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CORNEA (K HOUSER, SECTION EDITOR)

Immunosuppressive Therapy for High-Risk Corneal Transplant

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Abstract

Purpose of Review This review summarizes the efficacy, clinical utility, and adverse event profile of available immunosuppression agents used for high-risk keratoplasties. New studies are emphasized.

Recent Findings Recent studies have highlighted the use of different immunosuppressive agents in the setting of high-risk keratoplasty as well as supporting studies (e.g., immune privilege, panel reactive antibody, HLA matching, graft rejection, and large reviews on the topic). Specific agents studied were topical difluprednate, topical and systemic tacrolimus, topical and systemic cyclosporine, mycophenolate mofetil, methotrexate, and immunomodulatory anti-VEGF agents.

Summary Due to loss of protective factors, high-risk keratoplasties benefit from immunosuppression to prolong graft survival. Aggressive topical immunosuppression with periocular/systemic corticosteroids and immunomodulatory agents are useful for initial high-risk keratoplasties. Any history of rejection will likely benefit more from adequate systemic immunosuppression. Additional long-term studies in this population are needed.

Keywords Systemic immunosuppression · High-risk corneal transplant (keratoplasty) · Graft rejection · Graft failure · Corneal neovascularization

Introduction

Corneal transplantation or keratoplasty has become one of the most common transplantation procedures due to the advancements in efficacy, patient safety, and availability of corneal tissue [1]. The main types of keratoplasties include penetrating keratoplasty (PK, full thickness) and a few different partial thickness (lamellar) keratoplasties such as deep anterior lamellar keratoplasty (DALK) and endothelial keratoplasty (EK, e.g., Descemet stripping endothelial keratoplasty [DSEK], Descemet membrane endothelial keratoplasty [DMEK]). Rates of success for these allografts depend on donor and recipient factors. In general, the amount of transplanted tissue (PK vs. lamellar keratoplasty) and the particular layers transplanted (i.e., whether the endothelial layer is included, as endothelial failure results in an edematous cornea and need for a regraft) often dictate donor-related success rates. Compared to the 21–31% rejection rate for PKs, DALKs (0.8–10.9%), DSEKs (2.2–7.9%), and DMEKs (0.1–5%) carry a lower risk of rejection [2•]. Recipient-related factors for keratoplasty failure are centered around immunologic factors (e.g., risk of rejection, sensitization) and anatomic factors (e.g., presence of a glaucoma drainage device, synechiae, anterior chamber intraocular

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lens) [3••]. This review will focus on the former and how immunosuppression has been used to decrease immunologic-related failure in high-risk keratoplasties.

Immunological Privilege of the Cornea

There are several unique features of the cornea and anterior chamber that allow a high overall success rate for keratoplasty. Ocular immunological privilege has been delineated into 4 separate factors: (1) hemangiogenic and lymphangiogenic privilege, (2) immunogenicity and immune privilege of the cornea as a transplantation tissue, (3) tolerance related to anterior chamber–associated immune deviation and the involvement of regulatory T cells, and (4) neuro-immune interactions and microenvironment of the eye made up by the aqueous humor [4•, 5•, 6]. The avascular nature of the cornea limits access of the immune system, and the lack of corneal lymphatics minimizes high-volume delivery of antigens and antigen presenting cells (APCs) to T cell reservoirs such as lymph nodes. Additionally, there are limited targets for an immune response due to the low expression of antigens by the cornea. There are also minimal resident APCs (including Langerhans cells) present contributing to the cornea's immune privilege [3••].

High-Risk Keratoplasty Features

In an effort to prognosticate keratoplasty outcomes, recipients are often labeled as either low-risk or high-risk. Low-risk patients are characterized as having an overall healthy cornea, where human leukocyte antigens (HLA) I are only detected on the epithelial layer with zero HLA I and II antigens on the endothelial layer; additionally, there are minimal APCs in the epithelium and near the stromal limbus [7, 8]. The rejection rate of low-risk keratoplasties may be less than 10% at the 5-year follow-up. Conversely, high-risk keratoplasties can have a rejection rate as high as 40–70% of cases a year [9•]. Rejection is clinically characterized by a variety of symptoms, depending on the cornea layer that has rejected. Due to the risk of subsequent failure, the most consequential type of rejection is endothelial rejection which often presents with decreased visual acuity, pain/irritation, conjunctival injection, and keratic precipitates with or without edema (Fig. 1) [10].

