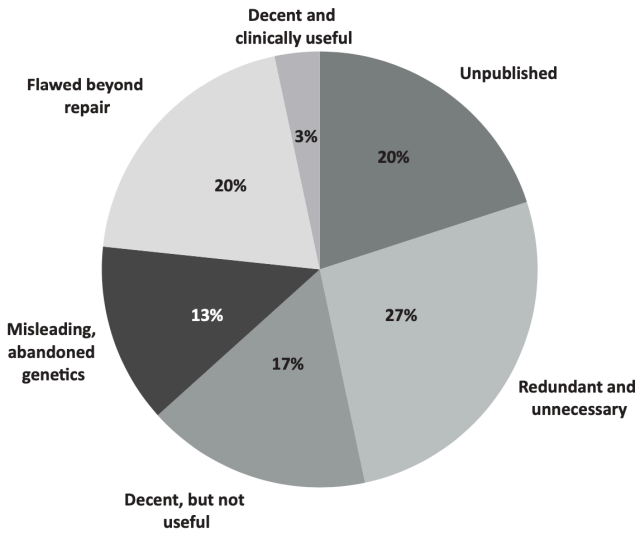

Figure 9 - Overview of Produced Meta-analyses
IOANNIDIS J. P. A - The Mass Production of Redundant, Misleading, and Conflicted Systematic Reviews and Meta-analyses. The Milbank Quarterly (2016) 94(3):485-514.

Uncritically accepting the results of a meta-analysis has risks. In the evidence-based healthcare literature is essential that meta-analyses have reproducibility and replicability. To evaluate the quality of meta-analysis, we can assess the methodological quality and the report quality of the reviews. For that, different guidelines have been projected to ensure transparency and reliability.[12,13,14] One of these guidelines is PRISMA, which is a guide for reporting systematic reviews. MOOSE is a guide for reporting systematic reviews of observational (non-randomised) studies and AMSTAR is a guide for conducting of a review.[12]

5.1.1. AMSTAR 2

To estimate the methodological quality of systematic reviews and meta-analyses of primary studies, we have some tools available, such as AMSTAR and its revised version, AMSTAR 2. This tool is available in a Stepwise checklist approach (Figure 10 a-d) and is intended to be used for reviews of healthcare interventions. This instrument allows rapid and reproducible assessments of the quality of conduct for systematic reviews of randomized controlled trials of interventions.[12,16]

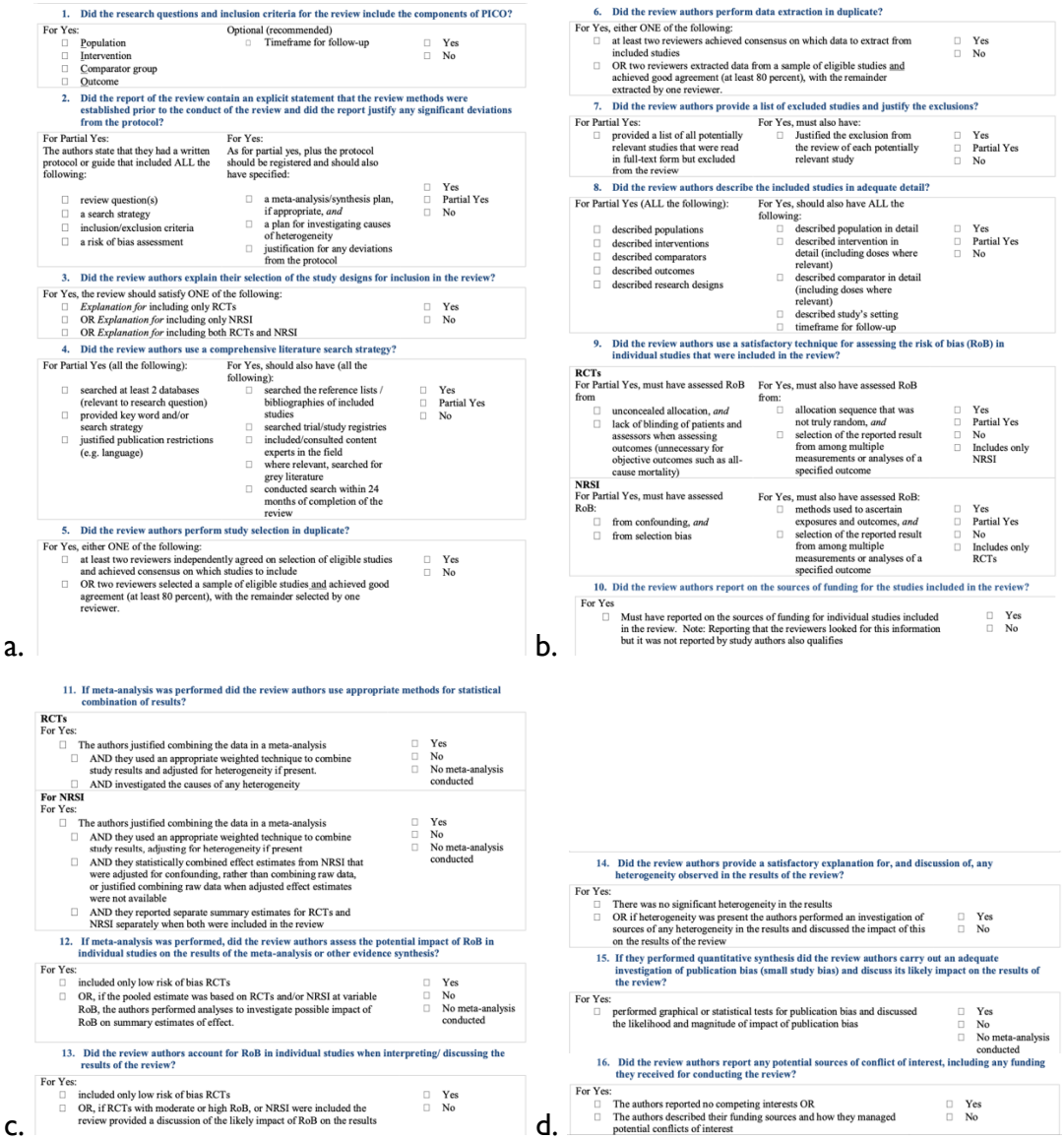

Figure 10 (a – d) - AMSTAR 2 checklist

SHEA B. J. *et al.* - AMSTAR 2: a critical appraisal tool for systematic reviews that include randomised or non-randomised studies of healthcare interventions, or both. BMJ (2017) 358:j4008.

