Review Article

A Review of the Potential Utility of Mycophenolate Mofetil as a Cancer Therapeutic

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Tumor cells adapt to their high metabolic state by increasing energy production. To this end, current efforts in molecular cancer therapeutics have been focused on signaling pathways that modulate cellular metabolism. However, targeting such signaling pathways is challenging due to heterogeneity of tumors and recurrent oncogenic mutations. A critical need remains to develop antitumor drugs that target tumor specific pathways. Here, we discuss an energy metabolic pathway that is preferentially activated in several cancers as a potential target for molecular cancer therapy. *In vitro* studies have revealed that many cancer cells synthesize guanosine triphosphate (GTP), via the *de novo* purine nucleotide synthesis pathway by upregulating the rate limiting enzyme of this pathway, inosine monophosphate dehydrogenase (IMPDH). Non-proliferating cells use an alternative purine nucleotide synthesis pathway, the salvage pathway, to synthesize GTP. These observations pose IMPDH as a potential target to suppress tumor cell growth. The IMPDH inhibitor, mycophenolate mofetil (MMF), is an FDA-approved immunosuppressive drug. Accumulating evidence shows that, in addition to its immunosuppressive effects, MMF also has antitumor effects via IMPDH inhibition *in vitro* and *in vivo*. Here, we review the literature on IMPDH as related to tumorigenesis and the use of MMF as a potential antitumor drug.

1. Introduction

Metabolic regulation in tumors is different from resting adult tissues. Tumor cells increase synthesis of proteins, lipids, and nucleotides to adapt to their rapid proliferation. Nearly, a century ago, Dr. Otto Warburg first described the altered metabolism of cancer cells, known as the "Warburg effect." The Warburg effect is the observation that the energy needed by tumor cells is mainly produced through high rates of glycolysis followed by lactic acid fermentation in the cytosol, as opposed to the low rate of glycolysis followed by pyruvate oxidation in the mitochondria of nonmalignant cells. The relationship between tumor and metabolism has just recently been a major focus of cancer biology. Growing evidence supports a pivotal role for oncogenic mutations and the resultant altered signaling pathways that dramatically change cell metabolism. For example, phosphatidylinositol 3-kinase (PI3K) and mammalian target of rapamycin (mTOR) signaling pathways are established oncogenic drivers in a variety of cancers [1]. These signaling pathways lead to increased glucose uptake and many anabolic processes, such as lipid biosynthesis and protein and nucleotide synthesis [2]. Potent inhibitors of PI3K and mTOR have been designed, and several of them are currently in clinical trials targeting
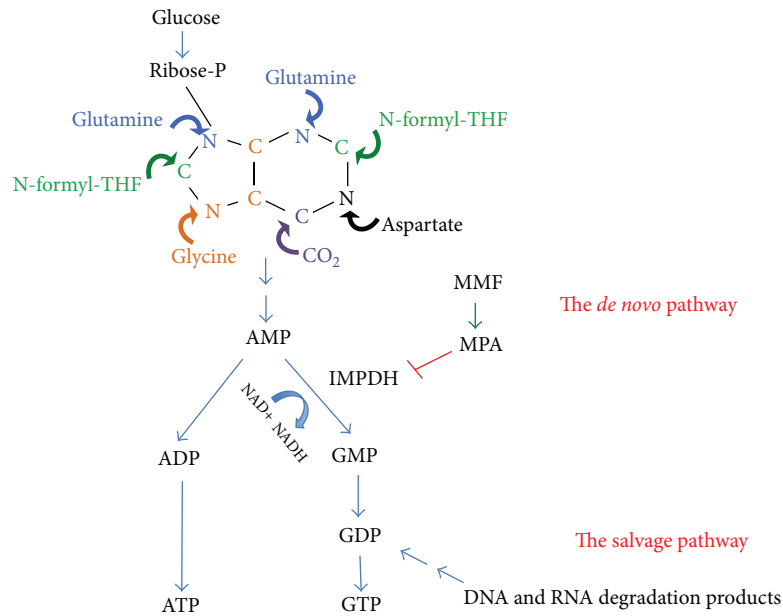
FIGURE 1: Purine nucleotide synthesis pathways. IMP: inosine monophosphate and IMPDH: inosine monophosphate dehydrogenase. XMP: xanthine monophosphate, AMP: adenosine monophosphate, and GMP: guanine monophosphate. GTP: guanosine triphosphate, ATP: adenosine triphosphate, and NAD: nicotinamide adenine dinucleotide. MMF: mycophenolate mofetil, and MPA: mycophenolic acid.

various cancers [3]. However, acquired resistance to these inhibitors via mutation of specific binding proteins or recurrent alteration of signaling pathways is an emerging problem [4]. Therefore, alternative approaches to target tumor cell specific metabolic pathways need to be considered.

