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## Review Article

# Metabolic reprogramming of myeloid-derived suppressor cells in the context of organ transplantation

Jasper Iske<sup>1,2</sup>, Yu Cao<sup>3</sup>, Maximilian J. Roesel<sup>1,2</sup>, Zhongyang Shen<sup>3</sup>, Yeqi Nian<sup>3,\*</sup>

<sup>1</sup> Department of Cardiothoracic and Vascular Surgery, Deutsches Herzzentrum der Charité (DHZC), Berlin, Germany

<sup>2</sup> Charité-Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin, Germany

<sup>3</sup> Research Institute of Transplant Medicine, Tianjin First Central Hospital, Nankai University, Tianjin, China

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## ABSTRACT

Myeloid-derived suppressor cells (MDSCs) are naturally occurring leukocytes that develop from immature myeloid cells under inflammatory conditions that were discovered initially in the context of tumor immunity. Because of their robust immune inhibitory activities, there has been growing interest in MDSC-based cellular therapies for transplant tolerance induction. Indeed, various pre-clinical studies have introduced *in vivo* expansion or adoptive transfer of MDSC as a promising therapeutic strategy leading to a profound extension of allograft survival due to suppression of alloreactive T cells. However, several limitations of cellular therapies using MDSCs remain to be addressed, including their heterogeneous nature and limited expansion capacity. Metabolic reprogramming plays a crucial role for differentiation, proliferation and effector function of immune cells. Notably, recent reports have focused on a distinct metabolic phenotype underlying the differentiation of MDSCs in an inflammatory microenvironment representing a regulatory target. A better understanding of the metabolic reprogramming of MDSCs may thus provide novel insights for MDSC-based treatment approaches in transplantation. In this review, we will summarize recent, interdisciplinary findings on MDSCs metabolic reprogramming, dissect the underlying molecular mechanisms and discuss the relevance for potential treatment approaches in solid-organ transplantation.

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## Introduction

Although clinical solid-organ transplantation has improved short-term outcomes, the procedure still faces limitations due to substantial side effects of immunosuppression and compromised long-term organ survival [1]. Calcineurin inhibitors, as the backbone of immunosuppression, are associated with nephrotoxicity, cardiovascular and metabolic side effects, as well as increased risk of opportunistic infections and *de novo* malignancies [2]. Moreover, current first-line immunosuppressive drugs have shown limited effects on chronic rejection, impeding long-term graft survival even under strict follow-up and maintenance immunosuppression [1,3]. To further improve the quality and expectancy of life of transplant recipients, innovative therapeutic solutions are required. Of note, inducing transplant tolerance has been considered as a desirable solution, as it enables organ acceptance without the need for long-term immunosuppression [2].

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of immature myeloid progenitor cells with potent

immunosuppressive activity, which has led the focus on investigating their therapeutic potential for allogeneic transplantation [4,5]. It is now generally accepted that MDSCs play an important role for the regulation of alloreactive responses [6,7]. MDSCs have been shown to expand early after transplantation [8], migrate to the allograft and suppress alloreactive T-cell responses [4]. At the same time, MDSCs were shown to facilitate allograft tolerance through promoting the development and recruitment of regulatory T cells (Treg) [9]. Of relevance, MDSC infusion has been demonstrated to prevent the rejection of allogeneic transplantation of pancreatic islets [10]. Similarly, adoptive transfer of *in vitro*-induced MDSCs prolonged allograft survival in a model of murine heart transplantation [11]. Moreover, targeted depletion of MDSCs leads to cardiac allograft rejection in recipient mice that had been treated with donor splenocyte transfusion and anti-CD40L, known to prevent transplant rejection [12], underscoring the essential role of MDSCs for induction and maintenance of transplant tolerance. In human studies, expansion of MDSCs has been observed in peripheral blood of kidney transplant recipients [8,13] (Table 1) [14–23]. Expanding the number of MDSCs and modifying their functional capacities to enhance their inhibitory effects on alloimmune responses is a promising approach for organ transplantation. The transduction of human monocytes with FADD-like

\* Correspondence: Yeqi Nian, Research Institute of Transplant Medicine, Tianjin First Central Hospital, Nankai University, Tianjin, China.  
 E-mail address: [nianyeqi0314@hotmail.com](mailto:nianyeqi0314@hotmail.com) (Y. Nian).

**Table 1**  
Key studies involving MDSCs in transplantation.

Author	Study	Findings
Lee et al., 2020 [4]	Animal	MDSCs expand after transplantation, increased numbers are associated with prolonged graft survival
Iglesias-Escudero et al., 2020 [8]	Human	Immunosuppressive therapy impacts MDSCs level in patients having received a kidney transplantation
Nakamura et al., 2016 [9]	Animal	MDSCs recruit CD4(+)Foxp3(+) regulatory T cells, thereby suppressing the immune response and promoting graft survival
Fujimoto et al., 2021 [11]	Animal	Recipient-derived monocytic MDSCs prolong graft survival via inhibiting T-cell response and nTreg infiltration
Luan et al., 2013 [13]	Human	MDSCs can promote the expansion of CD4(+) Foxp3(+) Treg, which suppresses the immune response and promotes graft survival
Lv et al., 2015 [14]	Animal	Monocytic and promyelocytic MDSCs contribute to immune tolerance in haplo-identical allogeneic HSC transplantation
Wu et al., 2016 [15]	Animal	mTOR is essential for immunosuppressive function, metabolism may impact MDSCs-mediated immune tolerance induction
De Wilde et al., 2009 [16]	Animal	Endotoxin-induced MDSCs can inhibit alloimmune responses via heme oxygenase-1 and promote immune tolerance
Dugast et al., 2008 [17]	Animal	MDSCs accumulate in tolerant grafts suppressing T effector cells and are involved in NO-dependent maintenance of tolerance
Yang et al., 2016 [18]	Animal	TNF- $\alpha$ -induced M-MDSCs have powerful immunosuppressive activity, which is mediated by an iNOS-dependent pathway
Wang et al., 2015 [19]	Animal	MDSCs are an integral player in controlling allograft survival after CsA or VIVIT treatment via the calcineurin-NFAT-IDO signaling axis.
Han et al., 2016 [20]	Animal	CsA treatment significantly increased the frequency of MDSCs and reduced the production of pro-inflammatory cytokines by MDSCs.
Nakamura et al., 2015 [21]	Animal	Rapamycin treatment prolonged cardiac allograft survival by inducing the expansion and activation of MDSCs
Okano et al., 2018 [22]	Human	MDSCs exert their suppressive activity on reactive T-cell through the production of arginase-1 and iNOS
Liao et al., 2014 [23]	Animal	Dexamethasone treatment increased the suppressive activity of MDSCs on T-cell responses by inducing the production of NO

Relevant studies are shown summarizing the effects of MDSCs in organ transplantation and immune tolerance.

CsA, cyclosporine; HSC, hematopoietic stem cell; IDO, indoleamine 2, 3-dioxygenase; iNOS, inducible nitric oxide synthase; MDSC, myeloid-derived suppressor cell; mTOR, mammalian target of rapamycin; NO, nitric oxide; TNF- $\alpha$ , tumor necrosis factor alpha.

interleukin (IL)-1 $\beta$ -converting enzyme-inhibitory protein (c-FLIP)-expressing lentivirus vectors, for instance, increased the suppressive activity on stimulated T cells and abrogated graft-versus-host disease in a xenograft model upon adoptive transfer, leading to improved long-term survival of recipient mice when compared with those receiving luciferase-expressing (control) monocytes. Strikingly, c-FLIP-transduced monocytes exerted more prominent suppression on graft-versus-host disease when compared with thawed human Tregs [24]. These rodent and human data encourage efforts to recruit MDSCs as a therapeutic approach in transplantation.

In addition to challenges due to their heterogeneous nature and limited expansion capacity, there are also transplant-specific challenges that need to be addressed. Common immunosuppressive drugs such as rapamycin (RPM), cyclosporine A (CsA) and tacrolimus interfere with complex metabolic and immunological signaling pathways affecting different cells, and MDSCs are no exception. Although CsA appears to promote the proliferation of MDSCs and thus enhance immunosuppression [19,20], RPM appears to inhibit the differentiation and proliferation of MDSCs as well as their immunosuppressive effect on T cells [15]. A detailed understanding of these interactions is needed for clinical application, but it seems worthwhile to investigate. MDSCs proliferate after transplantation and inhibit graft rejection, but durable graft tolerance has not been achieved in preclinical studies with MDSCs [4,17].

Given the advancements made in cancer research, cellular metabolic reprogramming represents a promising target for immunoregulation in transplantation [25,26]. Upon activation, immune cells reprogram the expression of critical metabolic enzymes and rewire metabolic pathways to overcome the bioenergetic and biosynthetic demand [26,27]. Notably, distinct features of different immune cells in metabolic reprogramming provide the opportunity to target specific subsets, allowing tailored immunosuppression [25], which constitutes a major goal of current transplant research in order to lower systemic side effects and provide long-term immune tolerance. Indeed, several studies have reported on targeted immune cell reprogramming towards an anti-inflammatory phenotype, setting the cornerstones for further investigations of this therapeutic approach. M1 macrophages, for instance, have been skewed towards M2 macrophages using dimethyl fumarate, metformin or TEPP-46 [28], whereas T cells have been successfully repolarized towards Tregs by dimethyl fumarate or RPM in multiple sclerosis [29].

