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POTENTIAL ANTI-CANCER FEATURES OF METFORMIN

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ABSTRACT

INTRODUCTION AND PURPOSE:

Metformin is one of the most frequently prescribed medications in the whole world. This lipophilic biguanide is widely used as a first-line medicine for patients suffering from type 2 diabetes mellitus because of its high effectiveness in monotherapy, and in connection with other antidiabetic drugs. Glucose-lowering properties of metformin were initially used only in the therapy of type 2 diabetes mellitus, but some data indicate that these properties might state an alternative in the prevention or treatment of some cancers both among diabetic and non-diabetic patients.

STATE OF KNOWLEDGE:

Metformin molecular mechanisms of action were thoroughly investigated, differentiated, and described, but in the context of the glucose-lowering effect. As a multiway drug, used mainly in diseases characterized by an increased level of glucose in the blood, numerous medical trials were conducted to find other treating properties of metformin. Recently, a few reports presented the potential connection between using metformin in the prevention and treatment of neoplasms in the same mechanisms. The scientists analyzed the influence of metformin's action on various cancers and drew conclusions. The research on potential anti-cancer features of metformin was conducted for a relatively short period and still presents a challenge to scientists.

CONCLUSIONS:

The aim of this review is to gather current knowledge and present the latest discoveries about potential anti-cancer features of metformin. We discuss the potential underlying molecular mechanisms of metformin's action in the human body and indicate the connection between the prevention and treatment of neoplasms. Additionally, we point out the exact cancers in which metformin might play a significant role.

KEYWORDS: metformin; cancer; AMPK; type 2 diabetes mellitus; prevention; treatment.

INTRODUCTION AND PURPOSE:

Neoplasms are the second cause of death in Poland after cardiovascular diseases. According to the survey conducted by Central Statistical Office in Poland in 2021, almost 20% of all deaths were caused by cancers. [1] It is estimated that by 2025 the number of neoplasms will increase due to demographic changes – the growing number of elderly people and the direct dependence between the risk of developing neoplasm and age. [2] One of the factors which increase the possibility of developing cancer is type 2 diabetes mellitus (T2DM). T2DM is a type of diabetes mellitus, which is connected to high levels of glucose in the blood, resistance to insulin, and relative lack of insulin. In most cases, T2DM occurs as a consequence of obesity and lack of exercise. Besides, T2DM increases the risk and mortality of almost every site-specific cancer, especially the liver and the pancreas. However, it was suggested that the probability of prostate cancer among patients with T2DM is decreased. [3] The basic treatment of T2DM involves following a proper diet, rich in vegetables and fruits, low in saturated fats, and choosing proper carbohydrates. Additively, regular exercise and body loss are desirable. That kind of lifestyle should be respected at least for 6 months. If obeying such rules is insufficient to maintain the proper levels of glucose in the blood, taking medications should be considered. The first-line medicine for patients suffering from T2DM is metformin. Metformin, a lipophilic biguanide, is one of the most often prescribed medications in the world. It was discovered in 1922, but it was widely examined by Jean Sterne in the 1950s, who had proved the anti-diabetic properties of the mentioned drug. [4] This drug decreases the glucose level in plasma by inhibiting gluconeogenesis. Besides, it enhances the vulnerability of the body tissues to insulin, improves the lipid profile, and has a neutral or even reducing impact on the body weight. [5] What is more, the mortality rate is decreased among diabetic patients who take this drug. Apart from this, metformin has also an anti-aging effect and has an impact on the gut microbiome. Additionally, metformin reduces cardiovascular risk and improves prognosis among patients with polycystic ovary syndrome (PCOS). [6] The first one who observed the connection between the decreased risk of cancer and metformin usage was Evans et al. in 2005. [7] After that discovery in recent years, much research was carried out on the topic which shows the association between metformin and cancer risk reduction among patients suffering from T2DM. Also, the surveys conducted by scientists presented the possible positive influence of the mentioned medication on the treatment of the neoplasms by inhibiting the growth of the tumor, survival, and metastases. This connection was particularly presented with colon and pancreatic cancer. [8,9]

The purpose of this review article is to focus on the current research about the potential metformin's anti-cancer features, including prevention and treatment of neoplasms, and gather state-of-the-art knowledge on the metformin's possible underlying molecular mechanisms of action.

