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Metformin: From diabetes to cancer to prolongation of life

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ABSTRACT

The metformin molecule dates back to over a century, but its clinical use started in the '50s. Since then, its use in diabetics has grown constantly, with over 150 million users today. The therapeutic profile also expanded, with improved understanding of novel mechanisms. Metformin has a major activity on insulin resistance, by acting on the insulin receptors and mitochondria, most likely by activation of the adenosine monophosphate-activated kinase. These and associated mechanisms lead to significant lipid lowering and body weight loss. An anti-cancer action has come up in recent years, with mechanisms partly dependent on the mitochondrial activity and also on phosphatidylinositol 3-kinase resistance occurring in some malignant tumors. The potential of metformin to raise life-length is the object of large ongoing studies and of several basic and clinical investigations. The present review article will attempt to investigate the basic mechanisms behind these diverse activities and the potential clinical benefits. Metformin may act on transcriptional activity by histone modification, DNA methylation and miRNAs. An activity on age-associated inflammation (inflammaging) may occur via activation of the nuclear factor erythroid 2 related factor and changes in gut microbiota. A senolytic activity, leading to reduction of cells with the senescent associated secretory phenotype, may be crucial in lifespan prolongation as well as in ancillary properties in age-associated diseases, such as Parkinson's disease. Telomere prolongation may be related to the activity on mitochondrial respiratory factor 1 and on peroxisome gamma proliferator coactivator 1-alpha. Very recent observations on the potential to act on the most severe neurological disorders, such as amyotrophic lateral sclerosis and frontotemporal dementia, have raised considerable hope.

Abbreviations: **ACLY**, ATP Citrate Lyase; **ALS**, Amyotrophic Lateral Sclerosis; **AMPK**, Adenosine Monophosphate-activated Protein Kinase; **ANGPTL3**, Angiopoietin-like 3; **ATP**, Adenosine Triphosphate; **BP**, Blood Pressure; **CD8**, Cluster of Differentiation 8; **ChREBP**, Carbohydrate Responsive Element-Binding Protein; **CNS**, Central Nervous System; **CRAC**, Calcium Release Activated Channel; **DARS2**, DNA-Reactivating Sequences 2; **DIPC**, Diffuse Intrinsic Pontine Glioma; **DNA**, Deoxyribonucleic Acid; **DNMT1**, DNA-Methyl Transferase 1; **DRP1**, Dynamin-Related Protein 1; **EDHF**, Endothelium-Derived Hyperpolarizing Factor; **EMT**, Epithelial-to-Mesenchymal Transition; **eNOS**, endothelial Nitric Oxide Synthase; **ER**, Endoplasmic Reticulum; **FBP1**, Fructose 1–6-bisphosphatase; **FIS1**, Mitochondrial Fission 1; **FDA**, Food and Drug Administration; **FMD**, Flow Mediated Dilatation; **FTD**, Frontotemporal Dementia; **GDF-15**, Growth Differentiating Factor-15; **GI**, Gastrointestinal; **GLP-1**, Glucagon-Like Peptide-1; **GLUT-1**, Glucose Transporter Protein Type-1; **GLUT-2**, Glucose Transporter Protein Type-2; **GLUT-4**, Glucose Transporter Protein Type-4; **GLUT-5**, Glucose Transporter Protein Type-5; **mGPDH**, Mitochondrial-3-Glycerol-Phosphate Dehydrogenase; **Gpx7**, Glutathione Peroxidase 7; **HbA1c**, Glycated Hemoglobin A1c; **HDL**, High-Density Lipoprotein; **HER2**, Human Epidermal Growth Factor Receptor-2; **HSF1**, Heat Shock Factor 1; **IF-1**, Interferon-1; **IL-6**, Interleukin 6; **IL-8**, Interleukin 8; **IR**, Insulin Receptor; **IRS-1**, Insulin Receptor Substrates-1; **IRS-2**, Insulin Receptor Substrates-2; **IIS**, Insulin-like Signaling; **KRAS**, Kirsten Rat Sarcoma virus; **LDL**, Low-Density Lipoprotein; **LINE-1**, Long Interspersed Elements-1; **lncRNA**, Long Noncoding RNA; **LPL**, Lipoprotein Lipase; **LPS**, Lipopolysaccharide; **MATE**, Multidrug and Toxin Extrusion Protein; **MFF**, Mitochondrial Fission Factor; **Mdivi-1**, Mitochondrial Division Inhibitor-1; **miRNA**, microRNA; MPC, Mitochondrial Pyruvate Carrier; **MRC**, Mitochondrial Respiratory Chain; **MS**, Metabolic Syndrome; **NADH**, Nicotinamine Adenine Dinucleotide; **NRF1**, Nuclear Respiratory Factor 1; **Nrf2**, Nuclear Factor Erythroid 2-related Factor; **NO**, Nitric Oxide; **OC**, Oral Contraceptive; **OCT1**, Organic Cation Transporter 1; **OGTT**, Oral Glucose Tolerance Test; **OXPHOS**, Oxidative Phosphorylation; **PAD**, Peripheral Arterial Disease; **PAI-1**, Plasminogen Activator Inhibitor-1; **PCOS**, Polycystic Ovary Syndrome; **PCSK9**, Proprotein Convertase Subtilisin/Kexin type 9; **PD-L1**, Programmed Death-Ligand 1; **PEN-2**, Presenilin Enhancer 2; PIK3, Phosphatidylinositol 3-Kinase; **PGC1α**, Peroxisome Gamma Proliferator Coactivator 1-alpha; **PGF2 α**, Prostaglandin F2 alpha; **PPAR**, Peroxisome Proliferator-Activated Receptor; **RAN**, Repeated Associated non-ATG; **RNA**, Ribonucleic Acid; **ROS**, Reactive Oxygen Species; **SASP**, Senescent Associated Secretory Phenotype; **SCFA**, Short Chain Fatty Acid; **SHP**, Small Heterodimer Partner; **SIRT**, Silent Information Regulator; **sVCAM-1**, Soluble Vascular Cell Adhesion Molecule-1; **TAME**, Target Aging with Metformin Study; **TBARS**, Thiobarbituric Reactive Substances; **T2DM**, Type 2 Diabetes Mellitus; **TNBC**, Triple Negative Breast Cancer subtype; **t-PA**, tissue-type plasminogen activator; **tRNA**, Transfer RNA; **VLDL**, Very Low-Density Lipoprotein; **vWF**, Von Willebrand factor; **ZEB1**, Zinc Finger E-Box-binding Homeobox 1.

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1. Introduction

Metformin is an old molecule. The synthesis of N-N dimethylbiguanide dates back to the work of Werner and Bell [1]. Interest in the molecule was revived after the introduction to therapy of phenformin (phenyl-ethyl biguanide) (Fig. 1). This drug proved to be a powerful hypoglycemic agent, but, after a few years, cases of lactic acidosis were reported. These became quite numerous and eventually the Food and Drug Administration (FDA) withdrew the phenformin authorization in 1977. In the period of phenformin availability, an idea by the French Company ARON was to retrieve and develop the simpler metformin (Fig. 1). Development progressed rapidly, and a clinical study was initiated by Professor J. Sterne, a diabetologist working in Morocco. The first publication, indicating a clear hypoglycemic effect of metformin, came out in 1957, providing data from diabetic inpatients [2]. This report indicated modest subjective adverse events and no apparent risk of hypoglycemia, a feared side effect with the, at the time, prevalent use of sulphonylurea medications. The later studies on insulin resistance and the detection of a novel mechanism consequent to the activation of adenosine monophosphate-activated kinase (AMPK) [3] have made metformin an antidiabetic drug with unique potential, thus justifying its very wide use.

2. Metformin absorption/disposition and gastrointestinal side effects

Metformin is among the most widely employed medications in terms of number of prescriptions and, since daily doses can frequently exceed 2000 mg/day, it is likely to be the most widely prescribed drug by weight. The high daily doses frequently associate with adverse gastrointestinal (GI) side effects, from abdominal discomfort to nausea, diarrhea, flatulence, and vomiting, both in diabetics and non-diabetics. These side effects occur in 25 % of all treated patients, resulting in discontinuation in 5 % [4].

While repeated administration and intake, e.g., during meals, may reduce adverse effects, a potential inhibition of digestive proteases has been described in animal models. Availability of extended-release tablets to be taken once daily before the evening meal or twice daily before breakfast and the evening meal, has improved tolerability and compliance [5].

