Accepted manuscript Article in Press

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Review Article

Pharmacotherapy of kidney transplant rejection: A narrative review on current therapy and future aspects

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ABSTRACT

Objectives: The current article was designed to review the main clinical and pharmacological data on the currently approved immunosuppressants used for the prevention and treatment of kidney transplant rejection.

Design: a narrative review manuscript.

Settings: a comprehensive search for the available literature on renal transplant pharmacotherapy on PubMed, Google, Medline Scopus and clinicalTrials.gov.

Subjects: Key related articles published until May.2020.

Intervention: non-interventional

Main outcomes and results: The induction therapy should be started with high doses of immunosuppressants immediately after transplantation, followed by gradual dosage reduction in maintenance therapy. It commonly involves thymoglobulin or Alemtuzumab. Basiliximab is also approved as an induction agent especially in recipients with low risk of rejection. thymoglobulin is superior in patients on a steroid-free maintenance regimen. The maintenance therapy should be started early after transplantation or even before. It can be made up of different combinations of calcineurin inhibitors (CNIs) like CsA/tacrolimus, mycophenolate mofetil (MMF), mTOR inhibitors (sirolimus/everolimus) and corticosteroids. Tacrolimus is considered a first-line agent in maintenance therapy and it is associated with a better allograft function. MMF is a major drug in the current maintenance therapy in combination with CNIs, and a main part in CNI-free regimens. Corticosteroids are still a main component in immunosuppressive regimens, despite the current interest in avoiding them due to their various long-term side effects. The rates of transplant loss are still unacceptably high mainly due to dose-limiting toxicities.

Conclusion: New drugs are being developed to improve the efficacy and safety profile of maintenance therapy. Some of them showed promising results.

KEYWORDS: thymoglobulin, alemtuzumab, tacrolimus, cyclosporine, sirolimus

INTRODUCTION

Chronic kidney disease (CKD) is defined as a decrease in kidney function indicated by a glomerular filtration rate (GFR) of less than 60 mL/min/1.73 m^2, presence of kidney damage markers, or both for more than 3 months, regardless of the underlying cause. The two main causes of CKD are found to be diabetes and hypertension.[1]

CKD is a major health care problem. According to a recent study, 13.4 % of the population worldwide are CKD patients, which is thought to be more than the prevalence of diabetes mellitus.[2] Treatment of CKD mainly aims to delay the progression towards end-stage renal disease (ESRD),[3] in which the only treatment becomes dialysis or renal transplantation when possible.[4] In the year 2016, renal transplants comprised around 66% of the different total transplants worldwide according to the Global Observatory on Donation and Transplantation (GODT).[5] However, this therapeutic option is challenged by the reaction of the immune system against the transplant, which necessitates a chronic control by immunosuppressants. [6]

Despite the continuous development of immunosuppressive agents over recent decades, renal graft loss as a consequence of rejection response cannot be totally prevented. The United Network for Organ Sharing (UNOS), reported that the probability of new graft rejection from a living donor at one-year post-transplantation is 3% and increases up to 14% at 5 years.[7] Generally, graft survival is affected by different factors including recipient age, the number of HLA mismatches, the onset of graft function after transplantation, donor type (deceased or living) and the presence of infections.[8-10]

This review discusses the current protocols, the new approaches and potential drug targets that might improve the prevention of renal graft rejection.

There are three types of graft rejection; hyperacute, acute and chronic rejection. Each one has a unique onset and mechanism.[11]

1.1. Hyperacute rejection

Hyperacute rejection appears only in the vascularized organs. It is a fast response that aims to eliminate foreign intrudes. It occurs within the first few minutes to hours of transplantation.[11, 12] In this reaction, the preexisting recipient antidonor antibodies bind to endothelial cells of the graft and activate the complement cascade, which recruits inflammatory cells such as polymorphonecular cells.[11-14] The recruited inflammatory cells secrete enzymes that cause injury to the endothelial cells, resulting in the release of von Willebrand factor that stimulates platelet aggregation and thrombus formation, which ultimately leads to graft loss.[11, 14] A high risk for hyperacute rejection occurs if the lymphocytotoxicity crossmatch was positive.[15]

1.2. Acute rejection

This is the most common type of renal transplant rejection. It reduces the graft half-life by around 34% and accounts for almost 6.2% of the causes of graft loss.[12, 16, 17] It is a combination of humoral and cellular responses.[11] The manifestations of this type begin within a week to three months after transplantation.[11, 12] It involves initially the activation of the innate response against the foreign grafts.[18] It is triggered as a consequence of a graft tissue injury caused by ischemic reperfusion or infection during transplantation,[11] where specific substances called damage-associated molecular patterns (DAMPs) are released from the site of injury, causing the activation of certain receptors expressed by innate immune cells called pattern-recognition receptors (PRRs).[19] Upon activation, these receptors mediate danger signals that initiate the innate response through the activation of dendritic cells (DCs) and the secretion of chemokines and cytokines.[11, 19] Activated DCs migrate to the secondary lymphoid tissues to activate T-cells and induce their migration to the graft area to initiate the adaptive response.[11, 17]