Since publication, some criticisms and feedback received from reviewers have pointed to the need to revise and update the original AMSTAR instrument. An expert group met to discuss and improve the value of this instrument and considered that revised instrument should function as a teaching aid and as a checklist. They believed that reviews should address all aspects of a systematic review conducting and also considered the challenges of including non-randomized studies. Therefore, ten domains were preserved from the original instrument, while two domains were expanded to provide more comprehensive coverage. Furthermore, four additional domains were introduced to enhance the overall scope of the instrument. The main modifications in this revised version include improved PICO framework for research questions, more details on selection of study designs for inclusion, more details

on the risk of bias of the included studies and more information on studies that were excluded.[12]

Another modification was to simplify response categories. The domain specific questions in AMSTAR 2 are structured with "Yes", "Partial Yes" and "No". A "Yes" answer denotes a positive result and a "partial Yes" response denotes partial adherence to the standard. The "not applicable" and "cannot answer" options of the original AMSTAR instrument have been removed from this revised version and if no information is provided to assess an item, the answer should be "No".[12]

AMSTAR2 focuses on methodologic methods, including statistical methods.[13] To use AMSTAR2, it is recommend that do not combined individual item ratings to create an overall score. Instead, the potential impact of a review item should be considered to create a score. Table 3 propose a plan to interpret weaknesses detected in critical and non-critical domains and assess the confidence of the review.[12]

Table 3 - AMSTAR2: Overall assessment of confidence in the review results

High confidence	No or one non-critical weakness: the review provides an accurate and comprehensive summary of the results of the available studies that address the question of interest.
Moderate confidence	More than one non-critical weakness: the review has more than one weakness but no critical flaws. It may provide an accurate summary of the results of the available studies that were included in the review.
Low confidence	One critical flaw with or without non-critical weakness: the review has a critical flaw and may not provide an accurate and comprehensive summary of the available studies that address the question of interest.
Critically low confidence	More than one critical flaw with or without non-critical weakness: the review has more than one critical flaw and should not be relied on to provide an accurate and comprehensive summary of the available studies.

SHEA B. J. *et al.* - AMSTAR 2: a critical appraisal tool for systematic reviews that include randomised or non-randomised studies of healthcare interventions, or both. BMJ (2017) 358:j4008.

In guiding a meta-analysis, all steps are important. According to Shea B. J. *et al* [12], there are some domains that can affect the consistency of a review:

- ◊ Protocol registered before commencement of the review (item 2)
- ◊ Adequacy of the literature search (item 4)
- ◊ Justification for excluding individual studies (item 7)
- ◊ Risk of bias from individual studies being included in the review (item 9)
- ◊ Appropriateness of meta-analytical methods (item 11)
- ◊ Considerations of risk of bias when interpreting the result of the review (item 13)

◊ Assessment of presence and likely impact of publication bias (item 15)

This classification is a suggestion and before starting an assessment of a meta-analysis, users must define which domains are or are not critical for the review. Identification of critical weaknesses or flaws, reduce the confidence in the results of a review.[12]

AMTAR 2 has a role as a teaching support and as a checklist for conducting s meta-analysis, however it doesn't explain in detail the rational and methods for conducting these studies.[12]

5.1.2. PRISMA 2020

To estimate the report quality of systematic reviews and meta-analyses, we have some tools available, such as PRISMA. This tool is available in a checklist approach and consists of a consensus on the minimum set of items that authors should report.[13,16,48] The PRISMA 2020 checklist contains 7 sections with a total of 27 items, some of which include sub-items (Figure 11 a-b). This checklist contain details of reporting recommendations for each item and can be exported to Word or PDF.

a.

Section and topic	Item #	Checklist item
Title		
Title	1	Identify the report as a systematic review.
Background		
Objectives	2	Provide an explicit statement of the main objective(s) or question(s) the review addresses.
Methods		
Eligibility criteria	3	Specify the inclusion and exclusion criteria for the review.
Information sources	4	Specify the information sources (e.g. databases, registers) used to identify studies and the date when each was last searched.
Risk of bias	5	Specify the methods used to assess risk of bias in the included studies.
Synthesis of results	6	Specify the methods used to present and synthesise results.
Results		
Included studies	7	Give the total number of included studies and participants and summarise relevant characteristics of studies.
Synthesis of results	8	Present results for main outcomes, preferably indicating the number of included studies and participants for each. If meta-analysis was done, report the summary estimate and confidence/credible interval. If comparing groups, indicate the direction of the effect (i.e. which group is favoured).
Discussion		
Limitations of evidence	9	Provide a brief summary of the limitations of the evidence included in the review (e.g. study risk of bias, inconsistency and imprecision).
Interpretation	10	Provide a general interpretation of the results and important implications.
Other		
Funding	11	Specify the primary source of funding for the review.
Registration	12	Provide the register name and registration number.

*This abstract checklist retains the same items as those included in the PRISMA for Abstracts statement published in 2013,[54] but has been revised to make the wording consistent with the PRISMA 2020 statement and includes a new item recommending authors specify the methods used to present and synthesise results (item #6).

b.

Section and topic	Item #	Checklist item	Location where item is reported
Title			
Title	1	Identify the report as a systematic review.	
Abstract			
Abstract	2	See the PRISMA 2020 for Abstracts checklist (table 2).	
Introduction			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	
Methods			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	
	13d	Describe any methods used to synthesise results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesised results.	
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	
Results			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram (see fig 1).	
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	
Study characteristics	17	Cite each included study and present its characteristics.	
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesised results.	
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	
Discussion			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	
	23b	Discuss any limitations of the evidence included in the review.	
	23c	Discuss any limitations of the review processes used.	
	23d	Discuss implications of the results for practice, policy, and future research.	
Other information			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	
Competing interests	26	Declare any competing interests of review authors.	
Availability of data, code, and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	

Figure 11 (a,b) - PRISMA 2020 checklist

a. PRISMA2020 Abstract checlist; b. PRISMA2020 checklist

PAGE M. J. *et al.* - The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ (2021) 372:n71.