In this review, we focus on metabolic pathways that are specifically upregulated in tumors. The aim is to target key enzymes in such pathways to suppress cell proliferation or induce differentiation of cancer cells. We focus on one of the major energy currency molecules, GTP. In addition to its well-known role as a building block of mRNA and DNA, GTP has distinctive roles as the cellular energy source. GTP is a critical energy molecule for protein synthesis and signaling via GTP-binding proteins. Most importantly, GTP levels are more elevated in several cancers than that of ATP [5] making the GTP synthesis pathway of particular interest in cancer biology. GTP upregulation may reflect the increased need for protein synthesis in tumor cells as opposed to resting, noncancerous cells. The knowledge of these two important aspects of GTP raises the following questions.

> How do cancer cells upregulate GTP synthesis?
>
> Is GTP upregulation important for tumor cell growth?
>
> Does targeting GTP synthesis pathway result in antitumorigenesis?

To address these questions, we review the current literature on the role of the rate-limiting enzyme of the GTP nucleotide synthesis pathway, inosine monophosphate dehydrogenase (IMPDH), in various malignancies. We also review the body of work that has explored the role of FDA-approved IMPDH inhibitor, mycophenolate mofetil (MMF), as an antitumor drug.

2. How Do Cancer Cells Upregulate GTP Synthesis?

2.1. Various Tumors Utilize the De Novo Purine Nucleotide Synthesis Pathway to Synthesize GTP. Cells use two distinct pathways to generate ATP and GTP: the "salvage pathway" and the "*de novo* purine nucleotide synthesis pathway." The salvage pathway is an energy efficient pathway that utilizes assembled parts of nucleotides formed during degradation of DNA and RNA to make ATP and GTP. The *de novo* purine nucleotide synthesis pathway on the other hand is an energy consuming, multistep pathway that uses glucose as the starting material and undergoes more than 20 steps of enzymatic reactions to become ATP or GTP (Figure 1). In order to generate one molecule of GTP from glucose, nine ATP-coupled reactions, a glycine, two folates, two glutamines, and an aspartate are required. Interestingly, the energy consuming *de novo* purine nucleotide synthesis pathway is often employed by rapidly proliferating cancer cells, while the energy efficient salvage pathway is predominantly used by quiescent, noncancerous cells. This may be due in part to the preferential increase in activity of the enzymes of the *de novo* purine nucleotide synthesis pathway in tumor cells including IMPDH [6, 7].

2.2. Various Tumors Increase IMPDH Expression. IMPDH is the rate-limiting enzyme of the *de novo* purine nucleotide synthesis pathway and a molecular determinant of the fate of IMP to GTP synthesis (Figure 1). The human genome encodes two different IMPDH genes [8]. IMPDH1 is believed to be ubiquitously expressed, whereas IMPDH2 is expressed in proliferative states where it has been linked to cell growth and malignant transformation [9, 10]. IMPDH1 is highly
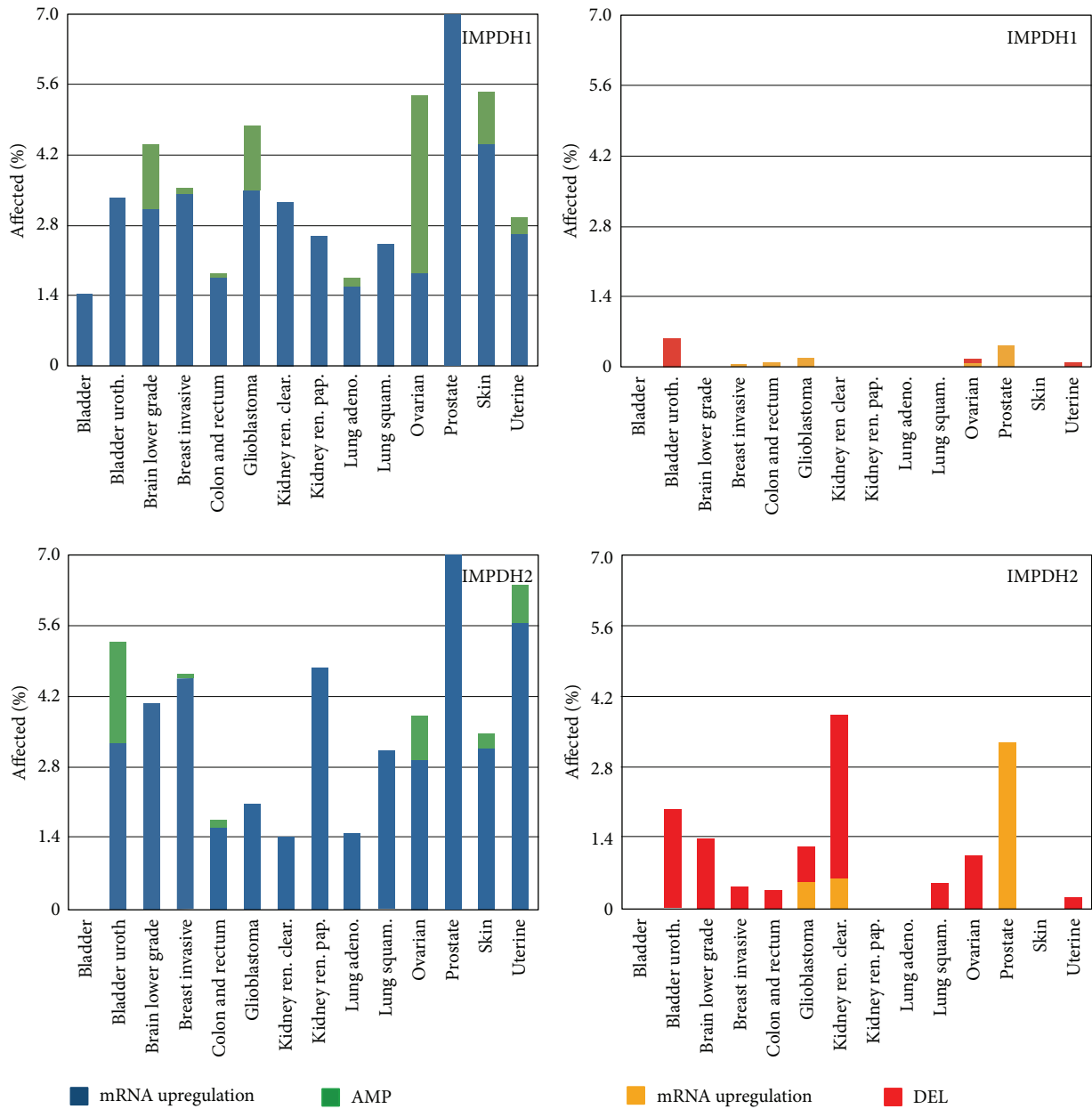
FIGURE 2: IMPDH gene alteration in cancer patients. AMP: amplification. DEL: deletion.