In rodents, studies on the activity of digestive enzymes in the duodenum indicated that enteropeptidase and trypsin were inhibited by metformin at concentrations in the dose range occurring during human daily intake [6]. In particular, trypsin activity in the intestinal content is significantly reduced in samples from metformin-treated mice [7]. A significant impact on protein digestion in a similar range is observed with the serine protease inhibitor camostat, a guanidine derivative acting as an inhibitor of both enteropeptidase and trypsin and with a similar activity on glycemic metabolism as metformin [8]. Changes in GI enzyme activity, both *ex-vivo* and *in-vivo,* may result in a reduced absorption of a number of proteins including transcobalamin [7]. A relatively impaired protein digestion may lead to the GI side effects, but also possibly to additional therapeutic outcomes such as weight loss [9].

Metformin undergoes a complex disposition, recently rated as a "40-year-old mystery" [10]. Being a highly polar cationic compound (logP −1.4 and pKa 12.4), it is unlikely to go through membranes by passive diffusion and thus requires transporters. The early kinetic studies by our group [11], indicated relatively rapid half-lives after i.v. injection, i.e., 1.5–4.5 h, however with a prolonged half-life (9–19 h) when calculated by urinary excretion rate, concomitant with some erythrocyte accumulation [12]. An incomplete urinary recovery is possibly indicative of a modest drug metabolism [13].

Metformin disposition is tightly regulated by renal function, the drug undergoing renal tubular secretion. Steady state levels are however dependent upon the individual genetics of drug transporters, in particular organic cation transporter 1 (OCT1) [14]. Variants associated with nonfunctional OCT1 are linked to elevations of plasma metformin and possibly to a raised hypoglycemic response [14]. Inhibition of OCT1, e.g., by lansoprazole, similarly lead will to raised metformin concentrations and prolonged half-life, although with non-significant glycemic changes [15]. More recently, other genetically regulated drug transporters such as the multidrug and toxin extrusion protein (MATE) have been found to potentially contribute to the wide variation in metformin kinetics [16]. Identification of these genetic variants in treated individuals requires complex and lengthy procedures, probably not justified. The Metformin Genetics Consortium examining polymorphisms in five candidate transporter genes, failed to detect a significant contribution of this variability to the glycemic responses [17]. A commentary by the International Transporter Consortium also concluded for the need of improved evaluation of kinetic changes in the presence of genetic variants or potential interactions [18].

2.1. Metformin and gut microbiota

Metformin, being concentrated at the distal GI levels, has attracted much attention as a possible modulator of gut microbiota thus leading to an early conclusion from pre-clinical studies of a possible benefit for metabolic and immune health [19]. An associated conclusion was that of an improvement of the dysbiosis linked to T2DM [20]. These conclusions were, and still are, weakened by high variability and different ethnicities of microbiota in T2DM patients [21]. A general conclusion from the clinical studies with metformin has been an enrichment of taxonomic units from Bacteroides and reduced taxonomic units from *Faecalibacterium* [22] with, however, conflicting data [23]. In both normoglycemic people and T2DM patients raised *Escherichia spp* and reduced *Intestinebacter spp* have been reported [24].

Most data on the microbiota changes after metformin may relate to the large intestine, but since metformin can reach high concentrations also in the small intestine [25], alterations of microbiota at this site may lead to modification of genes affecting glucose and fatty acid uptake, contributing to the metabolic effects of metformin [26]. Interestingly, metformin-treated microbiota transplants to the upper small intestine were followed by a restoration of glucose sensing mechanisms [27].

A major mechanism of the protective activity of metformin on the intestinal mucosal barrier was the detection of an increase relative abundance of *Akkermansia muciniphila*, of high interest today because of

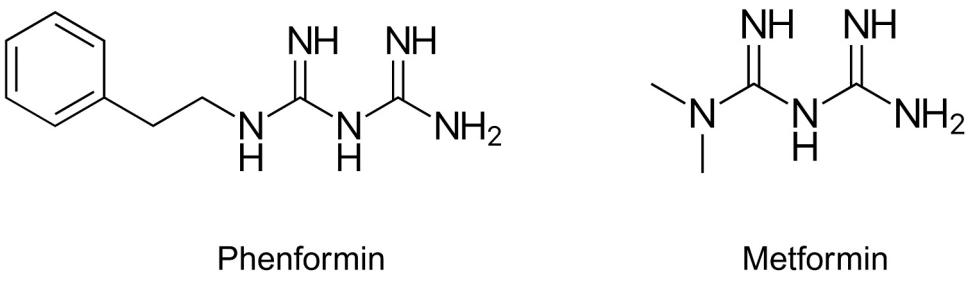

Fig. 1. Chemical structure of the biguanides: phenformin and metformin.

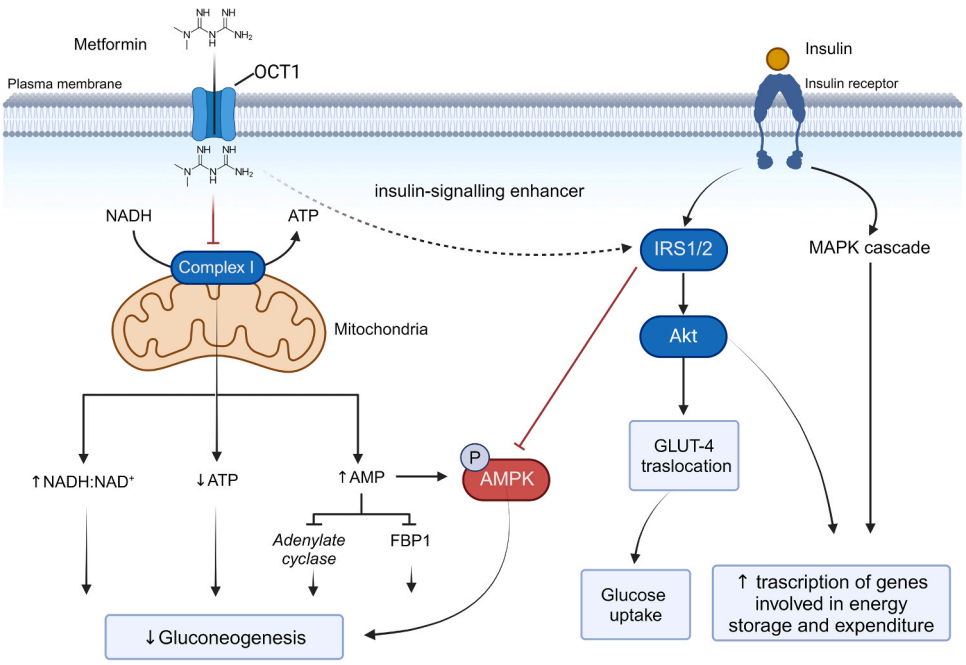

Fig. 2. Mechanism of action of metformin. In the liver, small intestine and skeletal muscle. Metformin inhibits mitochondrial respiratory chain (MRC) Complex I, leading to a decrease in ATP synthesis and concomitant increase in AMP. Raised AMP levels lead to the inhibition of AMP-regulated enzymes involved in gluconeogenesis, such as adenylate cyclase and fructose-1–6-bisphosphatase (FBP1), which contribute to reduced gluconeogenesis. The increased AMP/ATP ratio also activates AMP-activated protein kinase (AMPK). The inhibition of mitochondrial Complex I by metformin is also accompanied by an increase in cellular redox potential (NADH:NAD^+). The glucose-lowering efficacy of the drug requires the presence of insulin but involves both insulin-dependent and insulin-independent effects on cellular nutrient and energy metabolism. Metformin acts by reducing hepatic glucose output and improving insulin-mediated peripheral glucose utilization.

its potential aiding role in weight reduction [28]. Rise of *Akkermansia* and of Goblet cells leads to protective thickening of the mucosal layer [29].

The crosstalk between metformin and gut microbiota metabolism has major consequences, from changes in methionine production (mainly in model worms) to a shift to short chain fatty acid (SCFA) producing bacteria, with beneficial effects on glucose metabolism via multiple pathways involving glucagon-like peptide-1 (GLP1) and peptide YY [30]. Finally, alterations in gut microbiota may contribute to the antitumoral effects, as shown by the reduction in tumor growth after fecal transplants of microbiota from metformin treated donor mice, associated with increased abundance of SCFA producing bacteria [31]. Data on experimental inflammatory chronic disease also indicate benefit by way of activity on intestinal barrier integrity and on gut microbiota [32].