STATE OF KNOWLEDGE:

Mechanisms of metformin's action on cancer cells:

Nowadays at least 120 million people in the world use metformin as a first-line treatment for T2DM. The main principle of its action is lowering the level of glucose in the blood without causing hypoglycemia. Apart from this, metformin is also highly recommended for the treatment of T2DM because of the lack of certain side effects in comparison to other medications. [5] Metformin's anti-cancer mechanisms of action can be divided into direct and indirect effects. The indirect anti-cancer mechanisms of metformin's glucose-lowering effects involve suppression of hepatic gluconeogenesis and increase in the sensitivity of body tissues to insulin – it leads to higher peripheral glucose uptake, especially in skeletal muscles and, as a consequence, to modification of the insulin's level in the body. Considering the specific mechanism of inhibition of hepatic gluconeogenesis by metformin, which still presents a challenge to scientists, it is essential to comprehend the underlying basic mechanisms of hepatic gluconeogenesis. The following mechanisms of gluconeogenic regulation in the liver are distinguished: transcription of the genes, allosteric, substrate availability, and redox balance. [10] Metformin is supposed to suppress gluconeogenesis by the following mechanisms: inhibition of Complex-1, activation of 5' adenosine monophosphate-activated protein kinase (AMPK), inhibition of glycerol-3-phosphate dehydrogenase (GPD2), and cAMP response element-binding protein (CREB). [10]

Taking into consideration the indirect mechanism of metformin's action it is vital to point out the fact that insulin resistance and hyperinsulinemia might lead to carcinogenesis promotion – through insulin receptors, increasing levels of insulin-like growth factors (IGFs), steroid sex hormones, disrupting adipokines homeostasis and inflammatory processes. [11]

A vital role in the regulation of glucose homeostasis as well as in presenting a variety of clinical manifestations of cancer is played by insulin receptors. Insulin receptors are present in the body tissues as well as in the primary neoplasms. [12] The difference between the types of receptors is the following: body tissues express the isoform B of the insulin receptor whereas cancer cells express the isoform A of the insulin receptor. In addition, the mentioned receptors have a distinct affinity to different ligands. [13] The growth of cancer is believed to be a consequence of losing cancer cells' ability to down-regulate the number of insulin receptors on the surface in reply to the increased level of insulin. [13] Initially, insulin connects to the receptor and activates the signaling proteins - the insulin receptor substrates (IRS1) which enable the phosphoinositide 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK). These triggered signaling pathways activate the range of proliferative and antiapoptotic reactions. [13]

The liver is the main place of production of insulin-growth factors (IGFs), both IGF-1 and IGF-2, which are protein complexes of high similarity to insulin in molecular sequence. The elevated level of insulin, as well as this for IGFs, may lead to the progression of the neoplasm by activating signaling pathways connected with cell growth, proliferation, and metastasis. [14] Metformin down-regulates the level of IGF-1 indirectly – metformin activates the AMPK, AMPK can activate the phosphorylation of insulin receptor substrate 1 (IRS1), and as a consequence, IRS1 suppresses the IGF-stimulated activation of protein kinase B (AKT)/ tuberous sclerosis 1 (TSC1)/ mammalian target of rapamycin (mTOR) what results in decreased protein synthesis and proliferation of malignancy. [15] Metformin's feature to activate the AMPK interrupts the attenuated DNA synthesis and proliferation by the connection between insulin, IGF-1 receptors, and G-protein coupled receptor signaling in different neoplasms. [16]