The adaptive response is more specific than the innate one. It plays a major role in the rejection mechanism.[11, 18] This response occurs when T-cells recognize the peptide antigen presented by major histocompatibility complex (MHC), which is expressed on the surface of the donor antigen-presenting cells (APC), this process is called direct pathway.[11-13, 17, 18] Full activation of T-cells is not only achieved by binding with MHC but also requires other interactions between molecules on the surface of T-cells and their ligands on the APC, such as CD28: CD80L or CD 86L, CD2: CD58L, CD5: CD72L. APC secret IL-12 that

activates the helper T-cells ($CD4^+$ cells) and induce their differentiation into TH1 cells, which produce IL-2 and INFs, both can stimulate the differentiation of cytotoxic T-cells. On the other hand, when $CD4^+$ cells are activated by DCs expressing CD86, they will be differentiated into TH2 cells and thereby acquire the ability to secrete IL-4, IL-5, IL-9, IL-10, IL-13 and IL-14, which in turn stimulate B-cells to produce antibodies against the graft and to present antigens to T-cells.[14, 18]

1.3. The chronic rejection

It represents the most common reason for graft failure after one year of transplantation. The exact cause of developing chronic rejection is still unknown. It is thought to be a combination of different factors including infections, chronic diseases and immunosuppressant induced nephrotoxicity.[15] Forty-seven percent of graft loss cases are attributed to this type of rejection,[20] compared to 6.2% of graft loss by acute rejection as mentioned earlier. This difference in the rejection rate could be due to the response of acute type to current immunosuppressive therapy in contrast with chronic type, which is not adequately controlled by any of the available immunosuppressants until now.[17]

METHODS

Combinations of different related keywords were fed to PubMed, Google, Medline Scopus and clinicalTrials.gov. The keywords included terms like "kidney/renal transplant rejection", "renal transplant therapy", "Immunosuppressants", and know drug names like thymoglobulin, tacrolimus...etc. The suggested related articles by PubMed and Google were also investigated. The study considered only the articles published in peer-reviewed journals or websites and lay in the scope of the manuscript. The literature search was performed between October, 2019 to April,2020.

LITERATURE REVIEW

1.4. Conventional immunosuppressive agents

To ensure successful renal transplantation and long-term survival of the graft, two categories of immunosuppressive agents are used sequentially for induction and maintenance.[21] Induction agents include potent immunosuppressive drugs that are given immediately after transplantation and continued over about 1-10 days to reduce the risk of early acute rejection.[21, 22] Thereafter, maintenance therapy is continued life-long in lower doses to maintain a long-term survival with the least possible side effects.[23, 24]

1.4.1. Induction agents

1.4.1.1. Antithymocyte globulin (ATG): Atgam and Thymoglobulin

Antithymocyte globulins (ATG) are polyclonal antibodies produced in horse or rabbit by injecting them with human lymphocytes to act as an immunogen. So that anti-lymphocyte serum (ALS) is produced, from which the most potent portion, immune gamma-globulins, are purified.[23, 25] These antibodies mediate immunosuppression by targeting markers on T-cell surfaces that are responsible for the immune-activating signals such as CD2, CD3, CD4, CD8, CD16, CD18, CD25 and CD45.[21, 25] Consequently, these antigen-antibody interactions result in complement-mediated lysis of lymphocytes.[21, 24]

Equine ATG (also known as Atgam, Lymphoglobulin, and Thymogam) was indicated for treating and preventing acute rejection after kidney transplantation.[21, 25] While rabbit ATG (also known as rATG or thymoglobulin) was initially approved only for treating acute rejection, but later on it became the most commonly used agent for the induction in the United States,[21, 22, 25, 26] as it is associated with lower acute rejection rates and better graft survival.[21] A randomized prospective multicenter six-month-long study assessed the efficacy and safety of using thymoglobulin, as an induction agent in combination with tacrolimus or cyclosporine A (CsA), in comparison with tacrolimus-based therapy without induction with thymoglobulin. The patients (n=555) were randomly assigned to tacrolimus triple therapy, ATG induction with tacrolimus, or ATG induction with CsA. All three treatment groups also received azathioprine and corticosteroid. This study demonstrated that the rate of biopsy-proven acute rejection (BPAR) in the groups of thymoglobulin-tacrolimus was significantly lower than the other two groups. However, thymoglobulin treatment was associated with a higher incidence of serum sickness, hematological toxicities (thrombocytopenia, leucopenia), and cytomegalovirus infections [27]. Besides these side effects, other studies also reported increased risks for lymphoma and anaphylactic reactions.[21, 23] The symptoms of hypersensitivity reactions could be prevented by slow infusion of thymoglobulin (over 4-8 hours) and by pretreatment with antihistamines, glucocorticoids and paracetamol.[25] Recently, in 2017, thymoglobulin has gained US FDA approval for prophylaxis as an induction agent.[26]

1.4.1.2. Interleukin-2 receptor (IL2-R) antagonist: Basiliximab

Basiliximab is a chimeric monoclonal antibody developed by DNA recombinant technology. It is a fusion of the variable region of murine immunoglobulin with the constant region of human immunoglobulin to produce an antibody that binds with the alpha chain (CD25) of interleukin-2 receptor (IL-2R) presented on the surface of the activated helper T-cells so that it blocks its binding with IL-2 and consequently prevents helper T-cell proliferation without causing their lysis as in the case with the use of ATG.[21]