expressed in resting and activated peripheral blood lymphocytes as well as the retina [11, 12]. Mutations in unique isoforms of IMPDH1 expressed in retinal cells are associated with retina-specific photoreceptor degeneration suggesting that IMPDH1 is important for normal retinal development [12].

To gain insight into altered IMPDH expression in tumors, we analyzed the cBio database operated by Memorial Sloan Kettering Cancer Center (http://www.cbioportal.org/public-portal/cross_cancer.do) that provides gene expression profiles in various cancer patient tissues. Figure 2 demonstrates IMPDH1 and IMPDH2 gene amplification and mRNA upregulation in a number of tumors obtained from cancer patients including brain, ovarian, skin, bladder, renal cell,

and uterine cancers. It should be noted that some tumors have reduced IMPDH expression, though it is a minor fraction. This suggests that some tumors may use IMPDH-independent nucleotide synthesis pathways. *In vitro* studies have corroborated the preferential upregulation of IMPDH in tumors obtained from patients in that many tumor cell lines also carry high levels of IMPDH expression (summarized in Table 1). These analyses show that IMPDH gene expression is elevated in a number of tumors including brain, sarcoma, and leukemia, when compared to normal tissue counterparts. Specifically, Fellenberg et al. showed that IMPDH2 gene expression is significantly elevated in osteosarcoma patients with poor response to chemotherapy and that this elevated expression is associated with poor risk disease evidenced

TABLE 1: IMPDH gene overexpression in a number of malignancies.

Malignancy/cell lines	IMPDH	References
Osteosarcoma patients with poor response to chemotherapy	Elevated expression	Fellenberg et al., 2007 [13]
Osteosarcoma cells (SaO2)	Exogenous overexpression induced chemoresistance	Fellenberg et al., 2010 [9]
MTX-resistant erythroleukemia cells (K562)	Overexpressed	Peñuelas et al., 2005 [15]
MTX-resistant human colon cancer cells (HT29)	Overexpressed	Peñuelas et al., 2005 [14]
Leukemic cells (HL-60)	(i) Upregulated by induced proliferation (ii) Downregulated by induced differentiation	Nagai et al., 1992 [16]

MTX: methotrexate.

by decreased event-free survival [13]. Similarly, Peñuelas et al. showed that IMPDH2 is overexpressed in methotrexate-resistant erythroleukemia cells, K562, and human colon cancer cells, HT29 [14, 15]. IMPDH2 mRNA is also selectively overexpressed in leukemic cancer cells [16, 17]. In HL-60 leukemic cells, IMPDH2 mRNA expression is upregulated by 2.8-fold under conditions of increased proliferation and downregulated to less than 5% by induced differentiation [16]. These data demonstrate that both patient-derived tumor tissue and cultured cell lines result in elevated IMPDH expression levels.