Rejection in high-risk patients is often due to increasing levels of angiogenesis, inflammation, and lymphangiogenesis which disturb the homeostatic environment of the cornea's site for immunological privilege [11]. Recipient corneal neovascularization has been known to highly increase graft rejection risk, which is more likely secondary to the associated increased lymphatic neovascularization (compared to blood vessel neovascularization) [12]. Additionally, previous graft rejection and repeat transplantation

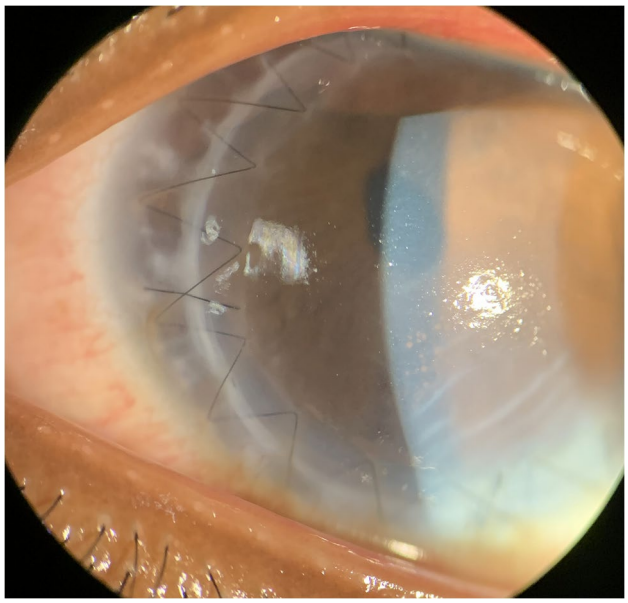

Fig. 1 Slit lamp photograph of high-risk penetrating keratoplasty (history of multiple grafts, large diameter) presenting with acute rejection. Note the conjunctival injection and inferior keratic precipitates and corneal edema

(i.e., multiple grafts) have been shown to be risk factors for rejection [13••]. Despite being one of the most common indications for PK, regrafts have significantly lower 5-year and 10-year survival rates, 53% and 41% for initial regrafts, respectively [14, 15]. Additionally, large-diameter grafts (more transplanted tissue and often closer proximity to the limbal vasculature) and decentered/peripheral grafts encroaching the limbus can have a higher risk of rejection. The presence of Langerhans cells in the peripheral cornea may increase the rejection risk when the graft-host-junction extends near the limbus. Host factors such as preoperative corticosteroid use that raises intraocular pressure or glaucoma are also associated with increased graft rejection [16]. Contact of the donor with the host vascular system via iris synechiae to the graft-host-junction and prior intraocular surgery can increase risk of rejection [17]. Furthermore, keratoplasties performed in the presence of active inflammation are more likely to reject than those performed in non-inflamed eyes [18]. Ultimately, the number of quadrants (typically more than 2) with neovascularization is the most important factor when considering the risk level of the eye [19].

Predictive Markers of Rejection

Solid organ transplantation specialists use serological markers like panel-reactive antibodies (PRA) to assess graft rejection risk. PRA is assessed by exposing the patient's serum to lymphocytes from a 100-donor panel. The patient's PRA

value is a percentage (0 to 100%), with a higher percentage correlated with a higher risk of rejection [20•]. While not often utilized clinically, there are several studies that have looked at PRA for corneal transplantation and found an elevated risk of rejection with higher PRA values, particularly posttransplant antibody development [21, 22]. Another study found that antibodies directed against donor class I HLA antigens following PK in high-risk patients were associated with immune graft rejection and can be an indicator of allograft rejection [23]. In contrast, however, it has been shown that anti-HLA antibodies after PK in rejecting patients was low [24].