In transplantation, preclinical research has demonstrated the feasibility of metabolic inhibitors in prevention of transplant rejection

and tolerance induction [30–32]. Most studies of these studies have focused solely on T-cell immunity as major drivers of allograft rejection. However, other critical immune cell components such as MDSCs remained largely unexplored. Although the metabolic phenotype and adaptations of MDSCs to hypoxic and inflammatory environments have been thoroughly characterized in cancer research, little effort has been made to translate this knowledge to the field of transplantation, where metabolic treatments are gaining increasing interest. Therefore, in the present review, we introduce MDSCs and their metabolic characteristics, dissect the underlying molecular mechanisms in MDSCs metabolic reprogramming and delineate the relevance for potential treatment approaches in solid organ transplantation.

### Phenotype and Function of MDSCs

MDSCs in both human and mice are heterogeneous and can be classified into two major groups according to their origin from the granulocytic or monocytic myeloid cell lineages, monocytic MDSCs (M-MDSCs) and polymorphonuclear MDSCs (PMN-MDSCs) [2]. Both MDSC subsets have been characterized by expression of CD11b, whereas their phenotype can be distinguished according to the expression of distinct surface markers. Hence, in mice M-MDSCs display a CD11b<sup>+</sup>Gr-1<sup>mid</sup>Ly6C<sup>hi</sup>Ly6G<sup>-</sup>CD49d<sup>+</sup> phenotype whereas PMN-MDSCs are defined as CD11b<sup>+</sup>Gr1<sup>hi</sup>Ly6C<sup>low</sup>Ly6G<sup>+</sup>CD49d<sup>-4,27</sup>. In humans, in turn, M-MDSCs are characterized as CD11b<sup>+</sup>CD14<sup>+</sup>HLA-DR<sup>low</sup>-CD15<sup>-</sup> and PMN-MDSCs as CD11b<sup>+</sup>CD14<sup>-</sup>CD15<sup>+</sup>(or CD66b<sup>+</sup>) [14,33].

Of note, human MDSCs also comprise another distinct group of myeloid precursor cells that are defined as Lin-HLA-DR-CD33<sup>+</sup> early-stage MDSCs which, however, only account for up to 5% of the total MDSCs population [5,34] (Table 2). In transplantation, alterations in MDSC subsets are rarely reported. In a murine cardiac transplant model, most posttransplant splenic MDSCs had been CD11b<sup>+</sup>Ly6C<sup>low</sup>Ly6G<sup>+</sup> PMN-MDSCs [4], whereas CD11b<sup>+</sup>CD33<sup>+</sup>HLA-RR<sup>-</sup> M-MDSCs in contrast had been the predominant subset in the peripheral blood of human kidney transplant recipients [8,13].

One of the key functions of MDSCs is T-cell suppression. Data from cancer research suggest that M-MDSCs and PMN-MDSCs share common mechanisms of immunosuppression but are somewhat different in their preference [35]. Hence, M-MDSCs have been shown to exhibit their major immunosuppressive effects through inducible nitric oxide synthase (iNOS), immunosuppressive cytokines and cell-surface molecules [35]. iNOS converts L-arginine to nitric oxide (NO),

**Table 2**  
Phenotype and function of MDSC subsets in humans and mice.

	M-MDSC	PMN-MDSC	e-MDSC
Mice	CD11b <sup>+</sup> Gr-1 <sup>mid</sup> Ly6C <sup>hi</sup> Ly6G <sup>-</sup> CD49d <sup>+</sup>	CD11b <sup>+</sup> Gr1 <sup>hi</sup> Ly6C <sup>low</sup> Ly6G <sup>+</sup> CD49d <sup>-</sup>	–
Human	CD11b <sup>+</sup> CD14 <sup>+</sup> HLA-DR <sup>low</sup> /CD15 <sup>-</sup>	CD11b <sup>+</sup> CD14 <sup>-</sup> CD15 <sup>+</sup> (or CD66b <sup>+</sup> )	Lin <sup>-</sup> (CD3/14/15/19/56)/HLA <sup>-</sup> DR <sup>-</sup> /CD33 <sup>+</sup>
Cell-specific function	Generation of NO, Production of immunosuppressive cytokines (IL-10, TGF- $\beta$ ), Inhibitory checkpoint (PD-1)	Generation of ROS, Depletion of L-arginine and cysteine levels from the environment	Poorly understood
General function	Blocking of T cell homing, Promotion of Treg development, ectoenzyme-induced adenosine production, Fas/FasL-dependent apoptosis induction of cytotoxic T cells, Methylglyoxal-mediated effector T-cell paralyzation		

Surface markers defining the phenotype of M-MDSCs, PMN-MDSCs and e-MDSCs in human and mice are shown. In addition, cell-specific function of each subset as well as general functions common in distinct subsets are outlined.

e-MDSC, early-stage myeloid-derived suppressor cell; IL-10, interleukin-10; M-MDSC, monocytic myeloid-derived suppressor cell; NO, nitric oxide; PD-1, programmed cell death protein 1; PMN-MDSC, polymorphonuclear myeloid-derived suppressor cell; ROS, reactive oxygen species; TGF- $\beta$ , transforming growth factor- $\beta$ .

which impairs T-cell proliferation by inducing apoptosis, suppressing T-cell mitogenic responses or inhibiting major histocompatibility complex class II expression [36–38]. In addition, M-MDSCs up-regulate the expression of immunosuppressive cytokines such as IL-10 and transforming growth factor- $\beta$ , as well as immune regulatory cell-surface molecules like membrane-bound programmed death receptor ligand 1 (PD-L1) [39]. PMN-MDSCs, in turn, have been characterized to exert their suppressive effects on immune responses promoting the generation of reactive oxygen species (ROS) and arginase 1. In detail, MDSCs express NADPH oxidase 2 (Nox2) catalyzing the production of ROS, whereas increased expression of arginase-1 causes a reduction of local L-arginine and cysteine levels, which constitute a key nutrition for T-cell proliferation and function [40,41]. Noteworthy, the described dichotomy does not imply an exclusive gene expression of either iNOS or arginase-1. Synergic expression of iNOS and arginase-1 has namely been characterized to constitute a cardinal feature of the immunosuppressive function of MDSCs, supporting the generation of reactive nitrogen species [42]. Reactive nitrogen species in turn impede protein–protein interactions and functions including chemotaxis, antigen recognition and activation of T cells [43]. In addition, MDSCs appear to mediate the development of Treg that suppress T-cell responses against transplanted grafts via interferon (IFN)- $\gamma$ -dependent pathways [6,12]. IFN- $\gamma$  seems to play a crucial role for tolerance induction in transplantation, as tolerance is not induced in IFN- $\gamma$ -deficient recipient mice due to active CD28 and CD40 ligand T-cell co-stimulation pathways, resulting in the proliferation of alloreactive effector T lymphocytes [44]. iNOS-expressing MDSCs seem essential for prolonging graft survival through stimulation of IFN- $\gamma$  secretion in Tregs [18]. In addition, CCL5 secreted by graft penetrating MDSCs has been shown to promote the accumulation of Tregs in tolerized kidney grafts [13,45,46]. Conversely, the increase in Tregs augmented the number of MDSCs in the peripheral blood of the recipient, in the spleen and in the graft itself [47]. Other mechanisms of MDSC-mediated immunosuppression that are mediated by both M-MDSCs and PMN-MDSCs include blockade of T-cell homing through reducing L-selectin expression on T cells [48], ectoenzyme-induced adenosine production [49] and Fas/FasL-dependent apoptosis of cytotoxic T cells [50]. Notably, a recent study showed a novel mechanism in which MDSC-derived methylglyoxal can be transferred to T cells via cell–cell transfer. Subsequently, methylglyoxal then paralyzed T-cell effector function through metabolic inhibition of these cells [51] (Table 2).

### Metabolic Reprogramming Orchestrating the Immunosuppressive Effects of MDSCs on T-Cell Immunity After Transplantation

Metabolic reprogramming is a general term initially derived from cancer research for the response of cells to crucial changes in the

surrounding microenvironment, involving altered expression in critical metabolic enzymes, metabolites and metabolic pathways, which in turn leads to altered functions [52,53]. Following transplantation, significant perturbation in both the systemic environment [54] as well as in the allograft microenvironment [55] can be observed deriving from ischemia–reperfusion injury (IRI), shear forces and allo-immune responses, which in turn affect the metabolic phenotype and function of immune cells, including MDSCs.