Meta-analyses serve many critical roles and create different types of knowledge for diverse users (such as researchers, healthcare providers and patients). To ensure that the systematic review has value, the author should write a transparent, complete and accurate report of why the review was done, what was done and what was found. For that end, a reporting guidance facilitates authors achieve this. In 2009, the published PRISMA checklist was designed to help authors report all of this important information. Advances in the methodology and terminology of studies led to an update of this guideline. To replace the 2009 statement, the PRISMA 2020 statement was created, which includes new reporting guidance with a different structure. To develop the revised version, PRISMA 2009 items that were often incompletely reported in published reviews and possible modifications to the PRISMA 2009 statement to improve de instrument were identified. For this, were invited systematic review methodologists and journal editors and the proposals for content and wording of the PRISMA 2020 statement were discussed.[48]

The PRISMA 2020 statement has been designed to apply in systematic reviews of health interventions studies, however it is also applied to reports of systematic reviews evaluating other interventions.

PRISMA 2020 is not projected to guide the conduct of systematic review, for which other tools exist. However, this checklist is useful for ensuring that all important information is reported when writing a systematic review. Furthermore, PRISMA 2020 is not planned to guide the reporting of systematic review protocols, for which a separate checklist exist. There are also PRISMA extensions that have been developed to guide reporting of other studies, such as network meta-analyses and meta-analyses of individual participant data.[48]

PRISMA 2020 only provides a model for how information might be organized, however the suggested location should not be seen as mandatory. This conducting principle is to ensure that the essential information is reported, which benefits many stakeholders.[48]

A complete report of a study allows readers to access information and access the accuracy of the methods, and consequently the reliability of the results. In this way, a complete report of meta-analysis allows healthcare professionals and policy makers to assess the applicability of the findings to their setting.[48]

6. Objective

Decision-makers can base their decisions on accurate, succinct, credible and comprehensible synthesis of evidence, such as meta-analysis. In this way, it is essential that meta-analyses provide robust outcomes, have reproducibility and can improve precision.

The aim of this research is to investigate the methodologic and report quality of published meta-analysis that associate the survival outcomes of CRC with Metformin use in patient diagnosed with CRC.

7. Methods

This review is reported follows PRISMA2020 guidelines. Ethical approval will not be required because this study extracts and synthesizes data from already published studies.

7.1. Search strategy

A literature search of meta-analyses investigating the effect of metformin treatment on survival outcomes of CRC was performed in PubMed (MEDLINE and PubMed Central), Scopus and Web of Science from inception to 31 March 2023. The search strategy used MeSH terms and related title and abstract words and no filters were applied. Potentially eligible studies were imported into EndNote for removal of duplicates.

MeSH terms and keywords selected for the search include drug repurposing, drug repositioning, drug rescue, drug reprofiling, meta-analyses, metformin, biguanide, colorectal neoplasm, colorectal tumor, colorectal cancer, colorectal carcinoma, colon cancer, colon neoplasm, colonic neoplasm, colonic cancer and colon adenocarcinoma. The detailed search strategy ir shown in Table 4.

7.2. Eligibility criteria

Included studies met criteria for (1) meta-analysis, (2) Patient diagnosed with CRC, (3) Metformin as treatment and (4) any survival outcome for CRC.

The exclusion criteria adopted are (1) studies that are not meta-analysis, (2) studies with patient diagnosed with CRC after starting treatment, (3) studies with a drug other than metformin as treatment, (4) haven't full article access and (5) studies in a language other than English.

7.3. Selection process

Two reviewers (ARO, AV) independently assessed all studies title and abstract to identify potential studies and discrepancies were resolved by consensus. Studies that met the inclusion criteria were retrieved for full-text assessment. To assess the degree of agreement between reviewers, we applied Cohen's kappa coefficient (κ).

For full text selection, one reviewer (ARO) carefully read the full text of relevant references and evaluated in detail to ascertain their eligibility.

7.4. Data extraction

Data extracted from each individual study was DOI, authors' name, title and abstract of the article and publication year. The characteristics of included studies were extracted in a predesigned table by one reviewer (ARO). From included studies, the data extracted was patients characterization, cancer type, CRC stage, time of CRC diagnosis, period of metformin treatment, metformin dose used, co-adjuvant chemotherapy protocols, Hazard Ratio and 95% Confidence Interval for the impact of Metformin on overall survival in CRC, RC and CC, subgroup analysis, number of primary studies included and authors' name, title and publication year of these studies.

7.5. Methodological and report quality assessement

Each included meta-analysis was assessed using the AMSTAR 2 and PRISMA 2020 checklists. AMSTAR 2 rates the validity of review results and measures 16 items and PRISMA 2020 measures a total of 42 items (27 items, some of which include sub-items) and provides a model for how information can be organized. A descriptive analysis of the obtained results was conducted.

Table 4 - Full search strategy

Data base	Search strategy
PubMed/ Medline	(Meta-analysis[TIAB] OR "Meta-Analysis"[Publication Type] OR "Network Meta-Analysis"[MeSH]) AND (Metformin[TIAB] OR Biguanide[TIAB] OR Dimethylbiguanidine[TIAB] OR Dimethylguanylguanidine[TIAB] OR Metformin[MeSH]) AND ("Colorectal Neoplasm"[TIAB] OR "Colorectal Neoplasms"[TIAB] OR "Colorectal Tumor"[TIAB] OR "Colorectal Tumors"[TIAB] OR "Colorectal Cancer"[TIAB] OR "Colorectal Cancers"[TIAB] OR "Colorectal Carcinomas"[TIAB] OR "Colon Cancer"[TIAB] OR "Colon Cancers"[TIAB] OR "Colon Neoplasm"[TIAB] OR "Colon Neoplasms"[TIAB] OR "Colonic Neoplasm"[TIAB] OR "Colonic Neoplasms"[TIAB] OR "Colonic Cancer"[TIAB] OR "Colonic Cancers"[TIAB] OR "Colon Adenocarcinoma"[TIAB] OR "Colon Adenocarcinomas"[TIAB] OR "Colorectal Neoplasms"[MeSH] OR "Colonic Neoplasms"[MeSH])
Scopus	TITLE-ABS ("Meta analysis") OR KEY ("Meta analysis") TITLE-ABS (Metformin OR Biguanide OR Dimethylbiguanidine OR Dimethylguanylguanidine) OR KEY (Metformin) TITLE-ABS ("Colorectal Neoplasm" OR "Colorectal Neoplasms" OR "Colorectal Tumor" OR "Colorectal Tumors" OR "Colorectal Cancer" OR "Colorectal Cancers" OR "Colorectal Carcinomas" OR "Colon Cancer" OR "Colon Cancers" OR "Colon Neoplasm" OR "Colon Neoplasms" OR "Colonic Neoplasm" OR "Colonic Neoplasms" OR "Colonic Cancer" OR "Colonic Cancers" OR "Colon Adenocarcinoma" OR "Colon Adenocarcinomas") OR KEY ("colorectal tumor")
Web of Science	(Meta-analysis) AND (Metformin OR Biguanide OR Dimethylbiguanidine OR Dimethylguanylguanidine) AND ("Colorectal Neoplasm" OR "Colorectal Tumor" OR "Colorectal Cancer" OR "Colorectal Carcinomas" OR "Colon Cancer" OR "Colon Neoplasm" OR "Colonic Neoplasm" OR "Colonic Cancer" OR "Colon Adenocarcinoma")