Hyperinsulinemia and insulin resistance might also promote carcinogenesis through sex hormones binding globulin (SHBG). SHBG is the glycoprotein, which function is to bind sex hormones such as testosterone and estrogens and to transport them in the blood. The mentioned mechanisms inhibit the production of sex hormones binding globulin in the liver. As a consequence, the free sex hormone levels are elevated and available – this can promote the development of sex-hormone-dependent neoplasms like breast cancer. [13]

Usually, the adipose tissue among patients suffering from T2DM is overdeveloped. The fatty tissue is known for secreting adipokines, which are also known for starting inflammation. Adipokines are signaling proteins, whose representatives are substances like leptin, adiponectin, tumor necrosis factor-a, and interleukin-6, which are released from adipose tissue by macrophages. The majority of the mentioned substances are thought to increase insulin resistance – and as a result the level of insulin. Consequently, these reactions lead to the increased inflammatory response. Persistent inflammation is known as an important factor in cancer's origin and development. [13,17] Metformin suppresses the inflammation by inhibition of mediators like tumor necrosis factor-alpha (TNF-alpha), hypoxia-inducible factor -1a (HIF-1a), von Willebrand factor (vWf), and plasminogen activator inhibitor-1 antigen (PAI-1a) probably by the means of suppression mTOR signaling pathway. In the same way, metformin inhibits angiogenesis. [18]

After considering the indirect anti-cancer mechanisms of metformin's action it is essential to take a closer look at the direct anti-cancer mechanisms of metformin's action. The direct anti-cancer action of metformin is divided into AMPK-dependent and AMPK-independent mechanisms. The AMPK-dependent mechanisms are connected with decreased levels of mTOR, foliate, nuclear factor kappa-light-chain-enhancer of

activated B cells (NF-kB), and the increased level of TP53 phosphorylating. The AMPK-independent mechanisms are associated with decreased levels of reactive oxygen species (ROS), cyclin D1, the increased level of mammalian target of rapamycin complex 1 (mTORC1), autophagy, and the apoptosis of cancer cells. [19]

The regulation of the energetic balance of the organism on the cellular level is done by AMPK. In addition, AMPK is an energetic regulator also of the whole human body. When the level of energy is low, fatty acid and glucose uptake and oxidation are activated by AMPK to generate some energy. A chain of reactions is needed to activate AMPK. AMPK is a cellular energy sensor, which is allosterically activated by the adenosine monophosphate (AMP) or adenosine diphosphate (ADP). The Thr172 phosphorylation by liver kinase B1 (LKB1) or by the calcium/calmodulin-dependent protein kinase kinase 2 (CAMKKB) is activated by the interaction between AMPK and adenine nucleotides. [20] The main potential anti-cancer mechanism of metformin's action is the inhibition of complex 1. Complex 1 is the largest and most complicated protein complex of respiratory chains. The role of complex 1 is to catalyze the transfer of nicotinamide adenine dinucleotide hydrogen's (NADH's) electrons to coenzyme Q10 and to transmit protons through the inner mitochondrial membrane in eukaryotes or the plasma membrane of bacteria. [21] Inhibition of complex 1 results in the reduction of ATP: ADP and ATP (adenosine triphosphate): AMP ratios. Consequently, there is greater availability of substrates for AMPK, and this way AMPK is activated. The activation of AMPK results in the inhibition of CREB-regulated transcription coactivator 2 (CRCT2). As a consequence, the forming of CREB-CBP-CRTC2 is prevented and as an implication, the transcription of gluconeogenic genes is downregulated. [22] Also, AMPK suppresses acetyl-CoA carboxylase 1 (ACC1) and acetyl-CoA carboxylase 2 (ACC2), which results in lower lipogenesis and activation of fat oxidation. [23] Inhibiting ACC1 and ACC2 is the major purpose of the activation of AMPK. ACC1 and ACC2 are essential catalysts for the production of malonyl-CoA, which is the precursor for lipogenesis and affects mitochondrial fat oxidation. [24] As a result, body tissues' sensitivity to insulin is increased and hepatic lipid accumulation is decreased. In some cancers, like prostate, colon, or breast cancer, the ACC1, and ACC2 activation leads to the suppression of the development of the cancer cells. [25]