This IMPDH mediated GTP biosynthesis pathway is preferentially used by rapidly proliferating cells over the salvage pathway to support increased RNA production and DNA replication needed by transformed cells [7]. It is not yet clear how IMPDH levels are upregulated in cancer cells. In experiments using yeast, the researchers have found that expression level of yeast IMPDH homologues mRNA and protein are increased upon depletion of cellular GTP by mycophenolate treatment and reduced by GTP replenishment [18, 19]. Clearly, this induction of IMPDH genes observed in yeast is a likely homeostatic mechanism to increase GTP production. While it remains elusive whether this regulation of human IMPDH genes is conserved throughout evolution, a potential model could be that cancer cells encounter GTP depletion due to the increase in anabolic processes, which leads to the induction of IMPDH genes.

However, the above model may not explain the observation that elevation of IMPDH is associated with elevated GTP levels in cancer cells [20, 21]. Furthermore, it has been postulated that IMPDH elevation in tumor cells may be due to upstream regulators including p210 bcr-abl protein [22]. Increased GTP levels in tumor cells can at least in part be due to elevated IMPDH levels [5].

3. Is GTP Upregulation Important for Tumor Cell Growth?

Alterations in IMPDH genes including amplification and deletion as well as varying mRNA expressions are seen in a wide variety of cancer patients' tissues and a number of cancer cell lines (Figure 2 and Table 1). In this section, we review studies that evaluate the role of increased GTP levels in tumor

cells. Many of these studies use a pharmacological inhibitor of IMPDH to modulate cellular GTP levels.

3.1. MPA, the Active Form of MMF, Is a Potent, Highly Selective, Pharmacological Inhibitor of the De Novo GTP Synthesis Pathway.
Mycophenolic acid (MPA) is the active form of MMF. MMF is hydrolyzed to its active form, MPA, in the gut wall, blood, and liver. MMF is the compound administered to patients and MPA is the compound more commonly used in *in vitro* and *in vivo* experiments. These names have been used distinctly within the paper depending on the type of studies that are described.

MPA is an inhibitor of IMPDH that inhibits conversion of IMP to XMP (Figure 1) and results in cellular GTP reduction. The bioavailability of MPA after oral administration of MMF is more than 90% [23]. MMF inhibits proliferation of both T and B lymphocytes and has been widely used in posttransplant medicine for prevention of acute and chronic allograft rejection since its introduction as an immunosuppressant in 1994. MMF is also used as an immunomodulator to treat an array of autoimmune conditions such as lupus nephritis, idiopathic thrombocytopenic purpura, and scleroderma [24]. Widespread use of MMF in the field of organ transplantation has demonstrated a favorable safety profile [25]. In addition to its effect on the immune system, the role of MMF as an antitumor drug has been investigated.

3.2. MPA Suppresses Tumor Cell Growth In Vitro and In Vivo.
The very first reports of antiproliferative effects of MPA were published in the late 1960s [26]. Since then, the antitumor effect of MPA has been studied in cell lines obtained from a number of different malignancies. In these studies, MPA treatment suppresses cell proliferation of leukemia, lymphoma, pancreatic cancer, non-small-cell lung adenocarcinoma, and colon cancer cell lines as summarized in Table 2 [27–37].

In addition, murine models have been studied to test the potential tumor suppressive effect of MMF *in vivo*. Immunodeficient nude mice were subcutaneously injected with human cancer cell lines, A3.01 (T-lymphoblast), Molt-4 (T-cell leukemia), CaPan-2 (pancreatic adenocarcinoma), CaLu-3 (non-small-cell lung adenocarcinoma), LS174T and T84 (colon adenocarcinoma), and Daudi (B-cell lymphoma).

TABLE 2: Antitumorgenicity of MMF in preclinical studies.

Cell lines	Animal models	Effects	Suggested pathways/regulators	References
T-lymphoblast (A3.01) T-cell leukemia (Molt-4) Pancreatic adenocarcinoma (CaPan-2) Non-small-cell lung adenocarcinoma (CaLu-3) Colon adenocarcinoma (LS174T and T84) Daudi (B-cell lymphoma)	Tumor xenografts	Inhibition of subcutaneous tumor growth		Tressler et al., 1994 [27]
Lymphoma cells (RAQ 117-H10)	Murine model of metastasis (BALB/c)	Increased survival of animals		Tressler et al., 1994 [27]
Prostate cancer (DU145)		Cell cycle arrest and induction of differentiation		Floryk and Huberman 2006 [28]
Neuroblastoma (LAN5, SHEP 5, and IMR32)		Induction of differentiation and apoptosis	(i) GTP depletion (differentiation) (ii) P53 (apoptosis)	Messina et al., 2004 [29]
Multiple myeloma (RPMI8226 and RPMI8226-HAMcl) Primary multiple myeloma cells		Induction of apoptosis	(i) GTP depletion (ii) Cytochrome c release (iii) Caspase activation	Takebe et al., 2006 [30]
Imatinib and nilotinib resistant chronic myeloid leukemia (K562)		Induction of apoptosis		Drullion et al., 2012 [31]
Breast cancer (MDA-MB-231 and MCF-7)		Induction of differentiation	PPARγ	Zheng et al., 2011 [32]
Mouse colon adenocarcinoma (CT26) Melanoma (B16) Human gastric adenocarcinoma (TMK1)		Inhibition of tumor cell growth and angiogenesis		Koehl et al., 2007 [33]
Colon carcinoma (HT-29) Prostate cancer (DU145)		Reduction of cell adhesion	Beta 1 integrin	Engl et al., 2005 [34]
WiDr colon adenocarcinoma T cells		Reduction of cell adhesion		Leckel et al., 2003 [35]
Glioblastoma cells (U87)	BALB/c nude mice	Inhibition of tumor cell growth in xenograft model but not *in vitro*	MYC signaling	Domhan et al., 2008 [36]
Pancreatic cell lines (MiaPaCa and Panc-1)		Sensitization of gemcitabine	TGF-beta-1	Tuncyurek et al., 2007 [37]