8. Results

A comprehensive search was conducted across PubMed/Medline, Scopus, and Web of Science databases. The search yielded a total of 36 articles in PubMed/Medline, 52 articles in Scopus, and 71 articles in Web of Science. Following the removal of duplicates, the abstracts and titles of the remaining articles were screened, resulting in the identification of 24 publications for full-text review[42,49-71]. Among these, 13 studies[56-58,60-69] were excluded from the analysis due to various reasons, including the absence of meta-analysis design, patients not being diagnosed with colorectal cancer (CRC) prior to treatment initiation, utilization of a treatment other than metformin, or limited access to the full article. Consequently, a total of 11 studies[42,49-55,59,70,71] that fulfilled our inclusion criteria were included in our study. The PRISMA study selection diagram is shown in Figure 12.

All 11 selected studies encompassed colorectal cancer (CRC) patients across all stages, irrespective of the presence or absence of Diabetes Mellitus, and utilized Metformin as the intervention. Specifically, MEI Z-B *et al.* (2014), TIAN S. *et al.* (2017), WINSTON NG C.A. *et al.* (2020), and WANG Q., SHI M. (2022) exclusively included CRC patients, whereas the remaining studies encompassed patients with cancers affecting multiple sites. Among the included studies, only LEGA I.C. *et al.* (2014) and YANG J. *et al.* (2022) provided information on the initiation time of Metformin treatment. Furthermore, YANG J. *et al.* (2022) also mentioned the use of adjuvant chemotherapy, and only one study among them reported the administered dose of Metformin. The detailed characteristics of the included studies are shown in Table 5.