Basiliximab is approved as a prophylaxis against the rejection following renal graft transplantation.[21] It is currently recommended to be used in recipients with low risk of rejection.[28] A systematic study emphasized on the effectiveness of using Basiliximab as an induction agent with an acceptable side effect profile.[29] However, a study targeted patients at The “Organ Procurement and Transplantation Network registry” between the years 2000-2012 evaluated the necessity of the induction therapy with an IL-2R antagonist in patients receiving living donor renal transplantation, who were treated with tacrolimus/mycophenolic acid with and without steroids. The study demonstrated that the induction with IL-2R antagonist had no advantage over no-induction group in patients receiving a maintenance therapy with tacrolimus/mycophenolic acid/steroid. The study also demonstrated that, in the no-steroid group, thymoglobulin resulted in lower rates of acute rejection compared to IL2-R antagonist.[30] From the author’s point of view, it appears that although current guidelines recommend the use of the induction agents regardless of the risk of rejection, corticosteroids use or the constituents of the used maintenance regimen, it seems that future guidelines might restrict the use of the induction agents for higher risk group or when steroids are to be avoided. In such cases, thymoglobulin would be the preferred agent. However, large randomized clinical trials in this concern are needed.

1.4.1.3. Anti-CD-52 antibodies: Alemtuzumab

Alemtuzumab is a humanized monoclonal antibody that triggers antibody-dependent cell lysis by binding to CD-52 molecule on the surface of T- and B-cells, macrophages and NK cells.[21] Although it has an FDA approval only for B-cell lymphocytic leukemia, its use for induction therapy in kidney transplantation is increasing since 1998.[31] For this purpose, it is administrated intravenously as a single dose of 30 mg intraoperatively.[32] Like other monoclonal antibodies, alemtuzumab might cause hematological side effects such as thrombocytopenia, neutropenia, and lymphopenia. It may also cause cytokine release syndrome and infusion-related reactions.[21]

Several trials were published that investigated alemtuzumab's efficacy and safety in kidney transplantation. Of these, alemtuzumab was compared with basiliximab and thymoglobulin in low and high-risk groups. All groups received tacrolimus, mycophenolate mofetil (MMF) and five-day steroid as maintenance therapy. The study showed that alemtuzumab was superior to basiliximab in reducing the acute rejection rate (10% vs. 22%), but almost similar to thymoglobulin (18% VS. 15%).[33] However, another study showed no difference between alemtuzumab and basiliximab on graft and patient survival.[31] Recently, a systemic review and meta-analysis of randomized controlled trials reported superior beneficial effects of alemtuzumab over thymoglobulin, however, the comparison was statistically insignificant due to the limited number of the trials.[34] Therefore, more controlled comparative studies are required to determine the superiority of these drugs.

1.4.2. Maintenance agents

1.4.2.1. Calcineurin inhibitors (CNIs): Cyclosporin A (CsA) and tacrolimus

Cyclosporin A (CsA) and tacrolimus are two of the most widely used immunosuppressive agents. They are considered the mainstay of renal transplant maintenance therapy. They are called calcineurin inhibitors due to their ability to inhibit the activity of a protein called calcineurin,[35] which is a calcium/calmodulin-dependent serine-threonine phosphatase, consisting of two subunits, a catalytic subunit, calcineurin-A and a regulatory calcium-binding subunit, calcineurin-B.[36, 37] It plays a major role in T-cell activation. When an alloantigen is recognized by T-cell receptors, the intracellular calcium concentration increases, this, in turn, activates calcineurin-B. Once activated, it stimulates calcineurin-A to dephosphorylate a cytoplasmic transcription factor called the cytoplasmic nuclear factor of activated T-cells (NFATc), allowing its transfer along with the activated calcineurin to the nucleus, where it induces the expression of cytokines and co-stimulatory substances like IL-2, which in turn upon interaction with their receptors result in cell activation and proliferation.[37-39]

CsA and tacrolimus have the same pharmacodynamic activity although they differ in their structures.[35, 39] CNIs bind cytoplasmic proteins called immunophilins, cyclophilin in the case of CsA and FK-binding protein in case of tacrolimus [40]. This complex then binds to calcineurin leading to inhibition of its activity.[37, 41]

CNIs are associated with many adverse effects due to their narrow therapeutic window and the presence of their target (calcineurin) in many cell types other than immune cells. The most frequent adverse effect is nephrotoxicity, which can be acute and reversible or chronic and irreversible. Chronic CNIs-induced

nephrotoxicity may result in interstitial fibrosis, tubular atrophy or glomerulosclerosis, which contributes to the deterioration of the function of the transplanted kidney with time. Other side effects include new-onset diabetes, dyslipidemia, neurotoxicity, de novo cancers and infections.[42]

Different studies showed that tacrolimus is superior to CsA in terms of the rates of graft loss, acute rejection and hypercholesterolemia in addition to lower nephrotoxic potential. While CsA is more likely to cause gingival hyperplasia, hirsutism and increased levels of LDL and triglycerides, tacrolimus increases the risk of developing diabetes (dose-dependent) and gastrointestinal upsets. No differences in malignancy and infections were observed.[43, 44]

Currently, tacrolimus is considered a first-line agent in maintenance therapy in renal transplantation.[28, 45] Extended-release tacrolimus formulation is available now as an alternative to the immediate release one with comparable efficacy.[46]

1.4.2.2. Purine synthesis inhibitors: Azathioprine and mycophenolate mofetile (MMF)

Azathioprine

Azathioprine inhibits purine synthesis as it is converted within cells to its metabolites, mercaptopurine (6-MP) and thioguanine (6-TGN). 6-MP, which is involved in the formation of thioinosinic acid nucleotides, consequently blocks the purine biosynthesis pathway. Besides, these metabolites are incorporated into the DNA blocking its replication.[47]

Azathioprine, since its development in the 1960s, was used as a main part of the standard regimens for immunosuppression.[23] However, lately, it has been replaced by mycophenolic acid,[28] as this drug was found to be more effective in the prevention of acute rejection.[48] However, this claim has been challenged by later studies, as discussed in the following section of this manuscript.