MDSCs rapidly expand after transplantation exerting immunosuppressive effects through mechanisms described previously [56]. Triggered by external stimuli, metabolic reprogramming of MDSCs hereby alters the acquisition and processing of nutrients in order to meet the requirement of augmented energy supply and biosynthetic intermediates [57] for proliferation and generation of effector molecules [4]. Oxidative stress for instance, can be recognized by general control nonderepressible 2 (GCN2) acting as an environmental sensor crucially involved in the instigation of MDSC metabolic reprogramming through inducing the upregulation of ATF4, a transcription factor that regulates the expression of genes involved in amino acid metabolism, oxidative stress and the unfolded protein response [58]. During metabolic reprogramming glucose, lipid and amino acid metabolism undergo significant alteration tightly regulating MDSCs effector pathways (Table 3).

### Glucose Metabolism

Glucose is the major source of cellular energy and converted into ATP through glycolysis and oxidative phosphorylation [59]. Oxygen-rich environments cause immune cells including quiescent naïve T cells, B cells and monocytes to prioritize glucose flux for maximum ATP production through mitochondrial oxidation [60,61]. In an inflammatory microenvironment, various activated immune cells including T effector cells [62,63], M1 macrophages [64] as well as neutrophils [65,66] recruit aerobic glycolysis to generate energy and biosynthetic intermediates within a shorter time period when compared with oxidative phosphorylation.

Notably, dynamic metabolic flux analysis revealed that MDSCs recruit glycolysis as the major metabolic pathway of glucose regardless of oxygen availability and activation status [5,67,68]. Thus, MDSCs were found to upregulate glycolytic genes, increase glucose uptake and reduce oxygen consumption during differentiation and activation [67]. Thereby, hypoxia-inducible-factor-1-alpha (HIF-1 $\alpha$ ) constitutes a key transcriptional factor that regulates the expression of glycolytic enzymes and promotes anaerobic glycolysis in MDSCs [69]. In addition, the mammalian target of rapamycin (mTOR) pathway has also been identified to increase glucose uptake and glycolytic enzyme expression [70]. Inhibiting MDSCs derived glycolysis with 2-

**Table 3**  
Overview of metabolic pathways involved in MDSC-derived immunosuppressive function on T cells.

	Metabolic pathway/ product	Regulated effector function
A	Glucose metabolism	Differentiation and proliferation under hypoxia PD-L1 expression Inhibition of CD4 proliferation Inhibition of CD8 IFN- $\gamma$ production
	HIF-1 $\alpha$	
	Sirt1/HIF-1 $\alpha$	
B	Phosphoenolpyruvate	M2 polarization IL-10 and TGF- $\beta$ expression Restraining ROS self-damage
	Lipid metabolism	
C	FAO	IL-10, PD-1, iNOS expression CD8 <sup>+</sup> suppression
	FA, AA, rbose	
	FAT 2/CD36	
	Amino acid metabolism	
	Arginase/iNOS	
	Arg depletion	
	NO release	
	IDO/Kyn	
	Trp depletion	
	Kyn, 3-HK, 3-HAA QUIN	
	Decreasing CD3 Expression Inhibition of T-cell proliferation Blocking T-cell Trafficking	
	Cell cycle arrest of T cells in G1 Inhibition of T-cell-derived IFN- $\gamma$ expression Increasing Treg frequencies Suppression of T-cell proliferation TH2 polarization Inhibition of T-cell IFN- $\gamma$ and TNF- $\alpha$ production	
	3005.66 3005.61 2150 2260 2003.74 6011.22 6011.22 4300 2260 2003.74 33011.19	

Overview of metabolic pathways and their downstream metabolite products of (A) glucose metabolism (B) lipid metabolism and (C) amino acid metabolism. Every subtable lists the regulated effector function of MDSCs on T cells regulated by the respective metabolic pathway.

HIF-1 $\alpha$ , hypoxia-inducible-factor-1-alpha; IDO, indoleamine 2,3-dioxygenase; IFN- $\gamma$ , interferon- $\gamma$ ; IL-10, interleukin-10; iNOS, inducible nitric oxide synthase; Kyn, kynurenine; M-MDSC, monocytic myeloid-derived suppressor cell; PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1; QUIN, quinolinic acid; ROS, reactive oxygen species; TGF- $\beta$ , transforming growth factor- $\beta$ .

deoxyglucose, in turn, has been shown to significantly impede the differentiation of MDSCs *in vitro* [15].

Hypoxia is the most important inducer of HIF-1 $\alpha$  [71] and during organ transplantation, limited arterial blood supply causes an ischemic state with tissue hypoxia that induces an anaerobic metabolism with increasing lactate levels and ROS production [72]. Meanwhile, chronic transplant vasculopathy leads to abnormal blood flow patterns with subsequent local hypoxia in long-term surviving organs [73]. HIF-1 $\alpha$ , upregulated in response to tissue hypoxia, has thereby been delineated as a cardinal factor dampening the pathological consequences of IRI inevitably accompanying organ transplantation [74], as well as a key feature of intraorgan microenvironment in chronic allograft injury [73]. HIF-1 $\alpha$  has namely been shown to improve mitochondrial respiratory function through the phosphoinositide 3-kinase/AKT and Janus kinase 2/STAT3 pathways [75] while mitigating cellular oxidative stress and ROS production through frataxin [76]. Notably, HIF-1 $\alpha$  has also been characterized as a key transcriptional factor that regulates the expression of glycolytic enzymes as well as lactate transporters and promotes glycolysis in MDSCs [69], thus accelerating differentiation and proliferation under organ hypoxia. In addition, HIF-1 $\alpha$  has been shown to increase the expression of PD-L1,

an immune checkpoint receptor, which mediates the inhibition of T cells by binding to the PD-L1 receptor of T cells. Moreover, acidic microenvironment caused by hypoxia has been characterized to increase the proliferation of MDSCs and strengthen their suppressive activity on T cells, compromising CD4<sup>+</sup> T-cell proliferation and CD8<sup>+</sup> T-cell-derived IFN- $\gamma$  production via the HIF-1 $\alpha$  pathway [63]. Furthermore, local tissue hypoxia activates SIRT1 in MDSCs, which in turn exerts agonistic effects with HIF-1 $\alpha$  deacetylating several of its downstream targets, thus mediating the differentiation of MDSCs towards a regulatory and immunosuppressive M2-phenotype. Of note, SIRT1 deficiency was found to impair M2-MDSCs with compromised glycolytic activity and lower arginase activity as well as IL-10 and TGF- $\beta$ 1 expression. These studies indicate that hypoxia/HIF-1 $\alpha$  signaling is a potent inducer of immunosuppression by enhancing glycolysis in MDSCs. Of note, aerobic glycolysis does not only work as an energy generation pathway for MDSCs, but also constitutes a critical regulator of redox homeostasis. Thus, phosphoenolpyruvate generated through augmented aerobic glycolysis, for instance, has been identified as glycolytic intermediate with antioxidant activity that restrains self-damage by ROS generated during the effector response of MDSCs [77], which may also interfere with the impact of exogenous ROS, deriving for instance from IRI following transplantation, on MDSCs (Table 2, A).

### Lipid Metabolism

Lipid metabolism constitutes a cardinal pathway for the suppressive function of MDSCs on the local microenvironment. The major pathway of cellular lipid metabolism is fatty acid  $\beta$ -oxidation (FAO). Studies in cancer showed that MDSCs, upon infiltrating the tumor and becoming activated, increased the uptake of lipids by the upregulation of the scavenger receptor CD36 through STAT3 and STAT5 signaling [70,78]. Peroxisome proliferator-activator receptors signaling pathway sense the increased level of fatty acids and regulate downstream lipid metabolic pathways [79]. Thus, mitochondrial electron transfer complex and the key enzymes in tricarboxylic acid cycle are upregulated, promoting FAO and TCA. These metabolic reprogramming events shift the primary source of energy generation from glycolysis to FAO. Indeed, compared with peripheral MDSCs, tumor-infiltrating MDSCs showed increased mitochondrial mass, oxygen consumption and FAO-derived ATP generation [78]. Notably, in MDSC, FAO also displays the primary source of biosynthetic intermediates for the generation of FA, AA and ribose, which are essential for further synthesis of anti-inflammatory molecules such as IL-10, PD-1 and iNOS. IL-10, in turn, has been dissected to exert strong immunosuppressive effects on allo-immune responses following transplantation [80]. Of note, FAO also serves as the cardinal resource for the expression of pro-inflammatory cytokines such as IL-2. However, MDSCs have been delineated to display a primarily anti-inflammatory secretome in transplantation with IL-10 representing a cardinal cytokine [16]. The lipid metabolism of MDSCs has furthermore been associated with a suppressive function on dendritic cells (DCs). Accumulated lipids in PMN-MDSCs are hereby oxidized through ROS and MPO and subsequently transferred to DCs inhibiting their antigen cross-presentation and their orientation on major histocompatibility complex class II in cancer [81]. This, in turn, blocks antigen-mediated cross-presentation and inhibits T-cell stimulation, which is of translational relevance for transplantation as antigen cross-presentation represents a crucial step in the initiation of T-cell allo-immune responses [82]. Of note, extracellular oxidized lipids can also be taken up by T cells through CD36, subsequently leading to T-cell exhaustion mediated by p38 signaling [83]. Clearance of cellular oxidized lipids in T cells by overexpression of glutathione peroxidase 4, in turn, has been shown to restore the effector function *in vivo* [79]. Supporting the importance of lipid metabolism for the immunosuppressive function of MDSCs was the observation that an inhibition



of FA uptake blocking fatty acid transporter 2 [84] or CD36 [85] through pharmacological inhibition or genetic deletion, abrogated the suppressive effects of MDSCs on CD8<sup>+</sup> T-cells (Table 2, B).