The mice were treated with oral MMF either 24 hours prior to tumor injection or after tumors became palpable. MMF significantly inhibited subcutaneous tumor growth in both groups regardless of time of administration. The injected MMF dose was 30, 120, or 240 mg/kg/day in two daily doses. There was a dose response curve in the majority of cell lines tested with the doses of 120 or 240 mg/kg/day showing statistically significant reduction in xenograft tumor size. Additionally, MMF treatment in a murine model of metastasis, (BALB/c) injected with RAW 117-H10 lymphoma cells resulted in increased survival compared to controls [27]. Here, only the 240 mg/kg/day dosing was active in prolonging survival. The above studies demonstrate that MMF may be a feasible and efficacious anticancer therapy.

3.3. MPA Suppresses Tumor Cell Growth via Diverse Mechanisms. As shown in Table 2, MPA treatment leads to suppression of tumor cell growth in many cell lines. Interestingly, the mechanism of growth suppression by MPA shows significant diversity: (1) replication arrest (arrest at S-phase of cell cycle) in DU145 prostate cancer cell lines [28], (2) induction of tumor cell differentiation in neuroblastoma cell lines (LAN5, SHEP5, and IMR32) [29], (3) p53-mediated G1 cell cycle arrest and apoptosis of neuroblastoma cell lines (LAN5, SHEP5, and IMR32) [29], (4) attenuation of cell cycle progression and apoptosis following concomitant cytochrome c release and caspase activation of multiple myeloma cell lines and primary myeloma cells [30], and (5) apoptosis or cellular senescence of chronic myeloid leukemia cells [31]. Currently, the mechanism by which MPA treatment suppresses cell growth via such diverse pathways is unclear. It is likely that such differential MPA responses are attributed to the differential genetic mutations and/or cellular metabolism in each cancer type. It is important to clarify the molecular mechanism by which MPA exerts its cell growth suppressive effects to further test this drug in clinical trials.

4. Does Targeting GTP Synthesis Pathway Result in Antitumorigenesis?

Next, we review the literature investigating other antitumorigenic effects of MPA, including tumor cell differentiation, angiogenesis, and cell adhesion.

4.1. MPA Induces Tumor Cell Differentiation. Messina and colleagues showed that MPA induces differentiation of human neuroblastoma cell lines (LAN5, SHEP 5, and IMR32) by causing partial chronic guanine nucleotide depletion. They also demonstrated that MPA leads to cell cycle arrest and apoptosis through a p53-mediated pathway [29]. Similar studies were performed in breast and prostate cancer cell lines. MPA treatment of breast cancer cell lines (MDA-MB-231 and MCF-7) resulted in differentiation of adipose tissue as evidenced by arrest of breast cancer cells at the G1/G0 stage of the cell cycle, adipocyte-like morphological conversion, and lack of colony formation in treated breast cancer cells. Additionally, MPA resulted in upregulation of three adipocyte differentiation markers, PPARγ, adipsin D, and aP2. This differentiation was thought to be partly due to PPARγ

activation since the RNAi knockdown of PPARγ partially reversed the differentiated state of MDA-MB-231 cells after MPA treatment [32]. In the DU145 prostate cancer cell line, MPA induced expression of a number of differentiation markers including CD55, clusterin, granulophysin, glucose-related protein 78, vasoactive intestinal polypeptide, and prostate-specific transglutaminase [28]. Poorly differentiated status of tumor is associated with aggressive phenotypes. Thus, these data suggest that treatment with MPA may be an active anticancer therapy by inducing differentiation in tumor cells resulting in cell cycle arrest.

4.2. MMF Inhibits Angiogenesis. Neovascularization, also known as angiogenesis, is critical for tumors to maintain a high level of nutrient uptake and proliferation. MMF was discovered as a potent inhibitor of angiogenesis when Chong and colleagues screened a library of 1850 FDA approved drugs. They screened for inhibition of human umbilical vein endothelial cells (HUVEC) using H3 thymidine incorporation. MPA was discovered to be a potent inhibitor of HUVEC proliferation with an IC50 of about 100 nM. IMPDH1 was the predominantly expressed isoform in these endothelial cells and knockdown of its expression by RNAi was sufficient to cause endothelial cell cycle arrest in G1 [38].