The main side effects associated with azathioprine use are bone marrow suppression mainly in patients who take gout medications, allopurinol or febuxostat. Other side effects are nausea, joint pain and hepatotoxicity.[49] [47]

Mycophenolate mofetil (MMF)

MMF is a prodrug of the active agent mycophenolic acid. It is directed against the process of guanosine nucleotides production, mostly through the inhibition of the enzyme inosine monophosphate dehydrogenase (IMPDH) that is expressed in T and B lymphocytes, thus negatively affecting their proliferation rate.[50] However, MMF's ability to inhibit the recruitment of immune cells at the site of rejection and to decrease the proliferation of arterial smooth muscle cells are also considered main mechanisms by which MMF may improve graft survival.[51] MMF is considered a major drug in the current maintenance therapy of renal transplant along with CNIs, and a main part in CNI-free, toxicity sparing regimens that may take a place in the future.[52] Although many studies have shown that MMF may play a role in preventing tubular chronic graft rejection and prolong patient survival,[48, 53, 54] there is a need for additional clinical trials on a larger scales to confirm this effect.

In many systematic reviews and randomized controlled clinical trials, MMF was found to be associated with a superior benefit in preventing acute renal graft rejection compared with azathioprine, and with

comparable adverse effects profile.[48, 55] Therefore, MMF has largely replaced azathioprine in immunosuppression protocols, and it is considered now the first-line agent in renal transplant therapy.[28] However, this approach was opposed by a prospective, multicenter, randomized parallel group trial published in 2004 by Remuzzi and colleagues, Which demonstrated that MMF had no advantages over azathioprine in preventing acute rejection in kidney transplant patients, who were on CsA microemulsion and steroid over 6 months, followed by CsA alone over another 15 months. Interestingly, treatment with MMF was fifteen times more expensive than azathioprine. [56] A follow-up study by the same group followed the patients over 5 years demonstrated that the clinical outcomes were similar between MMF and azathioprine. In addition, based on the relatively high cost of MMF, they recommended the replacement of MMF with azathioprine in the standard immunosuppression treatment for kidney transplant recipients.[57] In agreement with these recommendations, another review of the respective clinical data in 2013 did not reveal any long-term benefits of MMF over azathioprine.[58] Therefore, we think that caregivers should balance the potential benefits and harms of MMF and AZA according to individual patient's risks and preferences.

1.4.2.3. Inhibitors of mTOR: Sirolimus and everolimus

Sirolimus (also known as rapamycin)

It is a potent antiproliferative immunosuppressive agent. It acts by blocking the action of a protein kinase called the mammalian target of Rapamycin (mTOR), which plays a major role in multiple signal transduction pathways. Sirolimus inhibits the proliferation of T- and B-cells. It also decreases the production of antibodies. This unique mechanism of action, as well as its adverse effect profile, make this drug one of the major agents used in the prevention of acute renal transplant rejection.[59]

A synergistic effect was observed when sirolimus was combined with CsA, that is, the risk of developing acute renal rejection was reduced.[59, 60] Furthermore, in different studies, when CNI was withdrawn and a CNIs-free maintenance regimen consisting of sirolimus and MMF was introduced, a comparable efficacy (in terms of graft and patient survival) with more safety was observed and maintained for 4 years post-transplantation.[61, 62]

The advantages of Sirolimus use include the lack of causing severe nephrotoxicity or major organ damage. However, gastrointestinal toxicity, Hypertension, hyperglycemia, hyperlipidemias, bone marrow suppression, infections, impaired wound healing, arthralgia, edema and increased risks for certain malignancies remain major side effects by sirolimus that require attention.[63, 64]

Everolimus

It is an oral sirolimus analog, with improved solubility and oral bioavailability but similar immunosuppressive efficacy and safety profile.[65, 66] As an advantage, everolimus may have an ability to lower the toxicity of CNI on the kidneys, in contrast to sirolimus which potentiates CNI-associated nephrotoxicity. However, this ability is claimed only by expert opinions and clinical trials on limited scales,[66, 67] and therefore further studies and randomized controlled clinical trials are needed to confirm this observation.