These findings, although not shared by all Authors and limited by microbiome diversities [33], by ethnicities and potentially by concomitant diet and drug treatments, point out, however, to the value of this approach in the still challenging understanding of the complex mode of action of metformin.

3. Metformin and the concept of "insulin resistance"

The hypoglycemic activity of metformin has been extensively evaluated from a practical and from a basic point of view. Unquestionably, the metabolic control in diabetics is well supported by the observation that metformin discontinuation will lead to a rapid rise of glycemia and glycosuria [34]. Use in clinical practice has also led to a reduction of postprandial insulin requirements in type 1 diabetics [35]. Comparative evaluation of metformin versus sulphonylureas has generally resulted in more favorable additional activities, among others, elevation of HDL-cholesterol as well as evidence of rare hypoglycemia [36]. Metformin typically lowers both glucose and insulin levels, thereby antagonizing the so-called "insulin resistance". It was therefore an exciting very early observation that treatment with metformin resulted in reduced insulin levels in hypertriglyceridemic patients [37].

"Insulin resistance" means an impaired biological response to insulin. This condition underlies deterioration of glucose homeostasis that leads to the typical forms of type 2 diabetes mellitus (T2DM) and to obesity [38]: insulin resistance initially meets with a compensatory increase in insulin secretion and hyperinsulinemia appears to contribute to disorders such as dyslipidemia, hypertension, atherosclerosis, hyperuricemia and procoagulant state [39].

Evolution of the concept of insulin resistance has led to the definition of clinical syndromes (syndrome X or Reaven Syndrome) [40], most frequently diagnosed as "metabolic syndrome". Metabolic syndrome (MS), the association of at least three of five elements, is characteristically linked to insulin resistance (hypertriglyceridemia, hypertension, low HDL-cholesterol, enlarged abdominal circumference and moderate hyperglycemia) [41]. A typical temporal trend of insulin resistance is the observation of declining insulin mediated glucose uptake, regulated by a "triumvirate" of muscle, pancreatic β-cell and liver, at the same time associated with raised plasma insulin during oral glucose tolerance test (OGTT), but with a progressive rise of glucose [42].

The mechanism of the intracellular signalling of insulin has been the object of extensive investigation and is of crucial value in the understanding of metformin activity. Insulin binds

to the α-subunit of the insulin receptor (IR) and this causes a conformational change leading to dimerization and activation of the kinase activity of the β-subunits, leading to their autophosphorylation [43]. Activated IR acts on adaptor proteins of the insulin receptor substrates-1 and 2 (IRS-1 and IRS-2); these last, through the phosphatidylinositol 3-kinase B/Akt (PI3KB/Akt) pathway, are responsible for the translocation of the glucose transporter protein type-4 (GLUT-4) into the plasma membrane and consequent stimulated glucose influx [44, 45]. The activity of metformin on the system (Figs. 2, 3), i.e., by raising ISR1 mRNA and protein expression, is clearly shown by investigating protein expression in human granulosa cell cultures [46]. Participation in glycogenesis and protein synthesis is by way of additional activation of Akt in the presence of insulin [47].

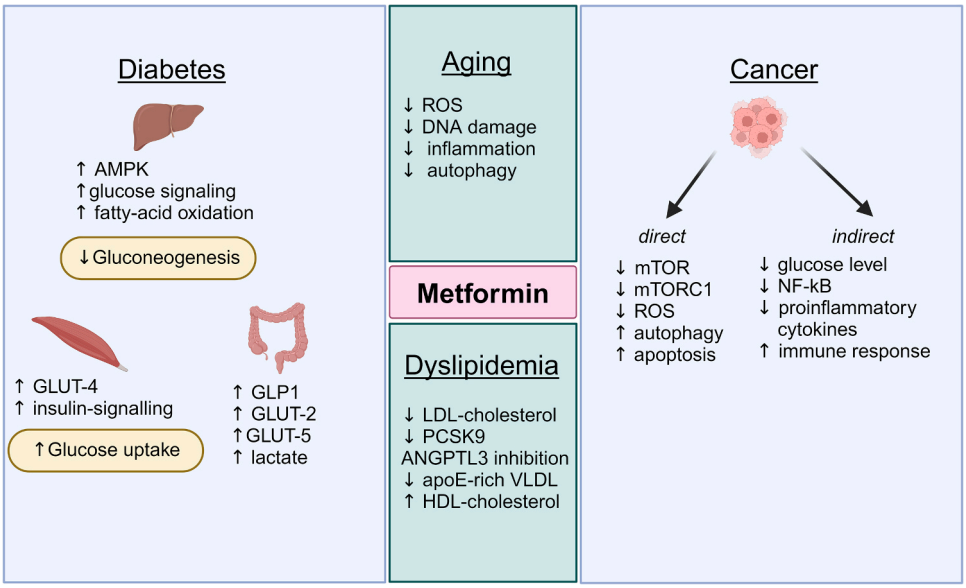

Fig. 3. Summary of the underlying major mechanisms of metformin in different diseases.

Improved knowledge of the mechanisms of insulin resistance has led to the identification of anti-diabetic agents also acting on insulin resistance. Glitazones, although associated with some weight gain, activate the PPARγ system [48] and may improve the response to metformin in the diabetic condition, also when given in fixed-dose combinations [49].

An exciting opening into the improvement of insulin sensitivity has been offered by the GLP-1 agonists, with a growing use mainly in overweight [50]. Metformin can by itself raise GLP-1 levels [51] and by this mechanism may increase pre-digestive satiation and consequent weight loss [52]. Combined use with GLP-1 agonists [53] shows efficacy both on body weight and on endothelial dysfunction. Insulin resistance may also occur following liver disease, being corrected by powerful anti-oxidants such as idebenone [54].

The intracellular mechanisms of glucose/insulin reduction by metformin definitely occur in the major site of action of metformin, i.e., the small intestine, where drug concentrations are higher than in blood [55]. Further, the hypoglycemic activity is less evident after intravenous versus oral administration [56]. In the small intestine, raised gene expression of hexose transporters, GLUT-2 and GLUT-5, has been reported [57], as well as intestinal glucose uptake and lactate production, by way of raised GLP-1 concentrations [4]. While a modulation of gut microbiota does not appear to play a role in the anti-hyperglycemic effect, very recently a direct activity on the basolateral glucose uptake was reported with a consequent reduced hepatic glucose production [58]. The gut-liver crosstalk, mediated by raised intestinal GLUT-1 and GLUT-2, lactate and bicarbonate, modulates liver pyruvate carboxylase, mitochondrial pyruvate carrier (MPC) 1/2 and fructose 1–6 bisphosphatase.

A more recent mechanism of improved insulin resistance has been proposed to be by way of an activated presenilin enhancer 2 (PEN-2, a subunit of the β-secretase complex) [59]: metformin-bound PEN2 activates AMPK at the lysosomal surface without altering cellular AMP levels. For this mechanism, however, critically low metformin concentrations are needed and it is thus unclear how this may occur at the GI level, exposed to concentrations well above 50 mM [60]. The link between improved glucose transport, reduced glycemia and improved insulin resistance thus appears to be quite a complex major target of metformin activity, still a constant object of extensive investigation.

3.1. *Weight loss*

Obesity is often a comorbidity of T2DM [61]. For this reason, weight loss is crucial in managing the disease, as T2DM patients who lose weight are more likely to avoid the complications associated with obesity and diabetes, thereby reducing morbidity and mortality [62]. However, achieving and maintaining weight loss is challenging; indeed, meta-analyses of clinical trials on non-pharmacological weight loss strategies have shown reductions of 1–6 Kg [63], which are difficult to sustain over time. Therefore, the use of antidiabetic drugs that not only help regulate blood glucose levels but also have significant effects on body weight is desirable. A recent review highlighted how antidiabetic drugs can contribute to significant weight reduction in diabetic patients, thereby improving glycemic control and reducing the risk of related complications [64]. Several studies demonstrated that metformin has a positive impact on body weight according to the specific mechanism of action of this drug. While studies on its impact on weight are mixed, many have found that metformin can lead to modest weight loss [65, 66], or prevent weight gain associated with insulin treatment [66]. Mechanisms of weight loss involve, in addition to the moderate GI intolerance, reduced appetite [67] apparently by a liver directed activity and, as above indicated, by raised GLP-1 levels, leading to elevated pre-ingestive satiation [52]. More recently, an increase of the appetite-suppressant Lac-Phe metabolite in humans has been reported [68]. In mouse hepatocytes, a stimulated secretion of the growth differentiating factor 15 (GDF-15) after metformin has been also reported [69]. The appetite suppressing effect is lost in GDF-15 null mice. Conversely, GDF-15 serum levels are raised, with associated weight loss, in metformin-treated T2DM [70].