### Amino Acid (AA) Metabolism

AA metabolism represents a critical mechanism of MDSC effector function mediating T-cell immunosuppression since arginine, tryptophan and cysteine are essential amino acids for T-cell function. MDSCs can deplete these AA from the microenvironment and thus starve T cells, leading to impaired T-cell survival, proliferation and function. Moreover, several products of AA metabolism inhibit T-cell immune responses through different signal pathways.

MDSCs metabolize arginine through arginase-I and iNOS. Upon activation through IFN- $\gamma$ , MDSCs have been shown to take up large amounts of Arg by inducing the cationic AA transporter 2, Arg-1 and iNOS. The reduction of local Arg levels in turn leads to decreased T-cell-derived CD3 $\zeta$  expression, thus compromising T-cell antigen-specific proliferation [86]. In addition, increased levels of NO released by iNOS in MDSCs further exert significant suppressive effects on T-cell function [32,87]. Thus, MDSCs have been shown to inhibit alloreactive T-cell expansion via iNOS, thus promoting allograft survival of kidney transplants [18]. Similarly, adoptive transfer of tumor necrosis factor- $\alpha$ -induced MDSCs prevented allograft rejection in male-to-female skin transplants through inhibiting T-cell proliferation that was abrogated in the presence of an NO-inhibitor or following genetic-depletion of NO [18]. Notably, PMN-MDSCs and M-MDSCs display divergent features of Arg metabolism with PMN-MDSCs expressing greater levels of Arg-1, and M-MDSCs exhibiting higher levels of iNOS [68].

Indoleamine 2, 3-dioxygenase (IDO) is the key enzyme of tryptophan metabolism in the kynurenine (Kyn) pathway. MDSCs have been shown to exhibit an increased IDO expression and augmented Kyn pathway causing a metabolization of Trp from the surrounding microenvironment associated with decreased Trp levels [88]. T cells in turn have been found highly sensitive for Trp shortage causing their cell-cycle arrest in the G1 phase [89], thus compromising T-cell-derived immune responses. Indeed, IDO expression was found to be critical, inhibiting T-cell-driven allo-immune responses with abrogated long-term cardiac allograft survival in CTLA4-Ig-treated mice deficient for IDO [90]. Moreover, genetically induced IDO overexpression has been shown to suppress allograft rejection of cardiac allografts [91] and small bowel transplants [92] in mice through inhibiting T-cell-derived IFN- $\gamma$  expression and increased Treg frequencies.

Noteworthy, recent studies have demonstrated an entwined pathway of arginine and tryptophan immunometabolism, leading to immunosuppressive function of DCs, which can be mediated by Arg1<sup>+</sup>-MDSCs. Hereby, IDO1 phosphorylation and consequent activation of immunosuppressive, non-enzymatic IDO1 signaling in DCs was dependent on previous expression of Arg1 and Arg1-dependent production of polyamines which in turn can be released by MDSCs [93]. Thus, joint regulation of arginine and tryptophan metabolism could represent an important target for regulating immunomodulatory effects of MDSCs.

Of additional relevance, downstream products of the Trp metabolism display bioactive compounds that further restrains T-cell derived immune response. Thus, Kyn, 3-hydroxykynurenine and 3-hydroxyanthranilic acid (3-HAA) were shown to strongly suppress T-cell proliferation in mixed leukocyte reactions leading to a prolonged rat skin allograft survival [94]. Similarly, 3-HAA also prevented the rejection of rat cardiac allografts diminishing the proliferation of T cells that could not be restimulated by donor-specific DCs [95]. Moreover, 3-hydroxykynurenine and 3-HAA were shown to compromise T-cell proliferation, thus preventing T-cell-induced kidney allograft rejection and protect the tubular epithelial cell from injury in pigs [96].

Quinolinic acid, another immunoregulatory metabolite from Kyn pathway [97], has been shown to shift the human immune balance towards a regulatory Th2-dominated phenotype while inhibiting Th1-cell-derived INF- $\gamma$  and tumor necrosis factor- $\alpha$  production through agonistic effects on D-methyl-D-aspartate receptors [98]. Notably, TH-1-cell-derived INF- $\gamma$  production has been identified as the major driver of acute rejection [99] whereas TH2-cells are accredited an immune regulatory function in transplantation [100,101] (Table 2, C).

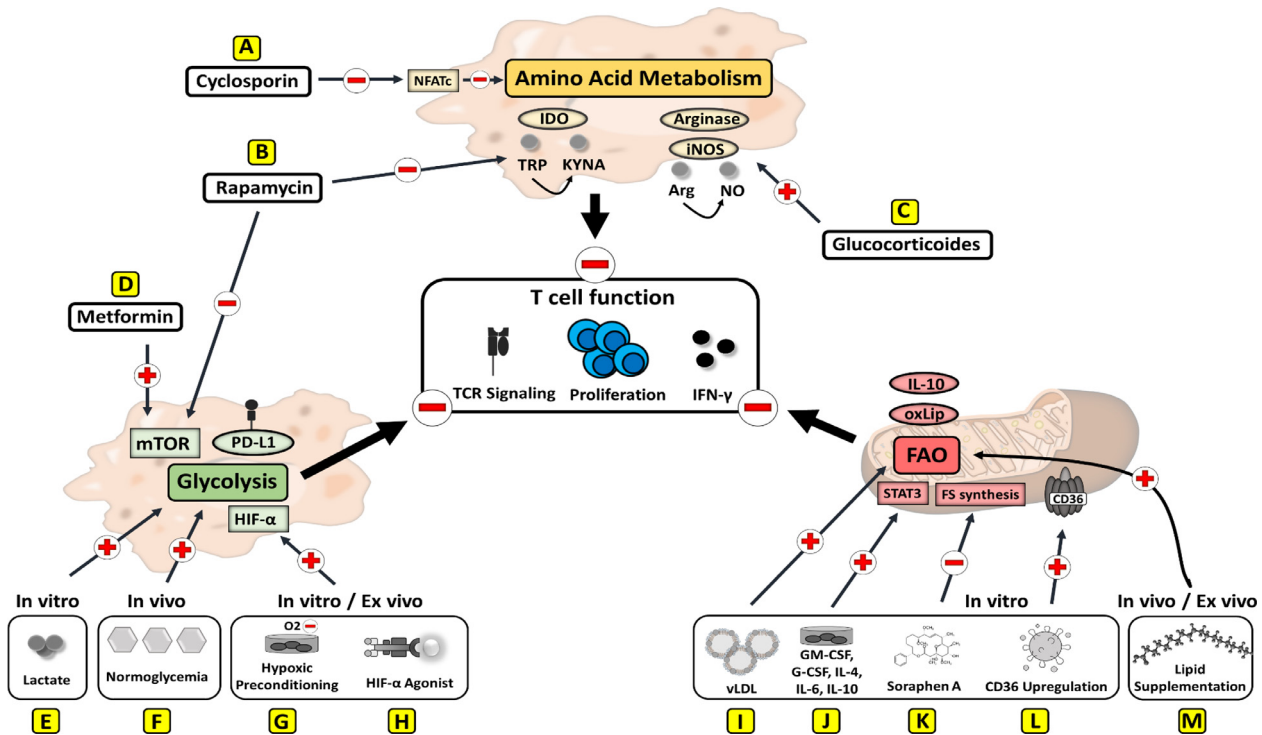
### Effects of Immunosuppressants on MDSC Metabolic Reprogramming

The calcineurin inhibitors, CsA and tacrolimus, constitute the backbone of the current first-line immunosuppressive regimen. Experimentally, *in vitro* and *in vivo* data from murine transplant models showed that CsA promoted MDSCs proliferation and enhanced their immunosuppressive function on alloreactive T cells [19,20]. Hereby, the calcineurin-NFATc pathway is the primary target of CsA regulating AA metabolism of MDSCs through regulating IDO and iNOS. Notably, inhibition of NFAT by CsA in MDSCs up-regulated IDO enhancing Trp metabolism as well as production the downstream immunosuppressive metabolite KYNA (Figure 1A). Thus, CsA treated MDSCs compromised both CD4<sup>+</sup>- as well as CD8<sup>+</sup>-T-cell-derived INF- $\gamma$  production, increased Treg differentiation and promoted allograft survival when compared to PBS treated MDSCs in a model of murine skin transplantation [19]. Of note, impeding IDO expression with an IDO-inhibitor reversed the augmented immunosuppressive capacity of CsA treated MDSCs on T cells [19].