1.4.2.4. Corticosteroids

For decades, corticosteroids have been used as a major part of immunosuppressive regimens due to their various inhibitory effects on inflammatory and lymphocytic activation processes.[68] Their anti-inflammatory effects include suppression of immune cell migration to inflamed tissues as well as inhibition of the production of inflammatory mediators such as prostaglandins. Steroids also decrease cytokines and interleukins production, thus affecting T- lymphocyte proliferation.[69]

Methylprednisolone and prednisolone are the main steroids used for immunosuppression preoperatively and as a part of the triple maintenance therapy consisting of CNI and MPA along with corticosteroids.[28] Intravenous methylprednisolone dose is used commonly at induction and tapered postoperatively. At 3-5 days post-transplantation, methylprednisolone is switched to a fixed oral dose of prednisolone.[68]

Unfortunately, long term use of corticosteroids is associated with unfavorable side effects that underlay several major health problems, such as dyslipidemia, hypertension, hyperglycemia and post-transplant diabetes mellitus (PTDM).[70] To obtain a better side effect profile, current protocols suggest that steroids could be withdrawn in patients with low risk of rejection during the first week after transplantation. However, if steroids are not avoided in the first month after transplantation, prednisolone should be continued with the lowest possible effective dose (5 mg daily).[28]

A recent retrospective cohort study included two groups of patients. All patients received a perioperative dose of basiliximab and a second dose on day 4. They also received a single perioperative dose of methylprednisolone as induction agents. The first group included low-risk patients in whom prednisolone was administered from day 1-7 and then maintained with tacrolimus and MMF. The other group included high-risk patients, who were maintained with tacrolimus, MMF and prednisolone for the first 3 months, and afterward, they were maintained with tacrolimus and prednisolone. The data demonstrated a close incidence of biopsy-proven acute rejection between the high and low-risk groups. Moreover, one-year post-transplantation, the rates of graft and patient survival, and the graft function were also similar between the two groups.[71] Interestingly, a systemic review and meta-analysis study advocated the withdrawal or avoidance of steroids, even in high-risk patients, as it was demonstrated in this study as being safe, in terms of graft survival and rejection, and associated with a reduced risk of death and post-transplantation diabetes mellitus. However, the authors recommended future prospective studies to further validate these results.[72]

1.5. Guidelines in renal transplant therapy (RTT)

Traditionally, the goal of therapy during the first six months after transplantation is mainly to prevent acute graft rejection and to prolong its survival. However, lately, the aim has expanded to also include the prevention/treatment of the major complications associated with the use of immunosuppressants such as malignancies and infections, and to improve patient's adherence and to ultimately preserve a good transplant function.[28, 73] The current guidelines recommend the use of multi-drug regimens in a way that produces a synergistic effect against acute rejection, starting with high doses immediately after transplantation followed by gradual dosage reduction in maintenance therapy. [45]

To prevent hyperacute transplant rejection, immunosuppressive therapy should be started at the time of transplantation. Antibody agents including ATG and IL-2 receptor antagonists (IL-2RA) represent the cornerstone of the induction therapy in all kidney transplant recipients.[28, 45] The use of ATG is preferred when there is a high risk for rejection or when corticosteroids are avoided early at induction.[73, 74]

Treatment with CNIs like CsA or tacrolimus should be started early after transplantation or even before.[28, 73] Tacrolimus is preferred as it is associated with a lower rate of rejection. The plasma trough level of tacrolimus is not recommended to exceed 4-8 ng/ml.[28] The anti-proliferative agent mycophenolic acid is the drug of choice to be used in combination with CNIs.[28, 45]

The mTOR inhibitors, sirolimus or everolimus are considered second lined drugs to be used when CNIs are causing serious adverse effects including chronic graft disease, tubular atrophy or interstitial fibrosis. CNI induced nephrotoxicity should be confirmed by biopsy.[28]

Despite the recent great interest in corticosteroids avoidance and withdrawal from RTT as better side effect profile could be obtained, corticosteroids, so far, remain a main part in immunosuppressive regimens. Studies showed that avoidance of steroids early after transplantation is associated with an increased risk for acute rejection. However, graft survival and mortality seem to be not affected by the current antibody induction and maintenance regimens used.[75, 76] A recently updated review article revealed that there is a significant increase in acute rejection associated with early steroids avoidance or discontinuation but graft survival seems to be similar in a time frame of 5 years. However, further clinical studies are required to assess longer corticosteroid withdrawal effects.[77]

1.6. New approaches in renal transplant therapy

1.6.1. Targeting specific cytoplasmic signaling proteins

Tofacitinib (also known as tositinib, CP-690,550) has a unique mechanism of action in immune suppression. It is directed against a family of cytoplasmic signaling proteins called Janus kinases (JAK) mainly JAK2 and JAK3, which mediate the signal from cytokine receptors, especially in this context the IL-2Rγ. The inhibition of this signaling pathway in B-cells, T- cells and NK cells results in their apoptosis.[78] In the year 2012, this drug gained FDA approval to treat Rheumatoid arthritis and recently, in 2018, it has been also approved for the treatment of moderate to severe ulcerative colitis.[78, 79]

Clinical trials are being conducted to evaluate the efficacy and safety of its use in renal transplantation especially as a part of CNIs- free regimens. Tofacitinib has successfully passed animal studies as well as phase 1 clinical trials regarding transplantation.[78] In a phase I study 28 stable kidney transplant patients were included and randomized to receive tofacitinib 5 mg BID, 15 mg BID, 30 mg BID or placebo (n=6, 6, 10 and 6 respectively). The study reported a dose-dependent decrease in hemoglobin, NK cell count and mild to moderate viral and bacterial infections, especially with the highest dose of tofacitinib. The study did not report any clinical rejection or renal toxicity.[80]