Additionally, metformin use has been shown to possibly results in improved body composition, by reducing visceral and abdominal fat in patients with T2DM. In routine use, patients may lose weight early in treatment, because of the associated GI side effects.

4. Metformin and lipid-lowering effects

A *cholesterol lowering activity,* specifically on the atherogenic low-density lipoprotein (LDL) fraction, has been reported for metformin from the early clinical studies, indicating a (-12 %) LDL-cholesterol reduction versus no change with the sulphonylurea glibenclamide [71]; the effect was well maintained over time [72]. Following a number of confirmatory studies [73], recently, Hu *et al.*, [74] reported in a mouse model treated with metformin, an association between total/LDL-cholesterol reduction and lower circulating proprotein convertase subtilisin/kexin type 9 (PCSK9) levels. Reduced transcription of

this well-known enzyme, responsible for LDL-receptor degradation. is apparently driven by the glucose sensor carbohydrate responsive element-binding protein (ChREBP), whose expression is inhibited by metformin [75]. Interestingly, metformin, by activating AMPK, increases expression of the sterol transporters ABCG5 and ABCG8 in mice, responsible of the hepato-biliary cholesterol transport [76]. This action, that apparently involves the enzyme ATP citrate lyase (ACLY), may provide a further mechanistic step in the anti-atherosclerotic activity of the agent. An alternative suggested mechanism is that of the inhibition of angiopoietin like 3 (ANGPTL3), a liver-derived endocrine protein antagonizing lipoprotein lipase (LPL) [77]. Inhibited expression of ANGPTL3 in the liver may suggest a potential indication for metformin in severe hyperlipidemias [78].

As early as the initial clinical investigations drug, an anti-atherosclerotic, lipoprotein-related activity of metformin was described and characterized by reduced cholesterol deposition in New Zealand rabbit aortic walls [79]. Our group revealed that, by a still unclear mechanism, metformin could directly reduce synthesis of apo-E rich VLDL, a characteristic lipoprotein abnormality in this animal model [80]. Studies on the mitochondrial function have significantly contributed to the understanding of this complex phenomenon. In particular, the major activity of metformin, i.e., protecting mitochondrial respiratory activity by activation of AMPK [81] appears to occur at the intestinal level, a major site of activity of metformin and can be reproduced in intestinal organoids, treated with TNF-α to induce intestinal injury [82]. Mitochondrial dysfunction in enterocytes may lead to inhibited chylomicron production and transport of dietary lipids to the peripheral organs, thus contributing to the understanding of lipid associated disorders, in this particular case, higher levels of apo-E rich VLDL [83]. A summary of the actions of metformin in dyslipidemia is reported in Fig. 3.

5. Mitochondrial activity of metformin

The major target of the pleiotropic effects of metformin and other biguanides are mitochondria. The early reports already indicated that the drug reduces cellular respiration by a specific inhibition of the mitochondrial respiratory chain (MRC) Complex I (NADH:ubiquinone oxidoreductase) without effects on other steps of the mitochondrial machinery [84]. As a consequence of this inhibition, the increase of both ADP/ATP and AMP/ATP ratios at the cellular levels, leads to phosphorylation of the AMPKα at Thr-172 and raised AMPK activity [85]. Activation of AMPK, a critical energy sensor of homeostasis, is the apparently major cellular mode of action of metformin [86] **(**Fig. 2**)**.

By targeting MRC Complex I, the drug may, e.g., reduce thrombosis induced by air pollution [87]. This action is mediated by the prevention of reactive oxygen species (ROS)-led IL-6 release and also by the inhibition of mitochondrial reactive oxygen species (ROS) to prevent calcium release activated channels (CRAC) and other mechanisms related the control of calcium influx [88], all potentially influencing the cardiovascular toxicity of <25 mM PM pollution [89]. These newer visions on the mitochondrial activity have mainly brought to light the inhibitory action on mitochondrial-3-glycerol-phosphate dehydrogenase (mGPDH) activity as well as on MRC Complex IV. The in vivo consequences of mGPDH inhibition have not led to major conclusions, since inhibition of the glycerol-phosphate shuttle may be insufficient to reduce gluconeogenesis [90] although low doses of metformin can raise the NADH to NAD^+ ratio [91,92]. The activity on MRC Complex IV instead may block the electron transport chain resulting, in addition, in mGPDH inhibition, that may raise redox potential-dependent ATP synthesis, leading to reduced cellular ATP levels [93]. At the end criticisms have been raised on the postulated activity on MRC Complex I, because the suppression of gluconeogenesis by metformin may occur at very low concentrations, without changes in the AMP to ATP ratio. Further, recent reports indicate that reduced gluconeogenesis may occur independent of detectable changes in the AMP to ATP ratio [94].

Mitochondrial activity has however become a crucial target also in the understanding of the anti-hyperglycemic and potentially anti-atherosclerotic effects of metformin. The deterioration of arterial function in diabetes is secondary to the promotion of ROS release [95]. This can damage sensitive components such as DNA, causing cell death and reducing nitric oxide (NO) availability resulting in endothelial dysfunction [96]. ROS generation is dependent upon systems, such as uncoupled endothelial eNO synthase (eNOS) and NADPH oxidases, dependent from mitochondrial dynamics [97]. The role of mitochondrial function in atherosclerosis is well exemplified by the atheroprotective effect observed after overexpression of thioredoxin I in mitochondria [98], and after administration of the mitochondria-targeted antioxidant MitoQ [99]. By a direct activation of AMPK, leading to reduced glucose production and raised fatty acid oxidation in hepatocytes [3] occurring in vitro [100,101], metformin can inhibit hepatic gluconeogenesis, among others through the regulation of the orphan nuclear receptor SHP [102].

Although contrasting data on the AMPK-dependent activity on the metabolic actions of metformin have been provided [103], a direct action on mitochondrial function and dynamics remains unquestionable. The beneficial cardiovascular effect of metformin is dependent on mitochondrial dynamics, by inhibiting mitochondrial fragmentation and preventing endothelial damage by processes such as apoptosis and inflammation [104]. Mitochondrial dynamics is characterized by *fusion and fission* [105]. Mitochondrial *fusion* is a canonical pathway, being followed by exchange of proteins, metabolites and DNA throughout the mitochondrial network. In contrast mitochondrial *fission* leads to fragmented mitochondria with impairment of the electron transport chain and raised ROS [106]. Mitochondrial fission is particularly sensitive to elevated glucose, which regulates dynamin-related protein1 (DRP1) expression [107]. Expression of the fission related DRP1, triggering mitochondrial division by binding to mitochondrial fission1 (FIS1) or mitochondrial fission factor (MFF) appears to be affected by the presence of metformin [105]. The drug reduces translocation of DRP1 in mitochondria thus preventing fragmentation, similarly to the selective mitochondrial division inhibitor 1 (mdivi-1), both reducing inflammation and suppressing atherosclerosis in diabetic mice, by an AMPK-dependent mechanism [107]. A similar mechanism appears to occur in patients, also showing reduced DRP1 and FIS1 after metformin treatment [108]. This last process is controlled by the mitochondrial aspartyl-tRNA synthetase (DARS2) [109]. DARS2-deficient enterocytes accumulate lipids derived mostly from undigested fat, concomitant with progressive alterations of the proximal enterocytes [83]. This potential novel mechanism of mitochondrial ROS production, resulting in increased autophagy in type 2 diabetics, is led by the Ser/Thr kinase PINK1 and the E3 ubiquitin ligase Parkin [108]. Metformin reduces the effects of both PINK1 and Parkin, in parallel to those of the mitochondrial regulatory protein PGC1α [110,111].