The biological processes which occur in the human body, both in the state of disease or health, in some spheres are connected to the reactive oxygen species (ROS). ROS play important roles in metabolism and the aging of living organisms. The main origin of ROS are mitochondria and their respiratory chain. Not only do ROS play a vital role in different chemical reactions, but also damage the various cell structures, such as proteins, lipids, and DNA. [26] ROS are produced by mitochondria predominantly by complex 1 and complex 3 of the electron transfer chain (ETC). Production of ROS by complex 1 is dependent on the availability of the substrates for ETC and might be realized by forward and reverse electron fluxes. [27] Metformin lowers the production of ROS by activating the reverse electron transfer (RET) without stimulating the production of ROS by the forward electron transfer. [28] Consequently, metformin prevents inflammation, which can lead to neoplasm genesis and development, by decreasing the production of ROS. Besides, metformin is also known as a substance that prevents the toxicity of ROS by activation in the internal repairing systems. [28]

Each cell of the human body undergoes a series of events, which is called the cell cycle, which results in the division of the primary cell into two daughter cells. To enter the cell cycle, certain conditions (like the availability of nutrients, growth factors, mitogens, and the proper size of the cell) must be fulfilled. The whole process is controlled by cyclins and cyclin-dependent kinases (CDKs). Cyclin D1 is a protein that is crucial in the regulation of mammalian cell proliferation. The role of the mentioned molecule is to control the cell cycle, especially the G1/S transition. Cyclin D1 adapts its level of expression as a reaction to the signals from the cell's environment and as a result, it regulates the cell cycle mechanism correctly. It activates the cyclin-dependent kinase 4/6 (CDK4/6) and leads to the phosphorylation of the retinoblastoma protein. This process conduces to the transcription of factor E2F, which is critical for the transcription of genes essential for the G1/S reaction. Metformin is capable of inhibiting G1/S transition by suppressing the cyclin D1 expression without the use of the AMPK pathway and as a consequence, decreases the growth of the tumor. [29]

Apoptosis, known also as programmed cell death, is the natural process that is present in every cell of the human body. This mechanism is crucial to regulate the development, and homeostasis and prevent the uncontrolled proliferation of the cells, which is the basic mechanism of cancer development. Apoptosis of cancer cells is provoked by metformin through the extracellular regulated kinases (ERK) signaling pathway, midline-1 transitional regulator complex, downregulation of androgen receptor or specificity protein (Sp) transcription factors, and Sp-regulated genes. The ERK signaling pathway is a complex of proteins that conduct a signal to the cell's nucleus DNA from a receptor localized on the surface of the cell. Inhibition of certain steps in the ERK signaling pathway was observed in breast cancer and led to cell apoptosis. [30] The inhibition of the midline-1 transitional regulator complex and downregulation of androgen receptors leads to cancer cell apoptosis in prostate cancer. [31]

Last but not least AMPK-independent anti-cancer effect of metformin is autophagy. Autophagy is one of the most principal mechanisms of homeostasis of the cell, which enables to alteration of the development of cancer cells. [32] What is more, metformin can have both pro-survival and pro-death roles towards neoplasms.

[33] Pro-death autophagy was observed among the cells from lymphoma, melanoma, retinoblastoma, endometrial cancer, and cervical cancer through various mechanisms. [34,35,36,37,38]