Another larger pilot study was carried out in the year after. It was a prospective, randomized study over 12 months and involved 61 kidney transplant patients, who received induction therapy with basiliximab and concomitant treatment with MMF and corticosteroids. The patients were distributed in three treatment groups; 15 mg tofacitinib twice daily, 30 mg tofacitinib twice daily and tacrolimus (control group). The

results showed that the combination therapy of 30 mg tofacitinib twice daily with MMF was associated with over-immunosuppression, while 15 mg tofacitinib twice daily showed efficacy and safety profile that was comparable to the control group (tacrolimus), except that it showed a higher risk for viral infections. A limitation of this study was that the treatment groups did not have equivalent demographics.[81]

Based on the outcome of this study, a larger prospective randomized multicenter phase-IIb trial was carried out on tofacitinib. The study included 331 renal transplant recipient patients with low to moderate risk of rejection. The patients were randomly distributed into three groups. All groups received basiliximab for induction and mycophenolic acid and corticosteroids for maintenance therapy. One group received CsA microemulsion, while the other two groups received tofacitinib with two different intensities; more intensive (MI) tofacitinib (15 mg twice daily (BID) in months 1–6, then 10 mg BID in Months 7–12), and less intensive (LI) tofacitinib group (15 mg BID in months 1–3, then 10 mg BID in months 4–12). After 12 months of post-transplantation therapy, patient and graft survival was similar among the different treatment groups. Despite the advantageous lower rates of chronic nephropathy associated with tofacitinib use as compared to CsA, patients treated with this drug developed more severe side effects in the context of anemia, leucopenia, viral infections and lymphoproliferative disorders, which was believed to be a result of excessive immunosuppression.[82] Similar results were obtained by a more recent study, which assessed the long term effect of tofacitinib in comparison with CsA over three years post-transplantation. The study also concluded an association between the increased risk of serious infections and the use of mycophenolic acid with tofacitinib.[83]

Possible future approaches need to be investigated for the aim of reducing the side effects profile of tofacitinib, which might include serum drug level monitoring to individualize the therapeutic levels of tofacitinib to achieve the lowest possible effective doses or testing tofacitinib-containing regimens that include lower therapeutic doses of MMF. Additionally, tofacitinib could be tested as a part of new CNI-free protocols.

1.6.2. Targeting co-stimulatory pathways

The interaction between the T-cell receptors (TCR) and the antigen presented on antigen-presenting cells (APCs) is not sufficient for full T-cell activation, but other co-stimulatory signals are also needed. A new insight for targeting these signals is currently being conducted to treat solid organ transplantation rejection and autoimmune diseases.[84] The inhibition of co-stimulatory pathways such as CD40-CD154 and CD80/CD86-CD28 negatively affects APCs activation, antibody-production and cytokine releasing processes[85].

1.6.3. Anti-CD40 monoclonal antibodies

The interaction between CD40, presented by APCs such as B-cells and dendritic cells, and CD40L (or CD154), presented by T-lymphocytes, plays a major role in T-cell activation.[17] Once CD40 interacts with its ligand, the activated APCs upregulate co-stimulatory proteins leading to T-cell proliferation and differentiation. Likewise, the activation of B-cells by the CD40-CD154 pathway causes their differentiation

into memory or plasma cells, which play a major role in chronic renal graft rejection as mentioned earlier in this article.[86]

Recently, different agents directed against the CD40-CD154 pathway were introduced to treat chronic graft rejection and auto-immune diseases, besides some kinds of cancers.Anti-CD154 antibodies were studied and they were considered to have a partial efficacy in renal transplant. They are suggested to be combined with B7/CD28 pathway inhibition for full efficacy in preventing chronic rejection.[87] Moreover, anti-CD154 antibodies are associated with thromboembolic events that are thought to be mediated by anti-CD154 Fc domain-platelets interaction.[88]

1.6.3.1. Bleselumab

Bleselumab (ASKP1240) is a fully-humanized IgG4 monoclonal antibody that binds with CD40 on B-cells and antigen-presenting cells to block its interaction with CD154 ligand on T-cells resulting in co-stimulatory pathway inhibition.[89] The mean maximal CD40 receptor occupancy is reached at a dose greater than 0.01 mg/kg and the receptor occupancy did not exceed 87%. After that, any increase in the dose only will prolong the duration of occupancy.[90, 91]

Regarding the safety of bleselumab, the most commonly reported side effects were procedure pain, hypophosphatemia, hypomagnesemia, nausea, tremor, edema, and BK viral infection.[89, 92] Unlike the some investigational chimeric anti CD40 monoclonal antibodies it does not cause cytotoxicity and immunogenicity. Also, there were not any thromboembolic events caused by this antibody.[91]

A phase 2 randomized open-label multicenter study was designed to assess the efficacy and safety of bleselumab combined with immediate-release tacrolimus (IR-TAC) or MMF in comparison with the standard of care treatment (SoC), which consisted of IR-TAC and MMF. Basiliximab as an induction treatment and corticosteroids were used in all recipients. The dose of IR-TAC was decreased gradually after 28 days of treatment in the bleselumab + IR-TAC group until a trough level of 2-5ng/ml was reached by 4 months. The biopsy-proven acute rejection after 6 and 36 months was 6.3 % and 14.6% respectively in SoC, while it was 37% and 41% in bleselumb + MMF and 9.1 % and 9.1% in bleselumab + IR–TAC. These results suggest the noninferiority of bleselumb plus IR-TAC in comparison to SoC in the prevention of biopsy-proven acute rejection. However, the incidence of new-onset diabetes mellitus was lower in bleselumab + MMF than other regimens. Finally, all regimens had no significant differences in eGFR and graft and patient survival rates.[89]

1.6.3.2. Iscalimab

Iscalimab, CFZ533A, is a fully-humanized Fc-silent anti-CD40 immunoglobulin that was recently invented. This drug can inhibit humoral immunity that is stimulated by helper T-cells without depleting peripheral blood B-cells.[93, 94] With its suspected efficacy in prolonging graft survival and no evidence for developing thromboembolic complication, this drug is now being studied to be considered in the main part of CNI-free renal transplant protocols.