A recent advancement in the understanding of the effects of metformin on mitochondria has emerged from the discovery of the plasticity factor zinc finger E-box binding homeobox (ZEB1) in macrophages [112]. ZEB1 expression plays dual roles in macrophages, influencing both inflammatory and immunosuppressive phenotypes. Intriguingly, mice with intact ZEB1 ($ZEB1^{WT}$) and those lacking ZEB1 in macrophages ($ZEB1^{\Delta M}$) exhibit divergent responses to bacterial lipopolysaccharide (LPS)-induced lethal endotoxemia [113]. Furthermore, the anti-inflammatory and ROS-inhibiting effects of metformin are contingent upon ZEB1 expression in macrophages, promoting an immunosuppressive-like state during inflammation [112]. Given the role of ZEB1 in inhibiting mitochondrial translation in immunosuppressed macrophages, metformin assumes a critical function in the mitochondrial transcription system. This pivotal role of the ZEB1 transcription system may also elucidate the anti-tumoral effects of metformin, as ZEB1 transcription has been implicated in maintaining cell plasticity in cancer cells, particularly through epithelial-to-mesenchymal transition (EMT) during cancer progression

[114].

ZEB1, being a promoter of cancer development in the transition from epithelial mesenchymal to malignant cells, may play a potentially detrimental role in metformin's mechanism. However, in this case, the major consequence apparently is a cell plasticity change, and a very recent crucial study [115] evaluated the role of ZEB1 in controlling the activity of metformin on *IL-1β-induced muscle atrophy*. The area of muscular/central nervous system (CNS) degenerative changes is of growing interest for metformin (see below). It appears that the IL-1β-induced atrophy is antagonized by metformin, and the drug, by raising levels of ZEB1, improves the expression of three differentiation proteins thus improving muscular rehabilitation. It thus appears that the raised expression of ZEB1 in this case is most likely a beneficial effect.

5.1. Endothelial dysfunction

Atherosclerotic cardiovascular diseases, in particular peripheral arterial disease (PAD), are the leading causes of morbidity and mortality in individuals with T2DM [116]. Subclinical endothelial dysfunction is one of the earliest significant contributors to atherosclerosis and can be identified at a young age in patients with diabetes. Endothelial dysfunction is generally defined as an altered endothelial dependent vasodilation, indicative of cardiovascular disease development [117]. Endothelial injury may represent the earlier phenomenon and hyperglycemia, together with other inflammatory mediators [118] is probably the main driver of endothelial injury in diabetes [119]. Clear endothelial dysfunction has been observed in diabetic children/adolescents [119] and in adults, it is frequently associated to platelet hyperactivity [120].

Endothelial dysfunction is a well described target of metformin. A number of clinical observations have reported a beneficial activity of metformin. The early results from clinical observations came from a double-blind study on arterial flow carried out in Italy on patients with PAD treated either with metformin or placebo, indicating a clear benefit from the drug [121]. In later years, studies made use of more advanced technologies, in particular forearm strain-gauge plethysmography. Following a 3-month treatment with 500 mg metformin bid versus placebo, an improved vasodilation following intra-arterial administration of acetylcholine [122] was reported, with no effects a non-endothelium-dependent (sodium nitroprusside) and nitrate-independent (verapamil) vascular mediators. Another study confirmed these effects by using flow-mediated dilatation (FMD) [123]. These findings were corroborated by a study in first-degree relatives of type 2 diabetics with the metabolic syndrome [124]. A more recent, larger, randomized, placebo-controlled study in patients with T2D treated for 52 months with metformin, showed a reduction of markers of endothelial dysfunction such as plasminogen activator inhibitor-1 (PAI-1), tissue-type plasminogen activator (t-PA), Von Willebrand factor (vWF) and soluble Vascular Cell Adhesion Molecule-1 (sVCAM-1) [125].

A similar benefit was found in type 1 diabetics who, while on the drug, had improvement in FMD, but not in metabolic parameters, including HbA1c, paradoxically raising prostaglandin F2α, a marker of oxidative stress, generally associated to vasoconstriction [126]. In order to clarify the mechanism of these paradoxical findings, these Authors evaluated the activity of vidagliptin, a newer GLP-1 agonist, noting that vasodilation markers [NO, endothelium-derived hyperpolarizing factor (EDHF)] were raised to a higher extent vs vasoconstriction markers. Interestingly, an improved microvascular insulin resistance of the skeletal muscle in carriers of the metabolic syndrome following metformin was recently described [127]. Finally, an ongoing double-blind study in the US (NCT05132439) will test in 200 surgical patients with PAD the activity of metformin on arterial function (Katherine Moll Reitz, University of Pittsburgh, personal communication).

Endothelial lipid accumulation occurring after high fat consumption also suppresses eNOS activity thus leading to blood pressure (BP) elevation [128]. By antagonizing lipid accumulation, metformin may associate to BP lowering, by AMPKα2 activation, resulting in reduced angiotensin II-induced endoplasmic reticulum (ER) stress [129].

Very recently another protective mechanism against hypertension was described, i.e., the induction of heat shock factor 1 (HSF1), improving mitochondrial morphology and the unfolding protein response [130] in spontaneously hypertensive rats. By this mechanism the drug can reduce cardiac hypertrophy and cardiomyocyte apoptosis. A prior small study in non-diabetic coronary patients had similarly described reduced left ventricular mass, office systolic BP, and thiobarbituric reactive substances (TBARS)-mediated oxidative stress after metformin [131].

6. Metformin and cancer

An unexpected anti-cancer activity of metformin was the result of an early epidemiological study [132], essentially indicating that metformin-treated diabetics had a significantly lower cancer burden versus diabetics treated with other agents. This observation was confirmed by several other reports [133,134]. This potential benefit of metformin is in contrast with the apparently raised cancer risk following insulin-based therapies [135,136]. Although non confirmatory data have been reported [137], two independent meta-analyses comparing metformin to other treatments reported 30–40 % reductions in cancer incidence in metformin-treated T2D [138,139].

Diabetes has a clear association with an increased cancer risk, particularly in insulin-treated individuals [140]. Metformin use has been shown to reduce the frequency of specific cancers, in particular breast cancer [141] and to be an effective radiosensitizer in the treatment of this most frequent tumor [142]. Metformin appears to provide additional benefit for the treatment of e.g., cisplatin-treated cancers [143] and the use of metformin has been associated with a clear reduction of cancer risk versus other antidiabetics [144].

The special sensitivity of breast cancer to metformin has been well exemplified in the HER2/Neu transgenic mouse cancer model, where treatment with the drug resulted in a reduced tumor burden and increased lifespan [145]. The mechanism of the anti-cancer activity of metformin is still generally attributed to the activation of the AMPK system, frequently associated to changes in transcription, phosphorylation or ubiquination [101]. AMPK-associated mechanisms may include among others improved response to the programmed cell death ligand (PD-1/PD-L1) checkpoint inhibitor therapy [146,147] for hepatocellular carcinoma. Metformin may raise immune destruction of these tumors by increasing motility of tumor infiltrating $CD8^+$ T cells, thus possibly resetting the polarization state of macrophages to an antitumoral M1-like phenotype [148]. Understanding the potential immune mechanisms of metformin resulting in anti-cancer activity has stimulated interest in additional pathways. These may thus provide an explanation for the still unclear mechanism of metformin, particularly in raising number and activity of $CD8^+$ tumor-infiltrating lymphocytes, protecting them from apoptosis and immune exhaustion, both counteracted by metformin [149]. This activity is possibly mediated by the expression of the plasticity factor ZEB1 (see above), a long noncoding RNA (lncRNA) in macrophages, essentially resolving inflammation by leading macrophages to an immunosuppressed state [150]. Metformin appears indeed to mimic the metabolic reprogramming of myeloid cells induced by ZEB1, possibly inducing massive cell death in preneoplastic cells treated with the promoter phorbol ester [151], also involving autophagy-related cytotoxin activity under stress conditions [152]. These and possibly other mechanisms underlying the still debated activity of metformin, not as yet fully supported by clinical findings, may be possibly linked to the apparent anti-aging activity of the drug. Indeed, the basic activity on the MRC Complex I appears to regulate tumor sensitivity. Tumors characterized by defective upregulation of oxidative phosphorylation (OXPHOS), possibly resulting from mutations in MRC1, appear to be most sensitive to biguanides [153].