Over a decade ago, our group first began incorporating PRA into our preoperative screening and donor selection process, not for corneal transplantation, but rather for ocular surface stem cell transplantation (OSST) in patients with limbal stem cell deficiency (LSCD). This was used first in our high-risk patients and now more routinely. We previously noted patients undergoing OSST with a PRA ≥ 80% had a relative risk of 2.8 of OSST graft failure and 0.6 of graft rejection [20•]. When looking at PRA in our patients with multiple keratoplasty grafts (most without LSCD), we found that patients who had undergone multiple PKs were associated with an elevated PRA [25•]. Specifically, patients with multiple PKs (range 2–12) had a mean PRA level of 34.2% as compared to single PK patients (mean PRA 6.3%). There was a moderately positive correlation between the number of PK grafts and PRA level [25•]. While PRA may serve as a prognostic factor in the preoperative assessment of repeat PK patients, it is not routinely covered by insurance in the USA.

Tissue Matching

HLA typing before surgical intervention is necessary for most organ transplants but is not commonplace among keratoplasties due to the cornea's immunological privilege and avascular status. Due to the original paper published by The Collaborative Corneal Transplantation Studies Research Group, it was deemed a non-factor to have matches when HLA typing [26]. However, there has been some controversy regarding the data interpretation and drawn conclusions [23]. Due to the burdensome cost of HLA typing tests and a minimal chance of rejection with low-risk patients, it is encouraged but not necessary for patients in this category to undergo HLA matching [27, 28]. With HLA matching, the graft survival rate is highly successful at around 90% with low-risk patients even after a 10 year follow-up [27]. Another study demonstrated low-risk patients are also at a high risk of rejection if tissue types are mismatched [29]. High-risk patients may have compromised the immune privilege of the eye, so HLA typing for this population may improve graft survival. HLA mismatches have been shown to increase the risk of rejection [21, 30]. More specifically, new data have concluded that HLA class I matching is associated with a higher success rate than HLA class II matching [31•]. While ABO blood type matching is not indicated for low-risk keratoplasty, ABO matching may be effective in reducing the risk of allograft rejection in high-risk corneal transplantations [32, 33].

Immunosuppressive Therapy

Immunosuppressive agents have been placed into the following categories: corticosteroids, T cell inhibitors, antimetabolites, biological response modulations, and immunomodulators. Here, we present a review evaluating the efficacy, clinical utility, and adverse event profile of immunosuppression agents in the context of high-risk keratoplasties.

Corticosteroids

Topical

While considered the mainstay for acute rejection prevention, topical corticosteroids may be used more frequently and for longer duration (indefinitely if no contraindications) postoperatively for high-risk grafts [34]. Dosing may start at every 2 to 4 h for the first few weeks with a gradual decrease over the next several months [35]. Due to its higher potency, difluprednate has shown efficacy and safety in graft rejection prevention, and it may be especially useful in high-risk grafts. High-dose difluprednate (initiated at every 1–3 h while awake) has been shown to treat PK endothelial rejection well, especially in non-high-risk grafts [36•]. Sorkin et al. found complete rejection resolution in 100% in non-high-risk grafts compared to 41.7% in high-risk grafts [37••]. Adjunct treatment (systemic or periocular corticosteroids) may be required in high-risk grafts. Monitoring for IOP elevation, toxic epitheliopathy, and cataracts is necessary.

Periocular/Intravitreal

Periocular injections include subconjunctival and subtenons corticosteroid injections (e.g., triamcinolone, dexamethasone). In a survey of cornea specialists in 2004, immediate postoperative subconjunctival injections of methylprednisolone acetate 40 mg/mL or dexamethasone disodium phosphate 0.1% were routinely used for the prophylactic management of graft rejection in high-risk patients [38•]. We have also judiciously used periocular corticosteroids 3–7 days prior to surgery or in the postoperative period to supplement topical corticosteroids. Intravitreal corticosteroid implants

may have a role in graft rejection refractory to topical, periocular, and systemic corticosteroids [39].