In phase-IIa randomized clinical trials conducted in 2018 (ClinicalTrials.gov Identifier: NCT02217410), 51 patients were studied to compare the therapeutic outcome between iscalimab and tacrolimus. All patients

received basiliximab and corticosteroids as induction therapy and MMF and steroids as maintenance therapy. Results showed that iscalimab has a better side effect profile and improved renal graft outcomes with no risk for nephrotoxicity.[95] Allograft biopsies were reviewed by blind pathologist and results were presented by American Transplant Congress (ATC), 2019. Chronic allograft damage index was lower in patients treated with iscalimab,[96] indicating that this novel drug could be a valuable add-on therapy to the current standard tacrolimus containing regimens. The results need to be confirmed by phase-IIb clinical trials (NCT03663335) that may be completed by 2021.[97]

1.6.4. Anti CD80/86 monoclonal antibodies

1.6.4.1. Belatacept

Belatacept is a CTLA4-Ig fusion protein developed from abatacept by substituting leu104 with glutamic **acid** and ala29 with tyrosine. It was approved in 2011 for acute rejection prophylaxis in kidney transplant recipients as an alternative for CNIs, to reduce their renal and cardiometabolic side effects. Belatacept interacts with CD80/86 on APCs to block its binding to CD28 on T-cells resulting in the prevention of T-cell activation.[98, 99]

Belatacept has a volume of distribution of 0.11L/kg, clearance of average 0.490_+13 mL/kg/h and a terminal half-life of almost 11 days. It is administrated intravenously over 30 mins with 10 mg/kg on days 1 and 5 and at the end of weeks 2, 4 and 8. After that, 5mg/kg is given on week 16 of transplantation and it is repeated every four weeks. It does not require therapeutic monitoring as its plasma concentration is not affected by renal or hepatic functions, therefore, the doses are adjusted only according to the factor of bodyweight.[100, 101]

The most common adverse effects related to belatacept are infusion-related reactions, anemia, headache, peripheral edema and infections [98]. Also, the FDA puts a black box warning about the post-transplant lymphoproliferative diseases especially in Epstein-Barr virus (EBV) seronegative recipients, therefore, contraindicates its use in this group of patients.[100, 102]

The two major clinical trials that evaluated belatacept efficacy and safety are “Belatacept Evaluation of Nephroprotection and Efficacy as a First-line Immunosuppression Trial” (BENEFIT) and the BENEFIT–Extended Criteria Donors Trial (BENEFIT-EXT) that followed the patients with extended criteria donor kidney transplant. In these studies, patients were randomized to three groups, more intensive belatacept (MI), low intensive (LI) belatacept, and CsA. All recipients were given induction with basiliximab and maintenance with MMF and corticosteroids. In BENEFIT, after 12 months both the graft and patient survival rates were not significantly different between the groups (95% MI vs. 97% LI vs. 93% CsA respectively).[98] Concerning safety, belatacept groups had a higher renal function than CsA [101] and lower chronic renal allograft nephropathy. Finally, belatacept approved its safety in reducing the cardiometabolic side effects related to CsA use, as it showed a lower incidence of hypertension, hyperlipidemia and new-onset diabetes mellitus after transplantation. However, in contrast to all of these advantages for belatacept over CsA, it remains associated with higher acute rejection rates. After the end of 7 years, the belatacept groups also remain non-inferior to CsA groups in the survival rates for patients and graft. It showed better results for kidney function and cardiometabolic effects.[98]

To compare between belatacept and tacrolimus, two studies were conducted. In the first one, basiliximab was used as an induction agent while MMF and steroids were used as maintenance agents. Both belatacept and tacrolimus resulted in the same renal function but the acute rejection rate was higher with belatacept (55% vs. 10%).[103] In the second study, the patients were divided into three groups, group 1 received tacrolimus and MMF as maintenance agents, both groups 2 and 3 received belatacept and MMF for maintenance, however, for induction ATG was used in groups 1 and 2 and tacrolimus with basiliximab in group 3. In this study, the belatacept groups had better eGFR and patient and graft survival. Nevertheless, the acute rejection rate was still the lowest in the tacrolimus group (3.4%, 34.5%, and 36.9%, respectively). This study has not been published yet.[104]

As discovered later, the CTLA4 pathway could play a role in the function of regulatory T-cells promoting their co-inhibitory signal. Therefore, the blockade of this pathway by belatacept might explain why belatacept is associated with increased rates of acute rejection when compared with CNI as well as a high incidence of lymphoproliferative disorders.[105, 106]

1.6.4.2. Selective CD28 blockade

In contrast to Belatacept, selective CD28 blockers inhibit CD28 co-stimulatory effects, while preserving the co-inhibitory effect of the CTLA-4 pathway. Therefore, they are considered to be more effective in inhibiting T-cell activation and proliferation.[107, 108]