The anti-cancer mechanisms of metformin appear also to be related

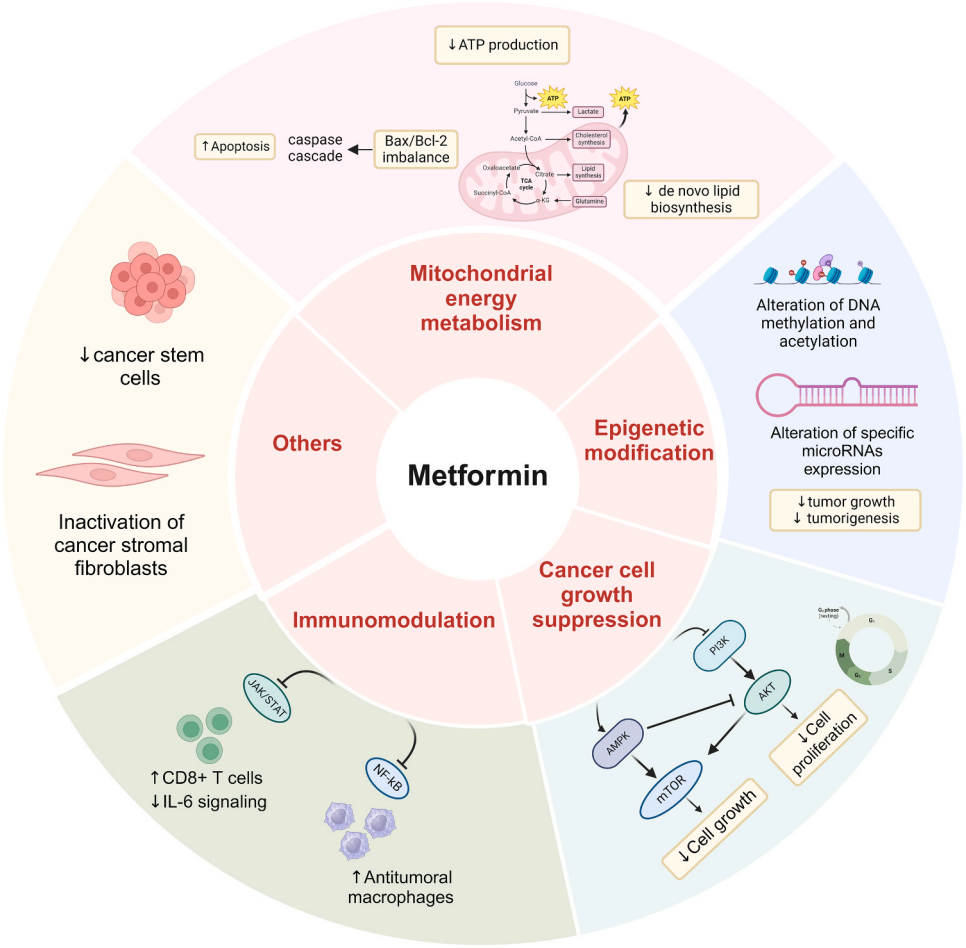

Fig. 4. Summary of the potential anticancer mechanisms of metformin.

to the peculiar pattern of drug absorption and elimination regulated by multidrug and toxin extrusion protein 1 (MATE-1) [154]. Colorectal cancers show apparently controversial responses to metformin [155]. However, they are metformin responsive when occurring in diabetic patients with *KRAS* mutations, in whom survival times are 37.8-month longer compared to those treated with other glucose lowering agents [156]. Mutated *KRAS* oncoprotein appears to hypermethylated MATE-1 by suppressing DNA methyl transferase 1 (DNMT1), thus antagonizing metformin extrusion from cells and leading to improved tumor regression.

Metabolic conditions associated with metformin use and apparently related to cellular glucose deprivation have been detected in a cell model of the triple negative breast cancer subtype (TNBC), i.e., with reduced expression of HER2 receptor and of both estrogen and progesterone receptors [157]. Metformin combined with glucose deprivation appears to suppress TNBC cells by reducing proliferation and increasing apoptosis and by stimulating the unfolded protein responses of the ER [158]. At present more than 400 ongoing clinical studies on the anti-cancer activity of metformin are listed in the *ClinicalTrials.Gov*. An excellent very recent review on the preventive activity of metformin on cancer is offered by O'Connor *et al.*, [159].

A possibly more exciting implementation of the metabolic properties of metformin is in the treatment of diffuse intrinsic pontine glioma (DIPC), a devastating disease of children, with essentially no effective treatment. By targeting the PI3K/mTOR pathway, the drug paxalisib achieves stable disease in some patients but, among others, with significant hyperinsulinemia and hypoglycemia [160]. Addition of metformin to the regimen results in reduced phosphorylation of the insulin receptor, the mechanism of PI3K-resistance, and a general improvement in anticancer response [161,162] The potential antitumor mechanisms of metformin are illustrated in Fig. 4.

7. Metformin and aging

The progressive loss of physiological functions, including chronic morbidities, metabolic, cardiovascular and neurodegenerative conditions, associated to frailty and reduced mobility lead to aging. Metformin may interfere with this sequence of events and this has led to a large number of trials in human aging [163], starting with the TAME study (Target Aging with Metformin Study) [164].

The hallmarks of aging were earlier provided by Lopez-Oti *et al.*, [165] and recently updated [166]. Nine major hallmarks were listed: from genomic instability, epigenetic alterations, loss of proteostasis, telomere attrition, deregulated nutrient-sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion and altered intercellular communication, to which they added disabled macroautophagy, chronic inflammation and age-associated dysbiosis.

The role of metformin in targeting biological aging is mainly consequent to its activity as an inhibitor of MRC 1, leading to multiple downstream effects on metabolic (e.g., a change in the AMP/ATP ratio with AMPK activation) and non-metabolic pathways in the aging process [167], including lysosomal pathways [168]. The activation of AMPK is most likely contributory to the direct activation of SIRT1 and other nutrient-sensing pathways, inhibition of m-TORC1, suppression of adipogenesis through the p70S6K pathway, activation of DNA-damage-like response via the ATM-Chk2 pathway and activation of nuclear factor erythroid 2 related factor (Nrf2) resulting in down regulation of inflammatory responses [164]. Further, a role of metformin on gut

microbiota, although not affecting branched chain aminoacids [169], could be crucial in intracellular signalling and attenuating inflammation [23]. This or a similar mechanism may help to explain the regulation of growth differentiation factor 15 (GDF-15), mediating body weight and energy production [170].

Identification of the hallmarks of aging allowed to identify those most sensitive to metformin. Among these, the activation of AMPK and SIRT1 and down-regulation of the insulin-IGF1 signaling and m-TORC1 are involved in the beneficial effects of metformin on energy metabolism [171]. Activation of AMPK via the liver kinase B1 (LKB1) mediates the prolonged lifespan in mice and *C. elegans* [172].

The inflammatory process is a major target of metformin. Suppression of the proinflammatory cytokines of the NF-kB pathway is associated to a reduced mortality in older diabetics treated with metformin [173]. The activity of metformin on dysfunctional mitochondria with aging is well predictable from the drug's mechanism on oxidative stress [174], possibly delaying mitochondrial biogenesis and senescence by AMPK mediated H3K79 methylation acting through the SIRT1-DOT1L axis [175].

Considerable progress has been made in the understanding of the drug's regulatory effect on transcriptional activity, a defensive mechanism against aging that has received important recent contributions [176]. Transcriptional activity regulation occurs via histone modifications, DNA methylation and miRNAs. Histone modifications may occur through phosphorylation of histone acetyltransferase, inhibition of histone acetylases and activation of SIRT1 or SIRT6 [177]. Histone demethylases modulate lifespan by key longevity routes such as the insulin-like signalling (IIS) pathway, a major target of metformin activity [176].

Dietary restriction and lowered IIS reverse most of age associated transcriptional changes. In particular, transcription speed of RNA polymerase II increases with age in all species with changes in splicing, including reduction of unspliced transcripts and formation of more circular RNAs. Reduction through dietary restriction and lowered IIS, acting as a mediating factor in intercellular communication, altered during aging, will lead to prolonged lifespan [178]. This protective effect also leads to reduced overexpression of protein inflammatory proteins, secondary to epigenetic dysregulation, deficient proteostasis [179] or disabled autophagy. In particular, a favorably reduced genomic instability in the context of aging or various types of cancers leads to genome protection [180] by raised endogenous ROS levels and associated DNA damage, and potentially mediated by reduced activation of ATM, a serine/proline kinase, marker of DNA double-stranded breaks and genomic instability [181].