Moreover, CSA treatment of MDSCs increased levels of iNOS which convert Arg to immunosuppressive NO, thus restraining CD8<sup>+</sup>- and CD4<sup>+</sup> T-cell differentiation while prolonging survival of murine skin grafts [20] (Figure 1A). Regarding tacrolimus, MDSCs isolated from tacrolimus-treated lung transplantation recipients were able to inhibit T-cell proliferation *in vitro* [8]. However, it remains to be investigated whether tacrolimus affects MDSCs AA metabolism via NFAT signaling similar to CsA.

RPM, an mTOR-specific inhibitor, has been broadly established for the treatment of allograft rejection following kidney transplantation [102]. In addition to its efficient effects on T-cell subsets [103], RPM has been found to regulate innate immune homeostasis also impacting MDSCs in transplantation. Thereby, RPM inhibited MDSC differentiation, proliferation and immunosuppressive effects on T cells through directly impeding glycolysis with down-regulated glycolytic enzymes hexokinase 2, phosphofructokinase 1, pyruvate kinase muscle as well as the transporter Glut1, both *in vitro* in sorted granulocyte-macrophage (GM)-colony-stimulating factor (CSF)-induced M-MDSCs and *in vivo* in induced M-MDSCs of AlloSkin-grafted mice [17] (Figure 1B). Similarly, genetically altered mice deficient for mTOR also showed decreased levels of M-MDSCs in skin-grafts as well as draining lymph nodes in addition to compromised inhibitory effects on CD4<sup>+</sup>- as well as CD8<sup>+</sup>-T-cell proliferation. Moreover, RPM also inhibited iNOS and Arg-1 as well as HO-1, IDO and NOX2 expression underlying the impaired immunosuppressive function of MDSCs on T-cells [17] (Figure 1B). Consistent with these experimental data, a clinical study reported that MDSCs isolated from kidney transplant recipients that had been treated with RPM failed to inhibit T-cell proliferation through decreased iNOS activity [8]. Of note, RPM also increased MDSCs recruitment to murine cardiac allografts leading to prolonged allograft survival [21].

Exogenous glucocorticoid (GC) doses positively correlated with the level of MDSCs in human transplant recipients [22]. In murine experiments, GC prolonged allograft by enhancing MDSCs recruitment and suppressive function [23]. Mechanistically, GCs augment the expression of iNOS and NO in MDSCs following binding of the GC receptor, thus impeding T-cell function [23] (Figure 1C). In a recent



**Figure 1.** Treatment approaches and targets of metabolic reprogramming of MDSCs in transplantation. The outlined three metabolic pathways involved in the immunosuppressive function on T cells (TCR signaling, proliferation and IFN- $\gamma$ ) and proliferation/differentiation of MDSCs can be upregulated through diverse approaches in the context of transplantation while immunosuppressive drugs may cause significant interference. AA metabolism: (A) Cyclosporin inhibits NFATc, which revokes its inhibitory effect on AA metabolism and promotes IDO and iNOS expression. (B) Rapamycin inhibits IDO expression and mTOR signaling, thus restraining glycolysis. (C) Glucocorticoids promote iNOS expression. Glycolysis: (D) Treatment with metformin exerts antagonistic effects on mTOR as rapamycin, promoting glycolysis both *in vitro* and *in vivo*. (E) Exogenous lactate increases MDSCs glycolysis and proliferation *in vitro*. (F) Following transplantation normoglycemia could ensure sufficient glycolysis of MDSCs. (G) Hypoxic preconditioning of MDSCs during cell culture or organs during *ex vivo* organ perfusion may promote HIF- $\alpha$ -mediated glycolysis. (H) HIF- $\alpha$  agonists can be recruited during *in vitro* cell culture of MDSCs or during *ex vivo* organ perfusion. Fatty acid oxidation: (I) Subjecting MDSCs to very low-density lipoproteins (VLDL) *in vitro* could augment FAO, leading to increased immunosuppressive effects on T cells. (J) Targeting STAT3 through optimizing culture conditions with different cytokines could augment FAO via increased lipid uptake. (K) Inhibiting fatty acid synthesis for instance with soraphen A may cause a compensatory upregulation of fatty acid uptake. (L) Viral transduction to artificially increase CD36 expression may promote lipid uptake. (M) Supplementing lipids as a diet to transplant recipients or during *ex vivo* organ perfusion may promote lipid uptake in MDSCs.

study, this effect was further attributed to an inhibition of HIF-1 $\alpha$ -driven glycolysis upon activation of MDSCs. However further insights on metabolic changes such as the lipid metabolism that is known to display the major energy source upon MDSCs activation had not been explored [104].

Costimulatory receptors are a class of molecules expressed by T cells regulating activation and generation of effector T-cell responses. Blockade of costimulatory signals represents a promising strategy to control T-cell responses. As the most recognized molecule, CTLA4-Ig has been shown to dampen activation of naïve T cells by blocking costimulation via CD28 [105]. Murine experiments and clinical trials have demonstrated the efficacy of CTLA4-Ig in the treatment of acute rejection [106–108]. Notably, in a randomized controlled trial in patients with advanced-stage melanoma, CTLA4-Ig treatment was found to decrease PMN-MDSC levels and compromise Arg1 expression [109]. However, the underlying mechanism of this effect remained unclear.

### Metabolic Optimization of MDSCs for Transplantation

Besides inhibiting the rejection of allografts following transplantation, mechanistic animal studies have further delineated MDSCs as essential component for the development and maintenance of immune tolerance [12]. Thus, MDSCs have emerged as a novel, cellular treatment opportunity to improve outcomes of transplant recipients. However, preclinical studies with MDSCs failed to achieve durable transplant tolerance [4,17] and haven't been any reports concerning MDSCs infusion in human transplant recipients.

Since metabolic reprogramming has been identified to crucially mediate differentiation, proliferative expansion and the immunosuppressive effects of MDSCs, manipulating or supplementing cardinal metabolic pathways may augment MDSCs function and improve treatment approaches. As a proof of concept, targeting metabolic reprogramming in other cellular therapies could improve the survival and expansion of *in vitro*-cultured cells [110–112].

Unlike cancer research, which aims to inhibit the suppressive function of MDSCs and enhance T-cell-derived anti-tumor immune responses through metabolic reprogramming, transplantation requires the opposite approach. Targeting MDSCs metabolism during transplantation can be achieved *in vitro* before adoptive transfer by altering the culture conditions or by genetic modification. Moreover, *in vivo* drug treatments and diet alterations could be recruited while *ex vivo* organ perfusion offers a treatment approach between ex- and implantation.

As described previously, MDSCs are highly dependent on increasing their glycolytic activity to differentiate and expand. In a tumor derived microenvironment hypoxia and excessive lactate levels force adjacent cells to adapt their metabolism with MDSCs upregulating their aerobic glycolysis. Thus, promoting MDSCs derived glycolysis *in vitro* may increase proliferation and survival before cellular therapy. Indeed, subjecting MDSCs to metformin that enhances the glycolytic pathway through augmenting glucose uptake while compromising mitochondrial respiration chain activity through an 5' AMP-activated protein kinase-dependent and -independent pathway [113] led to increased numbers of MDSCs during granulocyte (G)-CSF-mediated induction [15]. This mechanism has also been targeted *in vivo* with

translational relevance for clinical application. Namely, allograft-transplanted mice subjected to RPM displayed reduced MDSC frequencies with impeded immunosuppressive effects on T cells through compromised glycolysis, which was reversed upon treatment with metformin [15] (Figure 1D). Of note, metformin has been shown to improve immune homeostasis through reduced Th17-cell and increased Treg frequencies in kidney transplant recipients treated with tacrolimus [114]. Exogenous lactate as a product of glycolysis has been shown to increase the percentage of MDSCs derived from *in vitro*-cultured mouse bone marrow cells stimulated with GM-CSF and IL-6, which may improve culture conditions to expand MDSCs generation (Figure 1E). At the same time, MDSCs have also been shown to depend on sufficient glucose supply in order to exert glycolytic activity *in vivo*. Namely, depletion of glucose levels using a ketogenic diet to lower lactate production by glycolytic tumors resulted in smaller tumors, decreased MDSC frequency, and improved T-cell immune responses [115]. Diets for patients following transplantation should therefore ensure sufficient glucose supply with normoglycemia (Figure 1F). Notably, further studies have delineated stronger immunosuppressive capacity of MDSCs when stimulated with lactate via the HIF-1 $\alpha$  pathway [115].