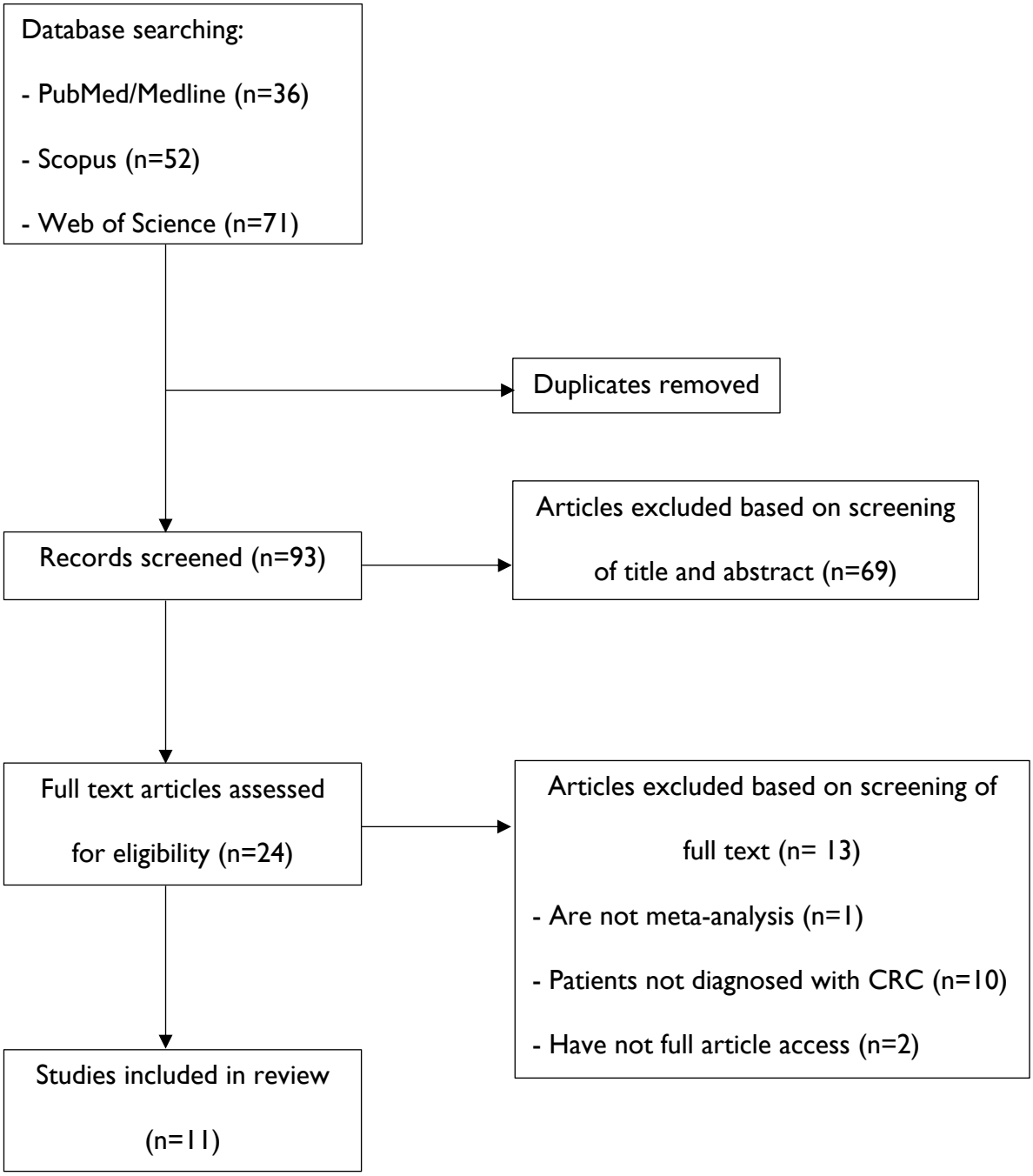

Figure 12 - The flow diagram of study selection

Table 5 - Characteristics of included studies

Author	Year	Patients	Cancer Types	CRC Stage	Nmb Primary Studies	Subgroup analyses
MEI Z. B. *et al*	2014	Cancer patients with concurrent DM II	CRC	All stages	6	N
ZHANG Z. J. *et al.*	2014	Cancer patients with or without concurrent diabetes	Any cancer, Breast, CRC, Ovarian, Prostate, Pancreatic, Lung, Endometrial, Liver, Laryngeal, Bladder	No information	28	N
COYLE C. *et al.*	2016	Cancer patients with or without concurrent diabetes	Prostate, CRC, Breast, Urothelial, Other types	I - III	27	N
CAO X. *et al.*	2017	Cancer patients with concurrent diabetes	Endometrial,, Bladder, Renal cell, Laryngeal, Breast, Pancreas, CRC, Hepatocellular, Ovarian, All sites, Prostate	No information	42	S
DU L. *et al.*	2017	Cancer patients with concurrent diabetes	CRC, Rectal, Colon	All stages	17	S
TIAN S. *et al.*	2017	Cancer patients with concurrent DM II	CRC	All stages	8	N
NG C. W., *et al.*	2020	Metastatic cancer patients with or without concurrent diabetes	CRC	IV	58	N
YANG J. *et al.*	2022	Cancer patients with or without concurrent diabetes	All sites, Bladder, Breast, Cervical, Colorectal, Endometrial, Gastric, Head and neck, Hepatocellular, Lung, Ovarian, Pancreatic, Prostate, Kidney	No information	80	S
SAKAMOTO K. *et al*	2022	Cancer patients and underwent NACRT	Rectal, Esophageal, Gastroesophageal	No information	5	S
WANG Q. and SHI M	2022	Cancer patients with concurrent diabetes	CRC	No information	10	S
LEGA I. C. *et al.*	2014	Cancer patients with or without concurrent diabetes	All Sites, Breast, Colon, Prostate, Pancreatic, Endometrial, Ovarian, Liver, Laryngeal, Lung	No information	21	S

8.1. Methodological quality assessement

To acess the metodological quality of the included studies, was used the 16-item AMSTAR 2 checklist. According to SHEA B. J. *et al.*[12], items 2, 4, 7, 9, 11, 13 and 15 of the checklist are some domains that can affect the consistency of a review. Thus, weakness in these items are considered "Critical Flaw" and weakness in remaining items are considered "Non-critical Flaw".

SHEA B. J. *et al.*[12] describe the review assessment of confidence as "high confidence" for studies with or without one non-critical flaw, "moderate confidence" for studies with more than one non-critical flaws, "low confidence" for studies with one critical flaw with or without non-critical flaws and "critically low confidence" for studies with more than one critical flaws with or without non-critical flaws. The results demonstrate that the meyhodological quality of the included studies is "low" and "critically low". Ten of the eleven studies have more than one critical flaws and more than one non-critical flaws, and one of the eleven studies has one critical flaw and more than one non-critical flaws (Table 6).

Table 6 - Results of quality assessment

Author (Year)	Critical Flaw (n)	Non-critical Flaw (n)	Quality Assessement
MEI Z. B. *et al* (2014)	2	2	Critically low confidence
ZHANG Z. J. *et al.* (2014)	4	5	Critically low confidence
COYLE C. *et al.* (2016)	5	5	Critically low confidence
CAO X. *et al.* (2017)	2	4	Critically low confidence
DU L. *et al.* (2017)	2	4	Critically low confidence
TIAN S. *et al.* (2017)	1	3	Low confidence
NG C. W., *et al.* (2020)	5	4	Critically low confidence
YANG J. *et al.* (2022)	3	5	Critically low confidence
SAKAMOTO K. *et al* (2022)	4	4	Critically low confidence
WANG Q. and SHI M (2022)	4	5	Critically low confidence
LEGA I. C. *et al.* (2014)	2	2	Critically low confidence

The most frequent critical flaws are usually in items 2 (8/11 – 72.73%), 7 (11/11 – 100.00%) and 15 (6/11 – 54.55%). And the most frequent non-critical flaws are usually in items 3 (11/11 – 100.00%), 10 (11/11 – 100.00%) and 12 (6/11 – 54.55%). Table 7 shows the frequency and percentage of flaw in each item individually.

Table 7 - Flaw assessment per item

Item	Description	Flaw (n)	Flaw (%)
1	Components of PICO	1	9.09
2*	Research protocol	8	72.73
3	Study designs for included reviews	11	100.00
4*	Search strategy	0	0.00
5	Study selection	3	27.27
6	Data extraction	4	36.36
7*	Excluded studies	11	100.00
8	Included studies	3	27.27
9*	Assessing risk of bias (RoB) in individual studies	2	18.18
10	Sources of funding for the studies included	11	100.00
11*	Methods for statistical combination of results	2	18.18
12	Assessing the potential impact of RoB in individual studies	6	54.55
13*	Discussing the potential impacts of risk of bias	5	45.45
14	Discussing the heterogeneity	4	36.36
15*	Publication bias	6	54.55
16	sources of conflict of interest	0	0.00

*- Critical Flaw

When considering both critical and non-critical flaws, the included studies presented varying numbers of flaws. MEI Z. B. *et al* (2014) reported a total of 4 flaws, ZHANG Z. J. *et al.* (2014) 9 flaws, COYLE C. *et al.* (2016) 10 flaws, CAO X. *et al.* (2017) 6 flaws, DU L. *et al.* (2017) 6 flaws, TIAN S. *et al.* (2017) 4 flaws, NG C. W., *et al.* (2020) 9 flaws, YANG J. *et al.* (2022) 8 flaws, SAKAMOTO K. *et al* (2022) 8 flaws, WANG Q. and SHI M (2022) 9 flaws and LEGA I. C. *et al.* (2014) exhibited 4 flaws.