In addition, MMF was shown to inhibit neovascularization in an aortic ring assay [33]. In this study, slices of rat aorta were cultured on matrigel-coated 24-well plates where sprouts from the aortic rings are normally visible after a few days. MMF at concentrations from 1 to 100 uM almost completely inhibited sprouting from the aortic rings. The same group showed that MMF causes a dose-dependent cell growth reduction in mouse CT26 colon adenocarcinoma, B16 melanoma, and human TMK1 gastric adenocarcinoma cells *in vitro* [33].

4.3. MMF Modulates Tumor Cell Adhesion. MMF prevents allograft rejection via blocking the proliferation of T and B lymphocytes. Another mechanism of preventing allograft rejection by MMF is via suppressing the expression of adhesion molecules and decreasing the recruitment of immune cells into sites of graft rejection [39]. Given that regulation of adhesion molecules is pivotal in the ability of tumor cells to metastasize, it is conceivable that MMF could affect invasion of tumor cells. It has been shown that MPA modulates adhesion properties of the colon carcinoma cell line, HT-29, and prostate tumor cell line, DU-145 [34]. Reduction in adhesion of HT-29 to endothelial cells by MPA is accompanied by downregulation of alpha subunits of beta 1 integrin—an adhesive protein whose varying expressions are associated with tumor cell growth and metastasis. On the other hand, reduction in adhesion of DU-145 cells is accompanied by upregulation of beta 1 subtypes. Upregulation of integrin subtypes in the face of decreased adhesion of cells in DU-145 cells could possibly be secondary to redifferentiation towards low-invasive phenotypes [34].

In another study, modulation of adhesion molecules by MMF was thought to increase the risk of tumor metastasis [40]. Blaheta and colleagues showed that MMF decreases

neural cell adhesion molecule (NCAM) receptors in neuroblastoma cells that self-aggregate by means of the homophilic-binding element. Decreased number of NCAM receptors is associated with increased migratory potential of neuroblastoma cells. It is inquiring that modulation of different cell adhesion molecules results in varying outcomes, redifferentiation of cells towards less invasive phenotypes versus promotion of migratory potential of cells. This likely depends on the specific cell adhesion molecules that are expressed on individual types of cancer cells.

Other groups have shown that MMF suppresses expression of additional adhesion molecules such as lymphocytic alpha4beta1 integrin [39, 41]. Additionally, MMF was shown to decrease T-cells and WiDr colon adenocarcinoma cell line binding to adhesion endothelium by 80% [35]. Here, MMF potently blocked both T-cells binding to ICAM-1, VCAM-1, and P-selectin and prevented WiDr cell attachment to E-selectin [35]. These results suggest that MPA could modulate cell adhesion via multiple molecular pathways. In addition, Dun and colleagues demonstrated that MPA has antimigratory and anti-invasive properties in gastric cancer cells via downregulation of large number of promigratory proteins such as AKT and MMP1 [42].

Consistent with the notion that MPA affects tumor microenvironment, Domhan and colleagues showed that U87 glioblastoma cells are resistant to MPA treatment *in vitro*, but their growth is markedly inhibited *in vivo* in BALB/c nude mice [36]. Genome-wide transcriptional analysis in U87, endothelial and fibroblast cells treated with MPA revealed a role for MYC signaling in endothelial cells.

In addition to the above-noted antitumor effects, MMF is effective as a chemotherapeutic agent in pancreatic cancer *in vitro*. In MiaPaCa and Panc-1 pancreatic cell lines, concomitant administration of MMF increased the EC50 response of gemcitabine treatment that is widely used in patients with pancreatic adenocarcinoma. In addition, treatment of Panc-1 cells with MMF inhibited TGF-beta-1 induced collagen I expression in a dose-dependent manner [37]. It has been shown that collagen I promotes metastasis in pancreatic cancer cells by activating c-Jun and upregulation of N-cadherin expression [43]. These studies concluded that MMF inhibition of collagen I overexpression may be responsible for the reduction seen in pancreatic cell line invasiveness.

5. Clinical Studies of MMF as an Anticancer Drug

Finding of MMF as an antitumor agent *in vitro* and *in vivo* has lead to its use in a number of clinical trials. MMF is already an FDA-approved drug, and its tolerability profile is superior to many other commonly used chemotherapeutic agents.

The first clinical trial involving MMF as an anticancer therapeutic was performed in multiple myeloma patients. In this phase I dose escalation study, MMF was well tolerated up to 5 g/day daily doses in 11 patients with relapsed and refractory disease [44]. Interestingly, the intracellular dGTP level in peripheral blood mononuclear cells was decreased in five patients with partial response or stable disease in comparison with the six patients that had progression of disease. This finding was at least in part consistent with the known mechanism of action of MMF: GTP depletion by MMF leads to alteration of intracellular guanine pool resulting in nucleotide imbalance and hampered DNA synthesis.