A possibly most exciting activity of metformin is the *antagonism to stem cell exhaustion*, typically associated to reduced tissue renewal at steady state together with impaired tissue repair upon injury. Although there is as yet no consensus on the association between changes in stem cell number in different tissues and aging, and the exact mechanism for reduced stem/progenitor cell capacity for repair is not known in detail, cumulative epigenetic changes may be in play [182]. Reprogramming processes of de-differentiation, acquisition of progenitor features and re-differentiation may lead to tissue rejuvenation [183]. Metformin, by activating the endoplasmic reticulum glutathione peroxidase 7 (Gpx7) and accumulating the nuclear factor Nrf2, binding the anti-oxidant response element in the Gpx7 gene promoter, can delay cellular attrition and prevent premature aging, thus increasing lifespan of mesenchymal stem cell [184].

While earlier studies indicated an activity of metformin in delaying cell aging in Drosophila intestinal stem cells [185], more recent data support an activity at the neural stem cell pool promoting neurogenesis and cognitive recovery with improved capacity to rejuvenate and differentiate oligodendrocyte progenitor cells [186].

Direct activities of metformin on DNA, leading to healthy aging and increased lifespan, have led to the identification of downstream targets, leading to increased *telomere length.* Telomere transcription may in fact occur by activation of AMPK by the NRF1 and PGC1α [187] as regulators of human telomere transcription. This is confirmed by studies in diabetics, where metformin monotherapy, in addition to improved glucose tolerance and insulin sensitivity led to prevention of leukocyte telomere shortening [188].

Associated mechanisms leading to improved longevity and more so in a reduced development of non-proliferative diseases, including kidney disease, liver steatosis type I and II and Parkinson's disease are grouped in the *senescence-associated secretory phenotype (SASP).* This is a heterogenous phenomenon characterized by cells with transcriptional retrotransposable elements (L1 or LINE-1), driving expression of interferon-1 (IF-1) and age-associated inflammation (*inflammaging*) [189]. While increased SASP may be linked to tissue repair processes in which senescent cells promote localized fibrosis and recruitment of immune cells, the killing of senescent cells (*senolysis*) has potentially beneficial outcomes and has led to the development of senolytics as a novel drug class [190]. SASP cells grow rapidly in normal mice and administration of the tyrosine kinase inhibitor, dasatinib, associated to quercetin, can significantly improve physical dysfunction, while raising post-treatment survival by 36 % and reducing mortality hazard by to 65 % [191]. A number of drugs, some of which approved for human use, have been tested in SASP conditions, e.g., rapamycin, targeting mTOR and extending lifespan in several species [192,193]. Chronic metformin at low doses may delay cell senescence and suppress SASP via upregulation of GPx7 through Nrf2 [184]. Metformin may also act in a DICER1-dependent mechanism, lowering p16 and p21 proteins and the RNA levels of SASP-associated IL-6 and IL-8 in human fibroblasts [194]. By down regulating SASP, the senescent cell burden will be lowered by metformin, exerting a protective effect against oxidative stress and leading to autophagy in conditions of chronic inflammation [195].

In the case of cancer and senescence, raised *autophagy* can potentially reduce proliferation of progenitor cells through the secretion of growth factors and alterations in the autophagy-lysosomal system [196] with detection of senescence-associated beta-galactosidase. The process of autophagy may have divergent outcomes in cancer, as well as in senescence, depending upon the individual conditions, a good case being the length of fasting in the intermittent fasting weight losing strategy [197]. For metformin, the anti-SASP and pro-autophagic activities may occur in essentially all conditions and provide an effective tool to suppress cellular senescence in age associated dysfunction (Fig. 3).

8. Metformin: other targets?

The wide use of metformin in diabetics allowed to indicate some potential areas of therapeutic interest. Among these, Alzheimer's disease, vascular dementia and Parkinson's disease have attracted the most attention. Metformin crosses the blood-brain barrier efficiently and is thus suited for these treatments, although cerebrospinal fluid levels are about 1/100 those in plasma [198]. Therapeutic reports on the effectiveness in Parkinson's disease have been inconclusive [199]. However, novel findings on the central nervous system activity of metformin have been met with enthusiasm. An inhibited translation of Repeated Associated non-ATG (RAN) occurs in the presence of expansion of multiple repeats near the cognate classical ATG translation [200]. RAN translation has been discovered in a number of CNS disorders [201]. Most recently, C9orf72 frontotemporal dementia and/or amyotrophic lateral sclerosis (C9orf72-FTD/ALS), were found to be associated to a number of mutant repeat expansion RNAs (e.g., CAG, CUG, CGG, G_4C_2 and G_2C_4) forming RANs that, in fact, activate the double-stranded RNA dependent protein kinase (PKR) [202]. Metformin reduces RAN protein levels, apparently improving ALS or FTD in this model, providing a potentially easy and low cost approach to these lethal diseases [203] and potentially opening the way to new approaches to the regulation of non-ATG RAN translation [204].

Finally, a classical endocrine disease characterized by insulin

Table 1
Most recent Phase III clinical trials using metformin registered on clinicaltrials.gov.

NCT Number	Study Title	Conditions	Interventions
Aging			
NCT04264897	Antecedent Metabolic Health and Metformin Aging Study	Aging	Metformin Placebo
NCT04221750	Diet and Exercise Plus Metformin to Treat Frailty in Obese Seniors	Frailty Sarcopenic Obesity Aging	Metformin Hydrochloride Placebo
COVID−19			
NCT04885530	ACTIV−6: COVID−19 Study of Repurposed Medications	Covid19	Metformin Ivermectin Fluvoxamine Fluticasone Placebo Montelukast
NCT06128967	A Multicenter, Adaptive, Randomized, doublE-blinded, Placebo-controlled Study in Participants With Long COVID−19: The REVIVE Trial	Long COVID−19 Syndrome	Metformin ER Fluvoxamine Maleate Placebo
NCT06042855	ACTIV−6: COVID−19 Study of Repurposed Medications - Arm G (Metformin)	Covid19	Metformin Placebo
NCT04510194	COVID-OUT: Early Outpatient Treatment for SARS-CoV−2 Infection (COVID−19)	Covid19|SARS-CoV Infection	Metformin Placebo Fluvoxamine Ivermectin
Cardiovascular disease			
NCT03507413	Metformin Therapy in Non-diabetic AAA Patients	Abdominal Aortic Aneurysm	Metformin 500 mg Placebo
NCT05305898	"MetCool ACS"- Metformin "Cooling" Effect on Metformin-naive Patients Treated With PCI Because of Acute Coronary Syndrome	Acute Coronary Syndrome	Metformin
NCT05708053	Cardio-protective Effect of Metformin in Patients Undergoing PCI	Elective Percutaneous Coronary Intervention	Metformin
NCT05182970	Metformin and Prevention of Cardiovascular Events in Patients With Acute Myocardial Infarction and Prediabetes (MIMET)	Acute Myocardial Infarction	Metformin
NCT05132439	MetfOrmin BenefIts Lower Extremities With Intermittent Claudication	Intermittent Claudication	Metformin ER Placebo
NCT03054519	Improve PAD PERformance With METformin	Peripheral Artery Disease	Metformin Placebo
Cancer			
NCT01905046	Metformin Hydrochloride in Preventing Breast Cancer in Patients With Atypical Hyperplasia or In Situ Breast Cancer	Breast Cancer	Metformin Hydrochloride Placebo
NCT01101438	A Phase III Randomized Trial of Metformin vs Placebo in Early Stage Breast Cancer	Breast Cancer	Metformin Hydrochloride Placebo
NCT01864096	The Metformin Active Surveillance Trial (MAST) Study	Prostate Cancer	Metformin Placebo
NCT05921942	The Impact of Metformin Administration on the Clinical Outcome of Stage IV Colon Cancer	Colorectal Cancer	Metformin
NCT05445791	Metformin Plus Tyrosine Kinase Inhibitors for Treatment of Patients With Non-small Cell Lung Cancer With EGFR Mutations	Non-Small Cell Lung Cancer	Metformin Hydrochloride Placebo
NCT01697566	An Endometrial Cancer Chemoprevention Study of Metformin	Endometrial Cancer	Metformin Placebo
NCT04792749	Clinical Effects of Metformin on Fertility-sparing Treatment for Early Endometrial Cancer	Endometrial Cancer	Metformin
NCT05292573	Longitudinal Follow-up in Women With Endometrial Hyperplasia Without Atypia	Endometrial Hyperplasia	Metformin
NCT05230758	Effect of Metformin on Behaviour and the Brain in Children Treated for a Brain Tumour	Medulloblastoma Childhood Cognitive Impairment	Metformin hydrochloride Placebo
Neurological disorders			
NCT04098666	Metformin in Alzheimer's Dementia Prevention	Mild Cognitive Impairment	Metformin ER Placebo
NCT02980276	A Randomized Placebo Controlled Trial of the Effectiveness of Metformin in Addition to Usual Care in the Reduction of Gestational Diabetes Mellitus Effects	Gestational Diabetes	Metformin Hydrochloride Placebo
NCT04826692	TEsting METformin Against Cognitive Decline in HD	Huntington Disease	Metformin Placebo
NCT05722951	Metformin Efficacy and Safety in Epileptic Patients	Epilepsy	Levetiracetam Metformin
NCT05838573	Metformin Treatment on Cognitive Impairment of Schizophrenia	Schizophrenia	Metformin Placebo
NCT03479476	A Trial of Metformin in Individuals With Fragile X Syndrome	Fragile X Syndrome	Metformin Placebo
Polycystic Ovary Syndrome			
NCT06142656	Efficacy of Vildagliptin Versus Metformin in Poly Cystic Ovary Syndrome	Polycystic Ovary Syndrome	Metformin Hydrochloride Vildagliptin 50 MG