These findings highlight the presence of various flaws in the methodological aspects of the included studies, indicating potential limitations in their overall quality. Careful consideration of these flaws is necessary when interpreting and applying the findings to ensure accurate and reliable conclusions.

8.2. Report quality assessment

To acess the report quality of the included studies, was used the PRISMA2020 checklist and PRISMA2020 abstract checklist. The PRISMA2020 checklist consists of 27 items, some of which include sub-items, and the PRISMA2020 abstract checklist consists of 12 items.

The results demonstrate that the report quality of the included studies is low, whit an average of 22 flaws per article (41.51%). Table 8 shows the number of flaws for each article.

Table 8 - Individual flaw assessment

Author (Year)	Flaw (n)	Flaw (%)
MEI Z. B. *et al* (2014)	17	32.08
ZHANG Z. J. *et al.* (2014)	27	50.94
COYLE C. *et al.* (2016)	20	37.74
CAO X. *et al.* (2017)	26	49.06
DU L. *et al.* (2017)	25	47.17
TIAN S. *et al.* (2017)	19	35.85
NG C. W., *et al.* (2020)	23	43.40
YANG J. *et al.* (2022)	18	33.96
SAKAMOTO K. *et al.* (2022)	23	43.40
WANG Q. and SHI M (2022)	22	41.51
LEGA I. C. *et al.* (2014)	18	33.96

The most frequent flaws are mainly in items 3 (11/11 – 100.00%), 5 (10/11 – 90.91%), 9 (10/11 – 90.91%), 11 (11/11 – 100.00%) and 12 (11/11 – 100.00%) of the PRISMA2020 abstract checklist and in items 13a (11/11 – 100.00%), 13b(11/11 – 100.00%), 13c (11/11 – 100.00%), 14 (10/11 – 90.91%), 15 (11/11 – 100.00%), 16b (10/11 – 90.91%), 21 (11/11 – 100.00%), 22 (11/11 – 100.00%), 24a (9/11 – 81.82%), 24b (10/11 – 90.91%) and 24c (11/11 – 100.00%) of the PRISMA2020 checklist (Table 9).

When considering flaws in the reporting of the reviews, the included studies exhibited varying numbers of flaws. MEI Z. B. *et al* (2014) reported a total of 17 flaws, ZHANG Z. J. *et al.* (2014) 27 flaws, COYLE C. *et al.* (2016) 20 flaws, CAO X. *et al.* (2017) 26 flaws, DU L. *et al.* (2017) 25 flaws, TIAN S. *et al.* (2017) 19 flaws, NG C. W., *et al.* (2020) 23 flaws, YANG J. *et al.* (2022) 18 flaws, SAKAMOTO K. *et al* (2022) 23 flaws, WANG Q. and SHI M (2022) 22 flaws and LEGA I. C. *et al.* (2014) exhibited 18 flaws.

Table 9 - Flaw assessment per item (studies with % of flaws higher than 50)

Item	Description	Flaw (n)	Flaw (%)
3*	Abstract: Eligibility criteria	11	100.00
5*	Abstract: Risk of bias	10	90.91
7*	Abstract: Included studies	6	54.55
9*	Abstract: Limitations of evidence	10	90.91
11*	Abstract: Funding	11	100.00
12*	Abstract: Registration	11	100.00
13a	Synthesis methods: Studies eligibility	11	100.00
13b	Synthesis methods: data presentation or synthesis	11	100.00
13c	Synthesis methods: display results	11	100.00
13f	Synthesis methods: assess the robustness of results	7	63.64
14	Reporting bias assessment	10	90.91
15	Certainty assessment	11	100.00
16b	Study selection: Excluded studies	10	90.91
20a	Results of syntheses: summarise the characteristics and risk of bias	7	63.64
21	Reporting biases	11	100.00
22	Certainty of evidence	11	100.00
24a	Registration and protocol: registration information	9	81.82
24b	Registration and protocol: protocol accessing,	10	90.91
24c	Registration and protocol: amendments to information	11	100.00
27	Availability of data, code and other materials	6	54.55

* - PRISMA2020 Abstract Checklist

These findings underscore the presence of various flaws in the reporting quality of the included studies. It is important create a complete study report that allows healthcare professionals and policy makers to assess the applicability of the findings to their setting.

8.3. Hazard Ratio and 95% confidence interval

Overall survival is a fundamental and widely utilized measure in medical research. It quantifies the duration between the initiation of a treatment or diagnosis and the occurrence of death from any cause. OS serves as a crucial endpoint in clinical trials and observational studies, providing valuable insights into the effectiveness of specific treatments or interventions in extending the lives of patients, particularly in the context of cancer research.

Overall survival HR and corresponding 95% CI were extracted from the included studies. However, it should be noted that not all of the studies provided specific HR and 95% CI. Table 10 presents the extracted results from the included studies regarding overall survival in CRC, as the studies focusing on CC and RC do not report specific results in this context.

Table 10 - Overall survival in CRC (HR, 95% CI) from the included studies

Author (Year)	Overall Sulvival (HR; 95% CI)
MEI Z. B. *et al* (2014)	0.49 (0.37; 0.66)
ZHANG Z. J. *et al.* (2014)	Not reported
COYLE C. *et al.* (2016)	0.69 (0.58; 0.83)
CAO X. *et al.* (2017)	Not reported
DU L. *et al.* (2017)	0.69 (0.61;0.77)
TIAN S. *et al.* (2017)	0.82 (0.77; 0.87)
NG C. W., *et al.* (2020)	0.60 (0.53; 0.67)
YANG J. *et al.* (2022)	0.81 (0.65; 1.01)
SAKAMOTO K. *et al* (2022)	Not reported
WANG Q. and SHI M (2022)	Not reported
LEGA I. C. *et al.* (2014)	Not reported

Among the studies examining the impact of Metformin on OS in CRC, Mei Z. B. *et al.* (2014) reported a HR of 0.49 (95% CI: 0.37; 0.66), indicating a significantly lower risk of survival events. Coyle C. *et al.* (2016), Du L. *et al.* (2017), and Ng C. W. *et al.* (2020) reported HRs of 0.69 (95% CI: 0.58; 0.83), 0.69 (95% CI: 0.61; 0.77), and 0.60 (95% CI: 0.53; 0.67), respectively, suggesting a favorable impact on overall survival. Yang J. *et al.* (2022) and TIAN S. *et al.* (2017) reported HRs of 0.81 (95% CI: 0.65; 1.01) and 0.82 (95% CI: 0.77; 0.87), respectively, indicating a potential positive effect on overall survival. However, it should be noted that some studies did not provide specific HR and 95% CI, which makes it challenging to accurately interpret their findings.