Rodríguez-Pascual and colleagues performed a high throughput screening for anticancer activity of FDA approved drugs and identified MPA as an active antiangiogenic agent. Here, MPA resulted in growth inhibition and reduced expression of vascular endothelial growth factor (VEGF) that was reversed by guanosine supplementation in the media *in vitro* [45]. Additionally, MPA was shown to have antitumor effects in a panel of six human derived pancreatic cancer xenografts in immunodeficient mice. They therefore tested MPA in a pilot clinical study in which MMF was administered to twelve patients with resectable pancreatic cancer prior to resection. However, assessment of VEGF levels and an apoptosis marker in resected specimens in resected specimens compared to six untreated patients showed no significant difference in expression [45]. Survival analysis was not reported. In this study, FDA-approved immunosuppressive doses were used, 1 or 2 gram per day of MMF divided into two daily doses. Higher doses might have shown more promising results, but the purpose of this study was to use dosages that would not require additional safety testing. Further studies will be required to determine efficacy of MMF in pancreatic adenocarcinoma.

6. Tolerability and Bioavailability of Mycophenolate

Two mycophenolate compounds are commercially available, mycophenolate mofetil (MMF) and enteric-coated mycophenolate sodium (EC-MFS). Mycophenolate compounds are the prodrug of MPA. MPA, the active form of mycophenolate, has a complex pharmacokinetic profile; it undergoes glucuronidation, enterohepatic recirculation, and protein-dependent clearance. The pharmacokinetics of MPA and MMF are not linear in transplant patients [46]. MMF, the ester prodrug of MPA, was developed to improve MPA bioavailability [47]. The increase in bioavailability was linked to increased gastrointestinal (GI) intolerance. However, more recent studies point towards improved GI complications (particularly diarrhea) with the use of enteric-coated mycophenolate sodium in posttransplant patients [48–50]. GI intolerance as well as other serious complications of MPA such as leukopenia and anemia seems to be dose dependent [51].

Bioavailability of MPA after MMF administration is about 90% versus 74% for EC-MFS [23, 51, 52]. MPA is highly protein bound (97–99%) and therefore its total and free concentrations are subject to interpretation in patients with hypoalbuminaemia (i.e., severe liver or renal disease as well as cancer patients with severe malnutrition or impaired liver synthetic function). Given that MPA undergoes glucuronidation, its effective dose may be reduced in the setting of induced expression of glucuronosyltransferases in the setting of high dose corticosteroids. Cyclosporine reduces enterohepatic recirculation of MPA and therefore lowers its effective dose. Most pharmacokinetic studies of MPA

have been obtained in posttransplant patients with varying albumin concentrations and immunosuppressant cotherapy [53–55]. Lessons learned from transplant literature call for careful monitoring of MPA total and free levels as well as potentially monitoring other biomarkers such as IMPDH levels when and if MMF is widely used in cancer patients.

7. Safety of MMF in Post-Transplant Patients

The introduction of immunosuppressive agents for treatment of acute rejection in post-transplant patients has led to prolonged survival and the advent of immunosuppressant-induced malignancies [56]. MMF was amongst the immuno-suppressive agents initially thought to be the culprit of posttransplant malignancy. However, transplant patients are very rarely treated with MMF monotherapy. Other immuno-suppressive drugs, most commonly calcineurin inhibitors, are usually part of the treatment regimen and have been shown to increase the rate of nonmelanocytic skin cancer and lymphoma in posttransplant patients [57].

The relative risk of post-transplant malignancy seems to be lower with the use of MMF than other immunosuppressive drugs such as calcineurin inhibitors and azathioprine [58, 59]. However, patients were on multidrug regimens rather than MMF monotherapy in these studies making it difficult to decipher the actual contribution of each agent. Robson et al. designed a prospective observational cohort study comparing 6751 patients from two different renal transplant registries receiving MMF with an equal number of matched controls receiving non-MMF immunosuppressants. This study did not show an association between the use of MMF and an increased risk of lymphoma with follow-up of 3 years. Inter-estingly, in the MMF group, there was a trend toward a lower risk of any malignancy [58]. Although prospective, this study was limited in that patients were only followed for 3 years after transplant for development of malignancy. This study was corroborated in a recent retrospective single-center cohort of 929 adult renal transplant recipients [60]. The latter study showed that MMF used for prevention of acute rejection after kidney transplant was not associated with an increased risk of solid tumors or posttransplant lymphoproliferative disorders. A similar study was performed in liver transplant patients. Using Scientific Registry of Transplant Recipients between 1995 and 2004, triple immunosuppressive regimen involving MMF, tacrolimus, and corticosteroids ($n = 9180$) was compared with dual therapy, tacrolimus and corticosteroids ($n = 10,099$). There was no significant difference in death due to malignancy between patients 4 years after transplant and the treatment groups. The incidence of posttransplant malig-nancy was also not significantly different. Therefore, addition of MMF to the dual therapy (tacrolimus plus corticosteroids) was not associated with an increased risk of malignancy in liver transplant recipients at 4 years [61]. Overall, as noted above, the analysis of data involving large groups of patients in posttransplant registries has failed to show an increased risk of malignancy secondary to MMF.