Besides the foregoing mechanisms of metformin's action explaining the independent anti-cancer effect of AMPK, the direct influence of AMPK activation is the inhibition of mTOR, folate, NF-kB, and increase of TP53 phosphorylation. mTOR pathway can be suppressed both by dependent and independent AMPK mechanisms. mTOR is involved in the regulation of the growth, proliferation, and movement of cells. Besides, mTOR also takes part in the transcription and translation processes of cells. mTOR is a base element of mTORC1 and mTORC2 protein complexes. mTORC1 is responsible for temporary cell growth because the complex is in charge of protein synthesis in ribosome biogenesis. mTORC2 performs a significant function in special control of cell growth, as it controls the actin skeleton. Metformin is an activator for AMPK and as a result mTOR pathway is inhibited. Tumor sclerosis complex 2 (TSC2) is phosphorylated by AMPK and as a result inhibits the mTORC1 at different stages, which results in inhibiting the synthesis of the proteins and the development of cells. [39] Apart from this, this phosphorylation dislocates the connection between mTOR and mTOR1, which prevents the mTORC1 activation. [40] It is vital to mention the fact that both mTORC1 and mTORC2 might be inactivated indirectly by the decreased level of insulin caused by metformin. [41] Besides, mTORC2 is known as an activator for AKT, which is a serine/threonine kinase. This kinase plays an important role in cell survival and glucose homeostasis. Three AKT kinases are varied – AKT1, AKT2, and AKT3, which phosphorylate forkhead box O3 (FOXO3). Inactivated and sequestrated FOXO3 leads to tumorigenesis. [42] Metformin maintains the activation of FOXO3 and as a result cancer cells might be reprogrammed into non-cancerous cells.

The inhibition of the mTOR pathway without the AMPK activation by metformin is potentially regulated by RAG GTPase. [43] RAG GTPase controls cell metabolism and growth dependently on the availability of the nutritional substrates in lysosomes. [44] It is activated by amino acids (AAs). RAG GTPase inhibition leads to the inhibition of the mTOR pathway. [43] Further clinical research is necessary.

Anti-folate drugs, such as methotrexate or pemetrexed, are widely used for the treatment of many neoplasms. These multi-targeted chemotherapeutic agents are extremely toxic to cancer cells, trigger the death of the neoplasm cells and prevent their proliferation of them by affecting the metabolism of folate. Metformin is also connected to the metabolism of folate as it mimics the mechanism of anti-folate drugs' action and is perceived as a potential anti-folate drug. [45] Metformin is known for increasing the level of homocysteine and decreasing the levels of folate and B12 among patients with T2DM using metformin as a medication. [46] Methionine synthase is considered to be the molecular target of metformin's mechanism of action. [47] The connection between the anti-folate and anti-tumor effect of metformin was observed especially in breast cancer.

The next AMPK-dependent mechanism of metformin's action is the reduction of the level of NF-kB. The NF-kB is a complex of proteins, which is involved in the production of cytokines and regulates DNA transcription and survival of the cells. This complex is in charge of regulating the immunological response to the infection and is present in almost every cell of the human body. [48] The abnormal level of NF-kB might promote the development of the neoplasm. The transcription of NF-kB, relocation of NF-kB to the nucleus, and phosphorylation of I kappa B protein (IkB) through I kappa B kinase a/b (IKKa/b) involved in the activation of the NF-kB pathway are inhibited by metformin. As a consequence, the inflammatory response is reduced, which prevents the development of a variety of cancers. [49]

Tumor protein p53 (TP53) is the transcriptional factor that is classified as a tumor suppression gene. This protein is involved in the regulation of many cell processes, especially in the activation of DNA repair and in the induction of apoptosis in response to DNA damage. The role of TP53 in cancer treatment is still under examination, but some data indicates the potential connections with cancer development and treatment. The decreased level of TP53 might promote carcinogenesis, but the increased level of TP53 might prevent the growth of cancer, as well as treat it. Metformin is an activator for AMPK. AMPK is known for phosphorylation at Ser15 of TP53, which activates the complex and increases the level of TP53. An increased level of TP53 induced by the action of metformin triggers the apoptosis, autophagy, and senescence of the neoplasm cells. [50] Besides, TP53 also activates the mTOR pathway regardless of AMPK, which is another crucial mechanism of the anti-tumor effect of metformin.