9. Discussion

Colorectal cancer is the third most common malignant tumor and accounts for approximately 916 000 deaths worldwide every year.[27,30] Diabetes Mellitus and Colorectal Cancer share many risk factors. In this regard, it has been reported that the intake of metformin is associated with a reduction in the risk of colorectal cancer and an improvement in survival among CRC patients.[27,42]

With the continuous advancement of medical science, drug repurposing has emerged as a concept. This concept pertains to drugs that have been on the market for a long period and have been studied for new medical treatments, as opposed to the development of entirely new medicines. Information regarding the formulation, mechanism, and safety profile of these drugs is already known. Therefore, repurposed drugs have the advantages of low research costs, low risk, and high success rates compared to de novo drugs.[27]

Metformin serves as an example of drug repurposing. It is a biguanide with hypoglycemic action and a good safety profile, which also exhibits potential antitumor effects. Multiple mechanisms contribute to the effect of metformin, including glucose uptake, gluconeogenesis, insulin sensitivity, secretion of GLP-1, and the AMPK signaling pathway.[23,26,27] Several studies have demonstrated that metformin plays a crucial role in reducing the incidence of cancer and improving the prognosis of patients, particularly in colorectal, pancreatic, and hepatocellular cancers.[7,27]

The precise effect of metformin on cancer pathways has not been fully elucidated. Some studies indicate that metformin influences the mTOR and AMPK pathways. Activation of the AMPK pathway promotes p53-dependent autophagy by upregulating the expression of the p53 gene. It also induces cell cycle arrest by inhibiting cyclin D1 expression, leading to G1 phase arrest. Additionally, AMPK activation mediates the mTOR pathway through the phosphorylation of TSC2, resulting in the inhibition of mTOR signaling and a reduction in protein synthesis in cancer cells. Another mechanism of metformin is its ability to lower serum levels of insulin and IGF-1, which are known to promote cell survival and proliferation. Consequently, metformin reduces activation of the mTOR cascade, thus inhibiting cancer growth.[5,23,27,42]

As a drug development strategy, drug repurposing has garnered significant interest from researchers, leading to the emergence of numerous new indications for existing drugs. Healthcare professionals and policymakers often rely on information from meta-analyses to make informed decisions. Hence, it is crucial for meta-analyses to provide accurate, concise, credible, and comprehensible synthesis of evidence. Meta-analyses summarize, synthesize, and

integrate evidence from the literature on a specific topic, enhancing precision and yielding robust outcomes.[10,13,14,15] However, some studies indicate that a notable number of sub-optimal and conflicting meta-analyses are published in the field of health sciences.[10]

In this review of meta-analyses reporting evidence of the effect of metformin on rectal colon cancer survival outcomes. The results of the statistical analyses reveal that the methodological quality of the meta-analyses reporting the relationship between metformin and rectal colon cancer survival outcomes is generally "low" and "critically low". These findings align with the conclusions of YU H. *et al*. (2019)[27] and NOWICKA Z. *et al.* (2023)[72], who also reported that the methodological quality of the studies was considered "low" and "critically low".

YU H. *et al* (2019)[27] conducted a comprehensive review encompassing 21 studies that investigated 11 major anatomical sites. The results of their analysis provided highly suggestive evidence indicating a positive association between metformin intake and improved survival outcomes in patients diagnosed with colorectal cancer. The summary random effect estimates yielded statistically significant results with strong magnitudes. Additionally, the authors assessed the methodological quality of the included studies and reached the conclusion that systematic reviews and meta-analyses in this field exhibit poor methodological quality. It was observed that all the studies included in the analysis displayed multiple critical flaws, specifically in items 2 (85.7%), 7 (100%), and 13 (100%). Furthermore, numerous non-critical flaws were also identified in items 3 (85.7%), 10 (100%), and 12 (90.5%), collectively indicating a "critically low quality" of the studies.

NOWICKA Z. *et al.* (2023)[72] conducted a study involving 11 studies with pancreatic cancer patients. The researchers arrived at the conclusion that the existing evidence linking metformin to a reduction in pancreatic cancer mortality is generally of low quality, and the methodological quality of the included studies is also notably "low" and "critically low". Despite promising findings from preclinical studies suggesting the potential of metformin as an antitumor treatment, no RCTs have confirmed such therapeutic effects. In terms of the methodological quality assessment, the researchers noted that 45% of the meta-analyses exhibited low quality, while 55% demonstrated a "critically low quality". Furthermore, when considering the publication year of the studies, those published after 2017 were generally rated as having a "critically low quality", whereas studies published in or before 2017 were deemed to have a "low quality".

Regarding reporting quality, this review revealed significant deficiencies in the reporting quality of the studies included. Out of the eight studies analyzed, only four adhered to the PRISMA2020 checklist, indicating a low rate of adherence to standardized reporting practices. These findings are consistent with the observations made by NOWICKA Z. *et al.* (2023)[72], who also reported poor reporting quality among the included studies.

Furthermore, NOWICKA Z. *et al.* (2023)[72] conducted an evaluation of the reporting quality and found that only four studies followed the PRISMA2020 reporting guidelines. Additionally, a significant majority of the meta-analyses (63.6%) exhibited incomplete or inaccurate reporting of data.

This finding underscores a concerning trend in the field, as the adherence to reporting guidelines plays a critical role in ensuring transparency, replicability and overall quality of meta-analyses. By following the PRISMA2020 guidelines, researchers are encouraged to present a comprehensive and transparent account of their study methods, results, and conclusions, thereby enabling a more accurate assessment of the evidence. The low compliance rate with the PRISMA2020 guidelines revealed in this review suggests potential gaps in the reporting of essential information in the included studies. Incomplete or inaccurate reporting has the potential to impede the reproducibility of research findings and can lead to biased interpretations or misinterpretations of the results. Additionally, it restricts the ability of other researchers and decision-makers to fully evaluate and apply the findings in clinical practice. To address these issues, it is crucial for researchers to prioritize adherence to reporting guidelines, such as PRISMA2020, which promote comprehensive and transparent reporting of study methods, results and conclusions. By providing detailed information regarding the study design, participant characteristics, data analysis methods and transparent reporting of the results, researchers can enhance the reliability and utility of their studies, ultimately advancing the quality and impact of research in the field.