It is worth noting that immunosuppressive reagents may increase the risk of oncogenic viral infections. For exam-ple, in the nontransplant literature where MMF is used as

monotherapy, there have been few reports of Epstein-Barr virus associated central nervous system lymphoma that were thought to be secondary to prolonged use of MMF [62–65]. However, it is reassuring that studies involving large post-transplant registries did not indicate an association between increased malignancy rate and the use of MMF directly.

8. Other IMPDH Inhibitors

In addition to MMF, other IMPDH inhibitors were tested in preclinical and phase I/II clinical trials as summarized in Table 3 [66–73]. A newer agent AVN-944, also known as VX-944, was developed due to concerns about MMF potency and gastrointestinal tolerability. This agent was well tolerated in normal volunteers in a phase I study, but it was not further developed due to financial issues [71, 73]. Most other IMPDH inhibitors such as tiazofurin were found to have robust antitumorigenic effects in preclinical and phase I/II clinical trials; however, further studies were limited due to their side effects [68] (Table 3).

9. Discussion

The *de novo* purine nucleotide synthesis pathway is upregu-lated in many tumors and is essential for their growth. Mod-ulation of the GTP *de novo* synthesis pathway by inhibition of IMPDH, the rate-limiting enzyme for GTP biosynthesis, seems to be a promising approach in our endeavors to inhibit cancer cell growth. MMF, an FDA-approved immunosup-pressant commonly used for prevention of acute transplant rejection and treatment of autoimmune conditions, has been studied as an antitumor drug in several tumor cell lines. Preclinical studies have suggested multiple antitumorigenic effect of MMF including (1) inhibition of cell proliferation, (2) inhibition of angiogenesis, (3) inhibition of cell adhesion, and (4) induction of cellular differentiation. These findings are likely due to the various roles of G-proteins in different mammalian cells. It is important to decipher the precise molecular mechanism of MMF to further guide its use in specific tumor types.

The safety and tolerability of MMF have been confirmed in multiple phase I clinical trials. We believe that it is oppor-tune time to further investigate MMF as an antitumor drug using mouse orthotopic tumor models and genetically engi-neered models that more precisely reflect the human tumor environment. Multiple preclinical trials have shown that MMF antitumor effects are reversed by addition of guanine to the cells. This suggests the possibility that MMF antitumor effects are related to its ability to inhibit the *de novo* purine synthesis pathway presumably via inhibition of IMPDH. A potential caveat of the use of MMF as an antitumor drug is that GTP can be produced via the *de novo* pathway as well as the compensatory salvage pathway. Tumor cell lines have the potential to develop resistance to MMF by upregulating the salvage pathway after prolonged treatment. Therefore, it would be beneficial to measure the cellular and blood guanine nucleotide concentrations in future studies that investigate the antitumor role of MMF *in vivo* and MPA *in vitro* and to potentially combine MMF with other treatment approaches.

TABLE 3: Antitumorigenicity of other IMPDH inhibitors.

IMPDH inhibitors	Study design: results	Suggested pathways	References
Tiazofurin	Preclinical: activation of erythroid differentiation program of human leukemia K-562 cells	Downregulation of c-ras gene	Gebeyehu et al., 1985 [66] Olah et al., 1988 [67]
	Phase I: • 16 patients with nonlymphocytic leukemia or myeloid blast crisis of chronic granulocytic leukemia. ∘ 8 patients showed improvement or complete remission.		Tricot et al., 1989 [68]
	Phase II: • 6 patients with bcr-abl positive AML or CML in blast crisis ∘ Transient hematological response		Malek et al., 2004 [69]
Benzamide riboside	Preclinical: induction of apoptosis in human lung cancer H520 cells	Increased caspase 3 activity and PAPR cleavage	Khanna et al., 2004 [70]
AVN-944	Preclinical: inhibition of cell proliferation in prostate cancer cell lines	Cell cycle arrest	Floryk and Thompson, 2008 [71]
	Phase I: well tolerated in 25 healthy males		Hamilton et al., 2009 [72]
VX-944	Preclinical: induction of apoptosis in multiple myeloma cell lines	Caspase-independent pathway	Ishitsuka et al., 2005 [73]

10. Conclusion

Mycophenolate mofetil is an FDA-approved immunosuppressant with antitumor effects. Its use as antitumor drug should be revisited in light of its potential for treatment of malignancies that overexpress IMPDH and its favorable safety profile in comparison with other chemotherapeutic agents. Careful research on the role of GTP regulation in tumor cells can aid further clinical investigations on the role of MMF as an antitumor drug.

Conflict of Interests

The authors confirm that there is no conflict of interests regarding this publication.

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