The direct effect of metformin on specific cancers:

In 1978 Biguanides, such as metformin were suggested to suppress the development of neoplasms among animals by Dilman and his co-workers. [51]. Afterward, the same researchers introduced the term "metabolic rehabilitation". [52] This therapy consisted of caloric restriction and treatment with the biguanides. Not only did this treatment result in the inhibition of the growth of the neoplasm, but also reduced the probability of occurrence of the metastases. [52] Especially this influence was observed in breast, colorectal or gastric cancers. Mentioned ideas were thoroughly examined in this century, even though they were first discovered almost 50 years ago.

According to the available data, metformin regulates the growth, survival, and metastasis of the various types of neoplasms. In addition, this biguanide controls angiogenesis, fibroblasts, macrophages, and immunosuppression, which results in changes in the microenvironment of cancer. Metformin especially has an influence on the tumor cells from colorectal, breast, liver, bone, cervical, endometrial, kidney, lung, prostate, ovary, blood cancers, and melanoma.

Colorectal cancer (CRC) is one of the most frequently encountered cancer connected with T2DM. Both have a lot in common. The risk factors for the occurrence of this neoplasm are metabolic syndrome and obesity. The clinical research indicates that metformin might prevent the risk of the development of CRC. Additionally, metformin reduces the probability of prevalence of colorectal adenomas among patients, who are treated for diabetes and previously suffer from colorectal cancer. [53] The data showed that among patients with T2DM treated intensely with metformin in comparison to the non-diabetic patients the lower mortality was observed in CRC with stages I-III. [54] For the patients with CRC who were treated with metformin, a 15% lower mortality rate was observed versus the patients treated only with insulin. [54] Increased overall survival among patients with CRC treated with metformin as a part of the treatment was observed. [55] Besides, metformin changes the microbiome and as a result has an impact on glucose, lipid, and energy metabolism. These features indirectly reduce the risk of occurrence and progression of CRC. [56]

The most common cancer among females in Poland is breast cancer, which has many risk factors, like BRCA-1 and BRCA-2 mutations, age, the early occurrence of period and late menopause, and obesity. Metformin reduces the level of glucose in the blood and as a result, there is reduced availability of the substrates for cancer cells. The research showed that there was no connection between metformin use and the risk of cancer, but there was a significant reduction of mortality with almost 40,4% among the patients exposed to metformin. [57] Triple-negative breast cancer (TNBC) is very difficult to treat and there is no effective low-toxicity chemotherapy or other targeted therapies. Therapy which consists of metformin and heme is very successful in treating TNBC as it suppresses the progression of the tumor. [58] Metformin is also known for being selectively toxic to cancer cells. Metformin as a single medication does not affect cancer cells, but connected with doxorubicin prevents the recurrence and growth of breast cancer. [59]

The liver is the most often place of metastases from the gastrointestinal tract, especially from colorectal cancer. Apart from this, in the liver may also occur primary cancer – hepatocellular carcinoma. T2DM is known for increasing the probability of liver neoplasm. Metformin is known for its protective effect on liver cells. The application of metformin reduces the risk of liver cancer by 48% among the patients treated with that substance in comparison to non-users. [60] Besides, better survival was observed among the patients suffering from liver cancer who used metformin as a primary medication for T2DM. A 53% reduction in mortality was also observed among the patients with liver cancer who were exposed to the action of metformin. [61]

Bone cancers are the most popular neoplasms among adolescents. Bone cancers might be classified into two groups: primary tumors, which origin is from bone and bone-derived cells and tissues, and secondary tumors, which originate from other parts of the body and are metastases. The second ones are more frequent and mostly breast, lung, and prostate cancers metastasize to the bones. Metformin through suppressing the AMPK and mTOR signaling pathway can inhibit the proliferation, translocation, and differentiation of bone cancer cells by suppressing receptor activator of nuclear factor kappa-B ligand (RANKL). RANKL is a protein complex that is involved in the metabolism of the bones. [62] RANKL is responsible for osteoblastic proliferation and formation, which is a fundamental mechanism of the development of bone cancers. Metformin is a suppressor for RANKL and as a consequence is an inhibitor for the growth of bone tumors.