HIF-1 $\alpha$ , in turn, constitutes a key transcriptional factor that regulates the expression of glycolytic enzymes and promotes glycolysis in MDSCs [69]. HIF-1 $\alpha$  can be induced *in vitro* through hypoxic preconditioning of cells, thus providing another opportunity to boost MDSCs differentiation and function (Figure 1G). Preconditioning of mesenchymal stroma cells in hypoxic conditions prior to transplantation for instance, has been shown to improve cell survival through a metabolic switch towards a more glycolytic state mediated by HIF-1 $\alpha$  activation [116]. Moreover, augmenting MDSCs derived HIF-1 $\alpha$ -mediated glycolysis could also be performed through pharmacological stabilization. In support, treating mice subjected to IRI with GSK360A that inhibits the prolyl hydroxylase domain-containing enzyme led to an increased expression of HIF-1 $\alpha$ -regulated genes such as pyruvate dehydrogenase kinase-1 and hexokinase II, thus comprising oxidative stress (Figure 1H). Equitable results were achieved *in vitro* treating a cardiac cell line [74]. Notably, drugs stabilizing HIF-1 $\alpha$  or exerting agonistic effects are already clinically available or tested in trials [117]. These drugs could either be applied *in vitro* to MDSCs before cellular therapy to boost differentiation and proliferation; to transplant recipients potentially exerting dichotomous, beneficial effects on both, IRI and MDSC function; or during *ex vivo* organ perfusion. Thereby, allograft resident MDSCs could be manipulated either through decreasing local oxygen delivery or treating organs with HIF-1 $\alpha$  agonistic drugs and via increasing HIF-1 $\alpha$  levels (Figure 1G and 1H).

At least, increasing FAO of MDSCs through altering culture conditions or genetic modification of MDSCs during *in vitro* expansion could display another approach to improve their immunosuppressive function. Strikingly, subjecting murine and human MDSCs to very low-density lipoproteins promoted iNOS expression and augmented the suppressive effects on T-cell proliferation [85]. The expression of the fatty acid translocase CD36 mediating FA uptake has hereby been identified as a crucial mediator [85]. Of note, CD36 expression is regulated through STAT3 [118]. Thus, augmenting MDSCs FAO could be orchestrated by expanding MDSCs in the presence of very low-density lipoprotein, which may also promote the immunosuppressive function of these cells (Figure 1I). Moreover, culture conditions could be further optimized through supplementing a combination of GM-CSF, G-CSF, IL-4, IL-6, IL-10 and vascular endothelial growth factor that are recognized as the most important inducers of STAT3 in MDSCs [119], thus inducing CD36 expression and improving lipid uptake (Figure 1J). Furthermore, inhibiting the *de novo* synthesis of fatty acids, conversely increasing FAO, with small molecules such as soraphen A that impedes the acetyl-CoA carboxylase 1 and 2 may present an additional option (Figure 1K). Of note, disruption of fatty

acid synthesis has been shown to prevent acute graft-versus-host diseases with diminished T-cell proliferation and augmented Treg frequencies [120]. At least genetic manipulation of MDSCs through lentiviral transduction could be employed to induce durable CD36 expression that may improve lipid uptake *in vivo* after adoptive transfer (Figure 1L). Supplementation of fatty acids to either transplant recipients or allografts during *ex vivo* organ perfusion may constitute a clinically implementable therapeutic approach to increase FA levels and improve recipient or donor derived MDSCs function, respectively (Figure 1M).

## Concluding Remarks

Metabolic reprogramming constitutes a mechanistic underpinning of MDSCs immunosuppressive effector functions in the microenvironment of solid malignant tumors. From a clinical perspective, the detrimental immunosuppressive effects of MDSCs in cancer translate into beneficial effects in transplantation regulating allo-immune responses and prolonging allograft survival. Metabolic reprogramming of MDSCs may thus also be crucially affected in the inflammatory microenvironment of allografts, IRI and immunosuppressive drugs. However, thus far experimental studies investigating the relevance of metabolic reprogramming of MDSCs in the context of transplantation have been limited, and further investigations are required to confirm the outlined mechanisms in both pre-clinical experimental models and human studies. Moreover, despite axiomatic similarities, the tumor microenvironment differs from that of a transplanted organ undergoing IRI or acute rejections episodes, which needs to be considered when evaluating the transferability of mechanistic insights deriving from cancer studies.

With a view on future perspectives, the metabolic characteristics of MDSCs in transplantation require further investigation to evaluate the potential of targeting those pathways for tolerance induction. Therefore, experimental animal transplant models with integrative omics combining transcriptomics, proteomics and metabolomics could serve to evaluate the expression of metabolic enzymes as well as metabolite levels and evaluate the proportional interplay of metabolic pathways during metabolic reprogramming in the context of transplantation.

Targeting metabolic reprogramming of MDSCs either *in vitro*, *in vivo* or during *ex vivo* organ perfusion may ultimately provide novel treatment approaches to improve MDSCs derived tolerance induction or MDSCs based cell therapy approaches.

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The authors have no commercial, proprietary or financial interest in the products or companies described in this article.

## Author Contributions

Conception and design of the study: JI and YN. Drafting and revising manuscript: YC, MR, SZS. All authors have approved the final article.

## References

- [1] Leventhal JR, Mathew JM. Outstanding questions in transplantation: tolerance. *American journal of transplantation* 2020;20:348–54.