In terms of the results of HR and 95% CI for overall survival in CRC patients using Metformin, the studies with less favorable values are Mei Z. B. *et al.* (2014) (HR 0.49, 95% CI: 0.37; 0.66), Ng C. W. *et al.* (2020) (HR 0.60, 95% CI: 0.53; 0.67), Coyle C. *et al.* (2016) (HR 0.69, 95% CI: 0.58; 0.83), and Du L. *et al.* (2017) (HR 0.69, 95% CI: 0.61; 0.77). On the other hand, Yang J. *et al.* (2022) (HR 0.81, 95% CI: 0.65; 1.01) and TIAN S. *et al.* (2017) (HR 0.82, 95% CI: 0.77; 0.87) reported results indicating a potential positive effect of Metformin on overall survival in CRC. It is important to note that some studies did not provide specific hazard ratios and confidence intervals, which poses challenges in accurately interpreting their

findings. This highlights the significance of consistent reporting standards in future studies to enhance the comparability and reliability of the results.

In general, the studies with less favorable values of HR and 95% CI for overall survival in CRC patients using Metformin are also the ones that exhibit a higher number of methodological and reporting flaws. However, there is an exception with Mei Z. B. *et al.* (2014), which has the lowest HR and 95% CI values (HR 0.49, 95% CI: 0.37; 0.66) and does not belong to the studies with the highest number of methodological and reporting flaws. Ng C. W. *et al.* (2020) reports an HR of 0.60 and 95% CI of 0.53, 0.67 for overall survival, along with 9 flaws in the assessment of methodological quality using the AMSTAR criteria and 23 flaws in the assessment of reporting quality using the PRISMA criteria. Coyle C. *et al.* (2016) presents results of overall survival with an HR of 0.69 and 95% CI of 0.58, 0.83, along with 10 flaws in the assessment of methodological quality using AMSTAR. Lastly, Du L. *et al.* (2017) reports HR values of 0.69 and 95% CI of 0.61, 0.77 for overall survival, along with 25 flaws in the assessment of reporting quality using PRISMA.

By analyzing the OS outcomes, researchers can evaluate the effectiveness of interventions in terms of patient survival. These findings enable informed decision-making regarding treatment strategies and patient care. Assessing the impact of interventions on OS provides crucial information on the potential benefits and risks associated with specific treatments or interventions. This knowledge is vital for healthcare professionals and policymakers when making decisions about the most appropriate and effective approaches to improve patient outcomes and quality of life.

Overall, the cumulative evidence from all studies consistently demonstrates a "low" and "critically low" methodological quality in the meta-analyses. Both studies identified flaws and inadequate reporting within the included studies, thereby highlighting the limitations and deficiencies in the methodological rigor of these meta-analyses. This finding raises significant concerns regarding the transparency and reproducibility of research within this field. Incomplete or inaccurate reporting has the potential to impede the interpretation and replication of study findings, which may result in biased conclusions and limited applicability in clinical practice. Enhancing the reporting quality in this field is of utmost importance to safeguard the credibility and validity of research findings. By encouraging researchers to adhere to reporting guidelines and promoting transparency in study reporting, we can foster the development of a more reliable and impactful body of evidence pertaining to the association between metformin and rectal colon cancer survival outcomes. This commitment to comprehensive and transparent reporting will enable a more accurate evaluation and

interpretation of research findings, thereby facilitating evidence-based decision-making in clinical practice and ultimately improving patient outcomes.

9.1. Limitations

Several limitations need to be acknowledged in this study. Firstly, while we have placed considerable trust in the accuracy of the data provided within the meta-analyses, it is important to recognize that potential issues within the published data could impact the results of the evidence-rating process, despite our rigorous statistical analyses. This reliance on existing data introduces an element of uncertainty that should be taken into account when interpreting the findings.

Secondly, it is worth noting that meta-analyses consisting of less than ten studies were unable to undergo statistical tests aimed at identifying small study effects and excess significance. This limitation restricts the ability to thoroughly investigate potential biases or inconsistencies arising from smaller studies, which may influence the overall conclusions drawn from the review.

Thirdly, it is crucial to highlight the "critically low" methodological quality of all the meta-analyses included in this study. The limitations inherent in the design and conduct of these studies raise concerns about the reliability and validity of their findings. Therefore, future research should aim to address these methodological shortcomings by adhering to the rigorous criteria outlined in AMSTAR 2.0, a widely recognized tool for assessing the quality of systematic reviews and meta-analyses. Only by conducting such high-quality studies can we obtain more reliable and robust evidence to confirm the findings presented in our study.

Finally, an additional limitation lies in the lack of information regarding the metformin treatment initiation time, the administered dose or the specifics of any adjuvant chemotherapy treatment. The absence of such crucial details restricts our ability to fully evaluate the potential impact of these factors on the outcomes of interest. Future studies should consider incorporating comprehensive information on treatment regimens to enhance the understanding of the effects observed and provide a more comprehensive analysis of the interventions under investigation.

10. Conclusion

By conducting an analysis of eleven studies, it has become apparent that the methodological quality of the included meta-analyses is generally "low" and "critically low". Therefore, caution must be exercised in interpreting these results and firm conclusions cannot be drawn due to the inherent limitations stemming from the poor methodological quality of the systematic reviews and meta-analyses.

These findings emphasize the urgent need for high-quality studies and rigorous methodological approaches in this field. In future research endeavors, it is crucial to prioritize adherence to reporting guidelines and the implementation of robust research methods. By adopting these measures, we can significantly enhance the reliability and validity of the available evidence. This transparency promotes reproducibility and facilitates the evaluation and comparison of research findings, thereby providing decision-makers with more reliable information.

11. Conflict of interests and funding

The author declare that there is no conflict of interests and that this research has not receive any specific grant.

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