The most frequent gynecological malignancies are cervical, endometrial, and ovarian cancers. Cervical cancer is the primary malignant neoplasm of the cervix and is caused by abnormal growth of the cancer cells in the cervix. The HPV infection is necessary for the development of the neoplasm. This neoplasm, especially in low-developed countries, is one of the most common causes of death by neoplasms. Metformin in combination with other drugs may increase the anti-cancer effects in cervical cancer. In addition, the side effects of the anti-cancer drugs might be decreased in presence of metformin. [63]

Endometrial cancer is frequent among females after menopause. One of the first noticeable symptoms of this cancer is abnormal vaginal bleeding, which is not connected to the menstrual period. The main risk factors for the development of this cancer are obesity and hyperglycemia. The two types might be differentiated: endometroid and non-endometroid subtypes. Metformin is believed to increase the survival rate among patients with DM who suffer from endometrial cancer. [64]. Some research states that the potential anti-cancer effect of metformin may vary depending on the histological type of the endometrial carcinoma and metformin is only beneficial for the non-endometroid subtype. [65]

One of the most lethal gynecological neoplasms is ovarian cancer. The risk of ovarian cancer increases among women which have the mutation of the genes BRCA1 and BRCA2 – adequately 54% and 23%. [66] There is no specific screening test to detect this neoplasm at an early stage. Frequently ovarian cancer is diagnosed in the advanced stage, which is complicated to treat and the final option is surgery. The association between metformin and ovary cancer was under examination, but there was no clinical evidence that metformin

improved the overall survival and prognosis. [67] Metformin in combination with cisplatin, which is widely used in chemotherapy, has a stronger suppressing effect than in the treatment with only a single of them. [68]

The kidney cancer is most often diagnosed cancer which is identified randomly during diagnostic imaging done due to other reasons. Usually, the first and the most significant symptom of kidney cancer is blood in the urine. The kidney cancer might be divided into 3 types: renal cell carcinoma (RCC), transitional cell carcinoma (TCC), and Wilms Tumor. Renal cell carcinoma is the most frequent and states almost 80% of kidney cancers. What is more, kidney cancer is more often observed among people who live in urban areas rather than in rural areas. Metformin is known for improving overall survival and cancer-specific survival, especially among patients with localized RCC. [69] Also, the risk of death was lower among the patients with kidney cancer who used metformin in comparison to the non-users of metformin.

In Poland, the most frequent cause of death among both groups females and males is lung cancer, which the most often cause is long-lasting tobacco smoking. Usually, the first symptom is a chronic cough and a noticeable change in the nature of the cough. When the patient reports symptoms that may indicate lung cancer the first investigative examination is the X-Ray of the chest. There is a large discrepancy between different ethnic groups in the risk of lung cancer. The risk of lung cancer was reduced among the Asian patients who took metformin, but among the European patients who received metformin, there was no relevant decrease in the risk of lung cancer. [70] The research states that taking metformin after diagnosis improves overall survival and progression-free survival among patients suffering from lung cancer. [71]

Prostate cancer is the second most common neoplasm among men in Poland. The manifestations of prostate cancer are connected with the urinary tract: difficulty in starting and maintaining a persistent stream of urine, painful urination, nocturia, frequent urination, or hematuria. The level of prostate-specific antigen (PSA) in the blood is used as a screening test among men without symptoms of the disease. Using this laboratory test is controversial, as the results might be inconclusive. The most popular way to certainly detect prostate cancer is to perform a transrectal ultrasound-guided prostate biopsy. Using metformin is connected with a decreased risk of occurring of prostate cancer. Also, the mortality rate among the patients treated with metformin who were diagnosed with prostate cancer was reduced. There is no significance in the used dose of metformin. [72]