- [2] Adams DH, Sanchez-Fueyo A, Samuel D. From immunosuppression to tolerance. *Journal of hepatology* 2015;62:5170–85.
- [3] Bhatti AB, Usman M. Chronic renal transplant rejection and possible anti-proliferative drug targets. *Cureus* 2015;7:e376.
- [4] Lee YS, et al. Myeloid-derived suppressor cells expand after transplantation and their augmentation increases graft survival. *Am J Transplant* 2020;20:2343–55.
- [5] Veglia F, Sanseviero E, Gabrilovich DI. Myeloid-derived suppressor cells in the era of increasing myeloid cell diversity. *Nat Rev Immunol* 2021;21:485–98.
- [6] Nakamura T, Ushigome H. Myeloid-derived suppressor cells as a regulator of immunity in organ transplantation. *Int J Mol Sci* 2018;19.
- [7] Scalea JR, Lee YS, Davila E, Bromberg JS. Myeloid-derived suppressor cells and their potential application in transplantation. *Transplantation* 2018;102:359–67.
- [8] Iglesias-Escudero M, et al. Myeloid-derived suppressor cells in kidney transplant recipients and the effect of maintenance immunotherapy. *Front Immunol* 2020;11:643.
- [9] Nakamura T, Nakao T, Ashihara E, Yoshimura N. Myeloid-derived suppressor cells recruit CD4(+)Foxp3(+) regulatory T cells in a murine cardiac allograft. *Transplant Proc* 2016;48:1275–8.
- [10] Marigo I, et al. Tumor-induced tolerance and immune suppression depend on the C/EBPbeta transcription factor. *Immunity* 2010;32:790–802.
- [11] Fujimoto K, et al. Analysis of therapeutic potential of monocytic myeloid-derived suppressor cells in cardiac allotransplantation. *Transpl Immunol* 2021;67:101405.
- [12] Garcia MR, et al. Monocytic suppressive cells mediate cardiovascular transplantation tolerance in mice. *J Clin Invest* 2010;120:2486–96.
- [13] Luan Y, et al. Monocytic myeloid-derived suppressor cells accumulate in renal transplant patients and mediate CD4(+) Foxp3(+) Treg expansion. *Am J Transplant* 2013;13:3123–31.
- [14] Lv M, et al. Monocytic and promyelocytic myeloid-derived suppressor cells may contribute to G-CSF-induced immune tolerance in haplo-identical allogeneic hematopoietic stem cell transplantation. *Am J Hematol* 2015;90:E9–e16.
- [15] Wu T, et al. mTOR masters monocytic myeloid-derived suppressor cells in mice with allografts or tumors. *Sci Rep* 2016;6:20250.
- [16] De Wilde V, et al. Endotoxin-induced myeloid-derived suppressor cells inhibit alloimmune responses via heme oxygenase-1. *Am J Transplant* 2009;9:2034–47.
- [17] Dugast AS, et al. Myeloid-derived suppressor cells accumulate in kidney allograft tolerance and specifically suppress effector T cell expansion. *J Immunol* 2008;180:7898–906.
- [18] Yang F, et al. TNF $\alpha$ -induced M-MDSCs promote transplant immune tolerance via nitric oxide. *J Mol Med (Berl)* 2016;94:911–20.
- [19] Wang X, et al. The calcineurin-NFAT axis controls allograft immunity in myeloid-derived suppressor cells through reprogramming T cell differentiation. *Mol Cell Biol* 2015;35:598–609.
- [20] Han C, et al. The effect of immunosuppressive drug cyclosporine A on myeloid-derived suppressor cells in transplanted mice. *Inflamm Res* 2016;65:679–88.
- [21] Nakamura T, Nakao T, Yoshimura N, Ashihara E, Rapamycin prolongs cardiac allograft survival in a mouse model by inducing myeloid-derived suppressor cells. *Am J Transplant* 2015;15:2364–77.
- [22] Okano S, et al. Myeloid-derived suppressor cells increase and inhibit donor-reactive T cell responses to graft intestinal epithelium in intestinal transplant patients. *American journal of transplantation* 2018;18:2544–58.
- [23] Liao J, et al. Dexamethasone potentiates myeloid-derived suppressor cell function in prolonging allograft survival through nitric oxide. *J Leukoc Biol* 2014;96:675–84.
- [24] Fiore A, et al. Induction of immunosuppressive functions and NF- $\kappa$ B by FLIP in monocytes. *Nat Commun* 2018;9:5193.
- [25] Bettencourt IA, Powell JD. Targeting metabolism as a novel therapeutic approach to autoimmunity, inflammation, and transplantation. *Journal of immunology (Baltimore, Md. : 1950)* 2017;198:999–1005.
- [26] Pålsson-McDermott EM, O'Neill LAJ. Targeting immunometabolism as an anti-inflammatory strategy. *Cell research* 2020;30:300–14.
- [27] Wei Y, et al. Metabolic reprogramming of immune cells at the maternal–fetal interface and the development of techniques for immunometabolism. *Frontiers in immunology* 2021;12:717014.
- [28] Kelly B, Tannahill GM, Murphy MP, O'Neill LA. Metformin inhibits the production of reactive oxygen species from NADH:Ubiquinone oxidoreductase to limit induction of interleukin-1 $\beta$  (IL-1 $\beta$ ) and boosts interleukin-10 (IL-10) in lipopolysaccharide (LPS)-activated macrophages. *J Biol Chem* 2015;290:20348–59.
- [29] Gross CC, et al. Dimethyl fumarate treatment alters circulating T helper cell subsets in multiple sclerosis. *Neurol Neuroimmunol Neuroinflamm* 2016;3:e183.
- [30] Lee CF, et al. Preventing allograft rejection by targeting immune metabolism. *Cell Rep* 2015;13:760–70.
- [31] Nian Y, et al. Targeting age-specific changes in CD4(+) T cell metabolism ameliorates alloimmune responses and prolongs graft survival. *Aging cell* 2021;20:e13299.
- [32] Cheng CH, et al. Targeting metabolism as a platform for inducing allograft tolerance in the absence of long-term immunosuppression. *Frontiers in immunology* 2020;11:572.
- [33] Hegde S, Leader AM, Merad M. MDSC: markers, development, states, and undressed complexity. *Immunity* 2021;54:875–84.
- [34] Bronte V, et al. Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. *Nat Commun* 2016;7:12150.
- [35] Guha P, et al. Monocytic and granulocytic myeloid-derived suppressor cell plasticity and differentiation are organ-specific. *Oncogene* 2021;40:693–704.
- [36] Jayaraman P, et al. Tumor-expressed inducible nitric oxide synthase controls induction of functional myeloid-derived suppressor cells through modulation of vascular endothelial growth factor release. *J Immunol* 2012;188:5365–76.
- [37] Raber PL, et al. Subpopulations of myeloid-derived suppressor cells impair T cell responses through independent nitric oxide-related pathways. *Int J Cancer* 2014;134:2853–64.
- [38] Harari O, Liao JK. Inhibition of MHC II gene transcription by nitric oxide and antioxidants. *Current pharmaceutical design* 2004;10:893–8.
- [39] Gabrilovich DI. Myeloid-derived suppressor cells. *Cancer immunology research* 2017;5:3–8.
- [40] Rodriguez PC, et al. Arginase 1-producing myeloid-derived suppressor cells in renal cell carcinoma are a subpopulation of activated granulocytes. *Cancer research* 2009;69:1553–60.
- [41] Srivastava MK, Sinha P, Clements VK, Rodriguez P, Ostrand-Rosenberg S. Myeloid-derived suppressor cells inhibit T-cell activation by depleting cystine and cysteine. *Cancer research* 2010;70:68–77.
- [42] De Sanctis F, et al. Interrupting the nitrosative stress fuels tumor-specific cytotoxic T lymphocytes in pancreatic cancer. *J Immunother Cancer* 2022;10.
- [43] De Sanctis F, et al. The emerging immunological role of post-translational modifications by reactive nitrogen species in cancer microenvironment. *Front Immunol* 2014;5:69.
- [44] Konieczny BT, et al. IFN-gamma is critical for long-term allograft survival induced by blocking the CD28 and CD40 ligand T cell costimulation pathways. *J Immunol* 1998;160:2059–64.
- [45] Meng F, et al. Clinical significance of myeloid-derived suppressor cells in human renal transplantation with acute T cell-mediated rejection. *Inflammation* 2014;37:1799–805.
- [46] Dilek N, Vuillefroy de Sully R, Blanche G, Vanhove B. Myeloid-derived suppressor cells: mechanisms of action and recent advances in their role in transplant tolerance. *Front Immunol* 2012;3:208.
- [47] Gong W, et al. Role of myeloid-derived suppressor cells in mouse pre-sensitized cardiac transplant model. *Clin Immunol* 2014;153:8–16.
- [48] Ku AW, et al. Tumor-induced MDSC act via remote control to inhibit L-selectin-dependent adaptive immunity in lymph nodes. *eLife* 2016;5.
- [49] Li J, et al. CD39/CD73 upregulation on myeloid-derived suppressor cells via TGF- $\beta$ -mTOR-HIF-1 signaling in patients with non-small cell lung cancer. *Oncimmunology* 2017;6:e1320011.
- [50] Zhu J, et al. Resistance to cancer immunotherapy mediated by apoptosis of tumor-infiltrating lymphocytes. *Nature communications* 2017;8:1404.
- [51] Baumann T, et al. Regulatory myeloid cells paralyze T cells through cell-cell transfer of the metabolite methylglyoxal. *Nature immunology* 2020;21:555–66.
- [52] Faubert B, Solmonson A, DeBerardinis RJ. Metabolic reprogramming and cancer progression. *Science* 2020;368.
- [53] Hu C, et al. Immune cell metabolism and metabolic reprogramming. *Mol Biol Rep* 2022; 1–13.
- [54] Stanimirova I, et al. Serum metabolomics approach to monitor the changes in metabolite profiles following renal transplantation. *Sci Rep* 2020;10:17223.
- [55] Wedel J, et al. The intragraft microenvironment as a central determinant of chronic rejection or local immunoregulation/tolerance. *Curr Opin Organ Transplant* 2017;22:55–63.
- [56] Zhang W, et al. Myeloid-derived suppressor cells in transplantation: the dawn of cell therapy. *J Transl Med* 2018;16:19.
- [57] Li X, et al. Metabolic reprogramming of myeloid-derived suppressor cells: an innovative approach confronting challenges. *Journal of leukocyte biology* 2021;110:257–70.
- [58] Jackson JJ, et al. Potent GCN2 inhibitor capable of reversing MDSC-driven T cell suppression demonstrates in vivo efficacy as a single agent and in combination with anti-angiogenesis therapy. *J Med Chem* 2022;65:12895–924.
- [59] Hui S, et al. Glucose feeds the TCA cycle via circulating lactate. *Nature* 2017;551:115–8.
- [60] Zhang X, et al. Metabolic substrate utilization in stress-induced immune cells. *Intensive Care Med Exp* 2020;8:28.
- [61] Pearce EL, Pearce EJ. Metabolic pathways in immune cell activation and quiescence. *Immunity* 2013;38:633–43.
- [62] Gubser PM, et al. Rapid effector function of memory CD8+ T cells requires an immediate-early glycolytic switch. *Nat Immunol* 2013;14:1064–72.
- [63] Chang CH, et al. Posttranscriptional control of T cell effector function by aerobic glycolysis. *Cell* 2013;153:1239–51.
- [64] Liu Y, et al. Metabolic reprogramming in macrophage responses. *Biomark Res* 2021;9:1.
- [65] Sharra AJ, Karnovsky ML. The biochemical basis of phagocytosis. I. Metabolic changes during the ingestion of particles by polymorphonuclear leukocytes. *J Biol Chem* 1959;234:1355–62.
- [66] Fossati G, et al. The mitochondrial network of human neutrophils: role in chemotaxis, phagocytosis, respiratory burst activation, and commitment to apoptosis. *J Immunol* 2003;170:1964–72.
- [67] Goffaux G, Hammami I, Jolicoeur M. A dynamic metabolic flux analysis of myeloid-derived suppressor cells confirms immunosuppression-related metabolic plasticity. *Scientific reports* 2017;7:9850.
- [68] Hu C, Pang B, Lin G, Zhen Y, Yi H. Energy metabolism manipulates the fate and function of tumour myeloid-derived suppressor cells. *British journal of cancer* 2020;122:23–9.
- [69] Corcoran SE, O'Neill LA. HIF1 $\alpha$  and metabolic reprogramming in inflammation. *The Journal of clinical investigation* 2016;126:3699–707.