(*continued on next page*)

Table 1 (*continued*)

NCT Number	Study Title	Conditions	Interventions
NCT03229057	Comparing the Effects of Oral Contraceptive Pills Versus Metformin	Polycystic Ovary Syndrome	OCP + Metformin OCP + Placebo Metformin + Placebo
NCT06140108	Empagliflozin vs Metformin on Metabolic Dysfunction in Polycystic Ovary Syndrome With or Without Comorbidity or Multimorbidity	Polycystic Ovary Syndrome	Metformin Empagliflozin Control
Chronic Kidney Disease			
NCT05373680	Efficacy and Safety of Metformin Versus Empagliflozin on Chronic Kidney Disease Progression	Renal Insufficiency, Chronic	Metformin Empagliflozin Control
NCT03831464	Metformin as RenoProtector of Progressive Kidney Disease	Chronic Kidney Diseases	Metformin Hydrochloride Placebo Oral Tablet
NCT04511416	Preventing Cognitive Decline With Metformin	Cognitive Decline	Metformin ER, 500–2000 mg
Diabetes, Obesity and Metabolic Syndrome			
NCT05280496	A Pragmatic Approach to Lower Diabetes Risk After Gestational Diabetes	Gestational Diabetes	Metformin
NCT02947503	Pregnancy Outcomes: Effects of Metformin Study (POEM Study)	Gestational Diabetes	Metformin 850 mg
NCT05098470	Effects of Modulators of Gluconeogenesis, Glycogenolysis and Glucokinase Activity	Type 2 Diabetes	Metformin Insulin Glargine Dorzagliatin
NCT03355469	Exercise Dose and Metformin for Vascular Health in Adults With Metabolic Syndrome	Metabolic Syndrome	Low Intensity Exercise + Metformin High Intensity Exercise + Metformin Low Intensity Exercise + Placebo High Intensity Exercise + Placebo
NCT04578652	Fiber and Metformin Combination Therapy in Adolescents With Severe Obesity and Insulin Resistance	Obesity Insulin Resistance	Metformin 850 mg bid DIETARY_SUPPLEMENT fiber mixture (35 g total) composed of 6 g of Oligofructose + 12 g of resistant maltodextrin + 12 g of acacia gum + 5 g of PGX.
NCT05629806	Effect of Acarbose and Mixture of Pioglitazone and Metformin in Patients With Type 2 Diabetes	Type 2 Diabetes	Metformin plus pioglitazone Acarbose
NCT04581447	Extending Time Without Diabetes After Bariatric Surgery: a Trial Comparing the Metformin Addition or Not to Standard Care	Diabetes	Metformin Standard Care
Sepsis			
NCT05979038	Clinical Evaluation of the Effect of Metformin in Sepsis	Sepsis	Metformin
NCT06181422	Metformin and Lactoferrin in Sepsis in Icu	Sepsis	Metformin Lactoferrin
Osteoarthritis			
NCT05638893	Metformin as Adjuvant Therapy in Obese Knee Osteoarthritis Patients	Knee Osteoarthritis	Metformin Placebo
NCT06367283	Metformin Treatment of Patients With Hand Osteoarthritis	Hand Osteoarthritis	Metformin Placebo
NCT06231758	Metformin Safety and Efficacy in Osteoarthritis.	Osteo Arthritis Knee	Celecoxib Metformin
Other			
NCT04649502	Metformin for the Treatment of Hidradenitis Suppurativa (HS)	Hidradenitis Suppurativa	Metformin
NCT06519786	Safety and Efficacy of Metformin for Treatment of Cytopenia in Children and Adolescents With Fanconi Anemia	Fanconi Anemia	Metformin
NCT04939935	Implementation of Metformin theraPy to Ease Decline of Kidney Function in Polycystic Kidney Disease (IMPEDE-PKD)	Autosomal Dominant Polycystic Kidney Disease	Metformin ER, Control
NCT06033131	PI4 - A Trial Assessing Metformin to Prolong Gestation in Preterm Preeclampsia	Preeclampsia	Metformin ER, Placebo
NCT05521633	Comparison of the Effects of Metformin and Pioglitazone on Liver Enzymes and Ultrasound Changes in Non-Diabetic Non-Alcoholic Fatty Liver	NAFLD	Metformin and Pioglitazone

ER, extended release

resistance is the polycystic ovary syndrome (PCOS), a disorder affecting approximately 10 % of adult women [205]. Being characterized by anovulation, oligomenorrhea and polycystic ovarian morphology, it is associated with reduced endometrial GLUT4 levels, raised by metformin treatment [206]. A number of studies confirmed this finding, whereas numerous others did not [207,208]. In view of the frequent combination treatment with oral contraceptives (OCs), a recent meta-analysis evaluated the effects of metformin versus OCs and the association of the two [209]. There appeared to be some benefit by the combined treatment on insulin levels, hyperandrogenism and insulin resistance versus OC alone, although there appeared to be no difference in clinical outcomes, indicating the need of individualized treatments in this syndrome. Table 1 summarizes the ongoing phase III clinical trials with metformin, listed on Clinicaltrials.gov.

9. Conclusions

The very large, still growing use of metformin in diabetes and other conditions is justified by a number of advantages: very low price, excellent tolerability (if taken with meals), minimal risk of hypoglycemia, weight loss, very easy combination with any other antidiabetics (today DPP4 inhibitors, SLGT-2 antagonists, etc.). The very recent availability of extended-release metformin has improved tolerability and overall effectiveness, also indicating use in gestational diabetes [210]. Metformin is in the list of WHO Essential Medicines-23rd Ed. 2023, thus making the use of this simple molecule, essentially imperative. The use of metformin has become particularly appreciated after the finding of ancillary properties of the molecule, some directly related to diabetes, such as weight loss and lipid reduction, some providing unexpected health benefits. The anti-cancer properties appear to be consequent to some of the basic mitochondrial activities and to the

mechanism of drug elimination by MATE1. While a therapeutic activity appears best in colorectal cancers with KRAS mutations, the very recent observations in the essentially untreatable diffuse intrinsic pontine glioma of children, as well as in combination to PD-1/PD-L1 inhibitors for hepatocellular carcinomas, have brought significant hope.

The apparent potential life-lengthening action of metformin appears to be linked to the mitochondrial activity of the agent, but also to anti-inflammaging properties, associated to the lysis of cells with SASP, possibly leading to autophagy and raised telomere length. The life prolonging activity has been recently found to potentially be linked to the antagonism of RANs, controlling expansion of multiple repeats of the classical initiation AUG in the CNS. This severe non-treatable condition may occur in CNS disorders, such as ALS and FTD, potentially targets of metformin.

CRediT authorship contribution statement

Sofia Castiglione: Writing – review & editing. **Chiara Pavanello:** Writing – review & editing, Writing – original draft, Visualization. **Cesare Riccardo Sirtori:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization.

Declaration of Competing Interest

None.

Data Availability

No data was used for the research described in the article.

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