The most advanced selective anti-CD28 agent is FR104, a novel humanized pegylated CD28 antagonist that is being developed for the treatment of graft rejection. A nonhuman primate study was conducted to evaluate its efficacy for the aim of allowing the minimization of steroids and CNI use. The results were promising as the combination of FR104 and low doses of tacrolimus with no steroid exposure significantly improved graft survival. FR104 was found also to be effective in preventing alloimmunization and in decreasing the development of donor-specific alloantibodies (DSAs). CNI-free regimens showed different results as (FR104/MMF/Steroids) was associated with increased risk for acute rejection but the combination between FR104 and sirolimus prevented acute rejection and showed improvement in graft survival.[109] Later evidence for synergistic effect between FR104 and mTOR inhibitors was demonstrated in a recent study that showed a significant inhibition for T-cell activation, resulting in more immunosuppression and more infectious complications.[110] The first-in-human phase 1 clinical study in 64 healthy volunteers to assess the efficacy and safety of FR104 was conducted in 2016. With all tested doses, FR104 was found to be safe and clinically efficacious supporting further studies to determine ideal dosage regimens.[111]

Summary

The prevention of kidney transplant rejection requires a short-term induction therapy, as prophylaxis against early acute rejection, and a life-long maintenance therapy against chronic rejection.

The induction therapy should be started with high doses immediately after transplantation followed by gradual dosage reduction in maintenance therapy. It commonly involves thymoglobulin (ATG), which targets several surface antigens on T-lymphocytes resulting in complement-mediated lysis of these cells. It

is also commonly used for the treatment of acute rejection. Alemtuzumab is another induction agent that causes antibody-dependent immune cell lysis. It demonstrated some statistically insignificant beneficial effects over thymoglobulin, therefore, more controlled comparative studies are required to determine the superiority of these two drugs. Both drugs are associated mainly with hematological toxicities. On the other hand, basiliximab is an IL-2R antagonist that targets activated helper T-cells and prevent their proliferation without causing their lysis. It is approved as an induction agent especially in recipients with low risk of rejection. However, thymoglobulin might be superior in patients on a steroid-free maintenance regimen.

The maintenance therapy should be started early after transplantation or even before. It can be made up of different combinations of CNIs (CsA/tacrolimus), MMF, mTOR inhibitors (sirolimus/everolimus) and corticosteroids. Tacrolimus is considered a first-line agent in maintenance therapy in renal transplantation. Studies showed that tacrolimus has lower nephrotoxic potential than CsA and it is associated with a better allograft function. CNIs are generally associated with cardiovascular, metabolic and immunosuppression-related side effects, as well as nephrotoxicity, which is the most frequent. MMF is also considered a major drug in the current maintenance therapy of renal transplant along with CNIs, and a main part in CNI-free regimens that may take a place in the future, although some studies recommended its replacement with azathioprine based on cost-effectiveness data. Sirolimus (rapamycin) and everolimus inhibit the proliferation of T- and B-cells, and they decrease the production of antibodies. They can produce a synergistic effect in combination with CNI and in CNI-free maintenance regimen along with MMF. They are considered second lined drugs to be used when CNIs are causing serious adverse effects. The inhibitors of mTOR are associated with cardiovascular, metabolic and immunosuppression related side effects. Until now, corticosteroids are considered a main part in immunosuppressive regimens, despite the current interest in avoiding them due to their various side effects.

New drugs are being developed to improve the efficacy and safety profile of maintenance therapy. A promising one is tofacitinib that inhibits JAK signaling pathways resulting in lymphocytes apoptosis. It showed comparable anti-rejection activity to CsA with a lower risk of nephropathy but more hematological toxicity and increased risk for infections and lymphoproliferative disorders. Other drug classes are being developed, some of them target co-stimulatory pathways, like bleselumab and iscalimab that targets CD40 antigens on B-cells, another one targets CD/8086 on APC like belatacept, and finally the recent selective CD28 blockers like FR104. These classes are still in clinical trials to assess their efficacy and safety profile in comparison to the currently approved drugs. Some of them showed some promising results.

CONCLUSION

Induction therapy is most commonly achieved by ATG and Alemtuzumab. The maintenance therapy can be made up of different combinations of CNIs, MMF, mTOR inhibitors and corticosteroids. Tacrolimus is considered a first-line agent in maintenance therapy in renal transplantation. MMF is another major drug that is used in combination with CNIs, and is being investigated as a main part in future CNI-free regimens. New promising drugs are being developed to improve the efficacy and safety profile of the maintenance therapy.

ACKNOWLEDGMENT

The authors have no conflict of interest to declare. We declare that all of the listed authors effectively participated in the preparation of this manuscript.

NK designated the idea of the manuscript, supervised the writing process, wrote the abstract and summary section, proofread and verified the scientific content, improved the scientific language of the text and approved the final version. **AH** wrote the introduction and the section on the induction agents. She also verified the scientific content and approved the final version. **LZ**, wrote the sections on the new approaches in renal transplant therapy and participated in the writing of the section on guidelines in renal transplant therapy. She also verified the scientific content and approved the final version. **IS** wrote the sections on maintenance agents and the guidelines in renal transplant therapy. **SH** proofread and verified the scientific content, improved the language of the text, assisted in the organization of the manuscript, verified the cited articles and approved the final version. This work did not receive any kind of funding.

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