Melanoma is the most aggressive skin cancer which occurs the most frequently in the skin, but also may be found in the intestines or the eye (uveal melanoma). This cancer develops from the melanocytes, which are pigment-producing cells. Mostly, melanoma is diagnosed among white people and depending on the gender may be generally found among females on the legs, but among males on the back. Metastases of this cancer can be found in the lymph nodes, lungs, brain, liver, and in bones. The most effective way of melanoma treatment is surgery, which can be completed with chemotherapy, immunotherapy, biological therapy, and radiation therapy. Metformin is known for stopping the progression in the cell cycle in the phase G0/G1 and activating apoptosis and autophagy in melanoma cells. [73] The synergic anti-tumor effect in causing melanoma cell death is observed in the connection between metformin and vemurafenib. [74] Apart from this, metformin strengthens cisplatin's toxicity in the treatment of melanoma. [75]

Hematological malignancies are caused by abnormal production or differentiation of the blood cells. These cancers have an influence on the function of the blood, bone marrow, lymph nodes, and lymphatic system. Blood cancers might be derived from one of the two major blood cell lineages: myeloid and lymphoid cell lines. The main cancers are chronic lymphocytic leukemia (CML), acute lymphatic leukemia (ALL), acute myelogenous leukemia (ACL), and multiple myeloma (MM). The basis of the diagnosis of hematological malignancies is the bone marrow biopsy to examine microscopically the type of the neoplasm. Mostly, blood cancers are treated with chemotherapy, radiotherapy, immunotherapy, and with bone marrow transplants. Metformin is known for increasing the cytotoxic effectiveness of fludarabine, used for treating CML, and should be largely used as an adjuvant in the treatment. [76] Besides, metformin inhibits the AMPK and mTOR signaling pathway and consequently, inhibits the growth of the B cells or T cells. [77] In the same way, metformin acts in MM and inhibits cell proliferation. In addition, metformin suppresses the glucose-regulatory protein 78 (GRP78) and increases autophagy and apoptosis. As a consequence, the anti-myeloma effects of bortezomib, which is largely used in the treatment of MM, are increased. [78]

CONCLUSIONS:

Metformin is a lipophilic biguanide, which is used as a medicament for almost 60 years. Initially, the treating features of metformin were undiscovered and thus the real properties were not fully revealed. Later, after the discovery of the anti-diabetic features of metformin, more research was done which showed the prospective anti-aging and anti-cancer effects of metformin - firstly the data was based on animal studies and later among the people. The scientist took a closer look at the potential mechanisms of action of metformin. The two main mechanisms were pointed out: the indirect and the direct effects of action. The direct mechanisms of action are closely related to the indirect effects of action, which contain the decrease of the glucose level by suppression of gluconeogenesis in the liver and the increase of the body tissues' sensitivity to insulin. The direct effects of action are connected to the AMPK-dependent suppression of mTOR, folate, NF-kB, and activation of TP53, but

also to AMPK-independent mechanisms like the decreased level of ROS, cyclin D1, apoptosis, and autophagy, which pose important mechanisms of anti-cancer effects of metformin. Many clinical trials revealed that metformin has a positive impact on many different cancers, like colorectal, liver, or breast cancer. The research showed that using metformin prevents the development and survival of neoplasm cells. In addition, the microenvironment of the tumor is changed and as a consequence, the growth of the cancer is inhibited. In some neoplasms, the overall survival was higher and the mortality rate was lower. In some clinical studies, metformin strengthens the toxicity of anti-cancer drugs and consequently increases the effectiveness of the applied chemical treatment. Many observational studies were carried out on the animals or additionally among the patients with DM who suffered from neoplasms. Still, there is a lack of data that was conducted on patients with cancer but without diabetes. As yet the available data is promising, but further research is needed to finally clarify the role of metformin in the prevention and treatment of different cancers, especially among patients who do not suffer from DM. Browsing many different research papers may lead to the impression, that metformin is a multiway drug, which might be used in T2DM, PCOS, and many different diseases. Metformin is an auspicious medication, which anti-cancer mechanisms of action are still needed to be further investigated as they could state a new promising way of cancer prevention and treatment.

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