- [70] Deng Y, et al. mTOR-mediated glycolysis contributes to the enhanced suppressive function of murine tumor-infiltrating monocytic myeloid-derived suppressor cells. *Cancer immunology, immunotherapy: CII* 2018;67:1355–64.
- [71] Ziello JE, Jovin IS, Huang Y. Hypoxia-inducible factor (HIF)-1 regulatory pathway and its potential for therapeutic intervention in malignancy and ischemia. *Yale J Biol Med* 2007;80:51–60.
- [72] Fernández AR, Sánchez-Tarjuelo R, Cravedi P, Ochando J, López-Hoyos M. Review: ischemia reperfusion injury—a translational perspective in organ transplantation. *Int J Mol Sci* 2020;21.
- [73] Bruneau S, et al. Key features of the intragraft microenvironment that determine long-term survival following transplantation. *Front Immunol* 2012;3:54.
- [74] Ong SG, et al. HIF-1 reduces ischaemia-reperfusion injury in the heart by targeting the mitochondrial permeability transition pore. *Cardiovasc Res* 2014;104:24–36.
- [75] Fuhrmann DC, Brüne B. Mitochondrial composition and function under the control of hypoxia. *Redox Biol* 2017;12:208–15.
- [76] Nanayakkara G, et al. Cardioprotective HIF-1 $\alpha$ -frataxin signaling against ischemia-reperfusion injury. *Am J Physiol Heart Circ Physiol* 2015;309:H867–79.
- [77] Jian SL, et al. Glycolysis regulates the expansion of myeloid-derived suppressor cells in tumor-bearing hosts through prevention of ROS-mediated apoptosis. *Cell death & disease* 2017;8:e2779.
- [78] Hossain F, et al. Inhibition of fatty acid oxidation modulates immunosuppressive functions of myeloid-derived suppressor cells and enhances cancer therapies. *Cancer immunology research* 2015;3:1236–47.
- [79] Liu Y, et al. The role of PPAR- $\delta$  in metabolism, inflammation, and cancer: many characters of a critical transcription factor. *International journal of molecular sciences* 2018;19.
- [80] Bromberg JS. IL-10 immunosuppression in transplantation. *Curr Opin Immunol* 1995;7:639–43.
- [81] Ugolini A, et al. Polymorphonuclear myeloid-derived suppressor cells limit antigen cross-presentation by dendritic cells in cancer. *JCI Insight* 2020;5.
- [82] Alegre ML, Lakkis FG, Morelli AE. Antigen presentation in transplantation. *Trends Immunol* 2016;37:831–43.
- [83] Xu S, et al. Uptake of oxidized lipids by the scavenger receptor CD36 promotes lipid peroxidation and dysfunction in CD8(+) T cells in tumors. *Immunity* 2021;54:1561–1577.e1567.
- [84] Veglia F, et al. Fatty acid transport protein 2 reprograms neutrophils in cancer. *Nature* 2019;569:73–8.
- [85] Al-Khami AA, et al. Exogenous lipid uptake induces metabolic and functional reprogramming of tumor-associated myeloid-derived suppressor cells. *Oncoimmunology* 2017;6:e1344804.
- [86] Norian LA, et al. Tumor-infiltrating regulatory dendritic cells inhibit CD8+ T cell function via L-arginine metabolism. *Cancer Res* 2009;69:3086–94.
- [87] Feng S, et al. Myeloid-derived suppressor cells inhibit T cell activation through nitrating LCK in mouse cancers. *Proc Natl Acad Sci U S A* 2018;115:10094–9.
- [88] Yu J, et al. Myeloid-derived suppressor cells suppress antitumor immune responses through IDO expression and correlate with lymph node metastasis in patients with breast cancer. *J Immunol* 2013;190:3783–97.
- [89] Munn DH, et al. Inhibition of T cell proliferation by macrophage tryptophan catabolism. *J Exp Med* 1999;189:1363–72.
- [90] Sucher R, et al. IDO and regulatory T cell support are critical for cytotoxic T lymphocyte-associated Ag-4 Ig-mediated long-term solid organ allograft survival. *J Immunol* 2012;188:37–46.
- [91] Yu G, et al. Gene delivery of indoleamine 2,3-dioxygenase prolongs cardiac allograft survival by shaping the types of T-cell responses. *J Gene Med* 2008;10:754–61.
- [92] Xie FT, et al. IDO expressing dendritic cells suppress allograft rejection of small bowel transplantation in mice by expansion of Foxp3+ regulatory T cells. *Transpl Immunol* 2015;33:69–77.
- [93] Mondanelli G, et al. A relay pathway between arginine and tryptophan metabolism confers immunosuppressive properties on dendritic cells. *Immunity* 2017;46:233–44.
- [94] Bauer TM, et al. Studying the immunosuppressive role of indoleamine 2,3-dioxygenase: tryptophan metabolites suppress rat allogeneic T-cell responses in vitro and in vivo. *Transpl Int* 2005;18:95–100.
- [95] Dai X, Zhu BT. Suppression of T-cell response and prolongation of allograft survival in a rat model by tryptophan catabolites. *Eur J Pharmacol* 2009;606:225–32.
- [96] Lassiter R, Merchen TD, Fang X, Wang Y. Protective role of kynurenine 3-mono-oxygenase in allograft rejection and tubular injury in kidney transplantation. *Front Immunol* 2021;12:671025.
- [97] Jennings MR, Munn D, Blazeck J. Immunosuppressive metabolites in tumoral immune evasion: redundancies, clinical efforts, and pathways forward. *Journal for immunotherapy of cancer* 2021;9.
- [98] Orihara K, et al. Neurotransmitter signalling via NMDA receptors leads to decreased T helper type 1-like and enhanced T helper type 2-like immune balance in humans. *Immunology* 2018;153:368–79.
- [99] D'Ellos MM, et al. Predominant Th1 cell infiltration in acute rejection episodes of human kidney grafts. *Kidney Int* 1997;51:1876–84.
- [100] Karczewski J, Karczewski M, Glyda M, Wiktorowicz K. Role of TH1/TH2 cytokines in kidney allograft rejection. *Transplant Proc* 2008;40:3390–2.
- [101] Tay SS, Plain KM, Bishop GA. Role of IL-4 and Th2 responses in allograft rejection and tolerance. *Curr Opin Organ Transplant* 2009;14:16–22.
- [102] Naik MG, et al. Determinants of successful use of sirolimus in renal transplant patients. *Transplant Proc* 2020;52:3103–11.
- [103] Augustine JJ, Bodziak KA, Hricik DE. Use of sirolimus in solid organ transplantation. *Drugs* 2007;67:369–91.
- [104] Lu Y, et al. Glucocorticoid receptor promotes the function of myeloid-derived suppressor cells by suppressing HIF1 $\alpha$ -dependent glycolysis. *Cell Mol Immunol* 2018;15:618–29.
- [105] Goenka R, et al. CTLA4-Ig-based bifunctional costimulation inhibitor blocks CD28 and ICOS signaling to prevent T cell priming and effector function. *J Immunol* 2021;206:1102–13.
- [106] Young JS, et al. Successful treatment of T cell-mediated acute rejection with delayed CTLA4-Ig in mice. *Front Immunol* 2017;8:1169.
- [107] Heinbokel T, et al. CTLA4-Ig prolongs graft survival specifically in young but not old mice. *Am J Transplant* 2020.
- [108] Vincenti F, et al. Belatacept and long-term outcomes in kidney transplantation. *N Engl J Med* 2016;374:333–43.
- [109] Pico de Coaña Y, et al. Ipilimumab treatment results in an early decrease in the frequency of circulating granulocytic myeloid-derived suppressor cells as well as their Arginase1 production. *Cancer Immunol Res* 2013;1:158–62.
- [110] Xu X, Gnanaprakasam JNR, Sherman J, Wang R. A metabolism toolbox for CAR T therapy. *Frontiers in Oncology* 2019;9.
- [111] Jenkins Y, Zabkiewicz J, Ottmann O, Jones N. Tinkering under the hood: metabolic optimisation of CAR-T cell therapy. *Antibodies (Basel, Switzerland)* 2021; 10.
- [112] Rigaud VOC, Hoy R, Mohsin S, Khan M. Stem cell metabolism: powering cell-based therapeutics. *Cells* 2020;9.
- [113] Pierotti MA, et al. Targeting metabolism for cancer treatment and prevention: metformin, an old drug with multi-faceted effects. *Oncogene* 2013;32:1475–87.
- [114] Lee SK, et al. Combination treatment with metformin and tacrolimus improves systemic immune cellular homeostasis by modulating treg and Th17 imbalance. *Front Immunol* 2020;11:581728.
- [115] Husain Z, Huang Y, Seth P, Sukhatme VP. Tumor-derived lactate modifies antitumor immune response: effect on myeloid-derived suppressor cells and NK cells. *J Immunol* 2013;191:1486–95.
- [116] Beegle J, et al. Hypoxic preconditioning of mesenchymal stromal cells induces metabolic changes, enhances survival, and promotes cell retention in vivo. *Stem Cells* 2015;33:1818–28.
- [117] Hasegawa S, Tanaka T, Nangaku M. Hypoxia-inducible factor stabilizers for treating anemia of chronic kidney disease. *Curr Opin Nephrol Hypertens* 2018;27:331–8.
- [118] Su YL, Banerjee S, White SV, Kortylewski M. STAT3 in tumor-associated myeloid cells: multitasking to disrupt immunity. *Int J Mol Sci* 2018;19.
- [119] Ko HJ, Kim YJ. Signal transducer and activator of transcription proteins: regulators of myeloid-derived suppressor cell-mediated immunosuppression in cancer. *Arch Pharm Res* 2016;39:1597–608.
- [120] Raha S, et al. Disruption of de novo fatty acid synthesis via acetyl-CoA carboxylase 1 inhibition prevents acute graft-versus-host disease. *Eur J Immunol* 2016;46:2233–8.