Systemic

Systemic corticosteroids are frequently used peri-operatively for high-risk grafts, especially in the setting of systemic inflammatory disease. Prednisone (1 mg/kg) can be started prior to or on the day of surgery with an individualized taper over the next 1–2 months [35]. Pulse intravenous therapy was equally effective in low- and high-risk grafts and may have a role [40]. While most of the described immunosuppression regimens below utilized concomitant early systemic (oral or intravenous) corticosteroids, long-term systemic corticosteroids should be avoided due to their adverse effects.

T Cell Inhibitors

Cyclosporine

Cyclosporine A (CsA) is a T cell immunosuppressant often used for solid organ transplants, although lower dosing is used for PKs [41]. By inhibiting calcineurin and IL-2 synthesis [42•], it limits the migration of T cells to the graft, suppresses neovascularization of the cornea, inhibits lymphocytic infiltration into lacrimal glands, and increases tear production [35].

Topical Cyclosporine

Based on a 2004 survey of the Cornea Society, 48% routinely managed high-risk grafts with topical CsA [34]. There are two commercially available topical CsA formulations, Restasis (0.05% CsA) and Cequa (0.09% CsA); recently, the 0.05% topical CsA has been available in a generic form. While both are used for dry eye, their efficacy in graft rejection prevention has demonstrated mixed results [38•]. Topical CsA with strengths ranging from 0.05 to 2.0% have been studied. Topical 0.05% CsA with topical corticosteroids has not shown better efficacy than topical corticosteroids alone for prevention and treatment of graft rejection [43, 44]. The use of a 0.1% solution in patients undergoing PK post-fungal keratitis revealed no recurrence of the fungal infection, with 50% of the patients having clear grafts post-op (compared to 14.3% of the control group) [45••]. Multiple studies have found topical 2% CsA useful in high-risk keratoplasties for both pediatric and adult patients, with lower rates of rejection although no significant difference in graft survival [46, 47, 48]. Although Belin et al. found 2% CsA had no significant difference in graft rejection incidence in high-risk cases, rejection reversal was seen more in the CsA group [49].

Systemic Cyclosporine

Studies have demonstrated CsA's effect on reducing corneal graft rejection in high-risk eyes but also a high incidence of adverse effects and subsequent need to discontinue the therapy [41, 50]. CsA tends to mitigate endothelial immune reactions from severe to mild [51]. Other studies noted only a moderate or no effect in these eyes [48]. A systematic review of 16 CsA studies found a mean 80.5% rejection-free rate and 85.3% clear graft survival rate at 1 year; at 3 years (6 studies), this fell to 67.6% and 54%, respectively [52]. Hill showed that longer treatment courses improved graft survival [50]. If utilized for high-risk keratoplasties, CsA likely needs long-term maintenance and not just 6–12 months to prevent long-term graft rejection [35]. Severity and incidence of rejection episodes will likely determine the necessary treatment duration and possible need for combination therapy with other agents such as azathioprine of MMF [35, 41]. Additionally, an intracameral CsA delivery system has been described for high-risk keratoplasties with long-term graft survival but needs further study for endothelial safety past 6 months [53].

There can be a high incidence of adverse events with even low dose CsA administration, reported as high as 81.8% in a retrospective study focused on adverse events [54]. Although most can be reversible, possible adverse events include hypertension, nephrotoxicity, hepatotoxicity, hirsuitism, gingival hyperplasia, neutropenia, and herpes keratitis [41].

Tacrolimus

Similar to CsA, this calcineurin inhibitor disrupts T cell activation by binding to the FK506 binding protein, decreasing the activity of calcineurin, and reducing the immunological response through NFAT and IL-2 signaling.

Topical Tacrolimus

Topical tacrolimus (both the 0.03% and 0.1%) has been a promising second-line immunosuppressant for high-risk keratoplasties [55••, 56–58]. Topical tacrolimus has been noted to be more effective for suppressing graft rejection than 1% topical CsA [55••, 57, 59, 60•]. In a randomized controlled trial, either 0.1% topical tacrolimus solution or topical 1% CsA was used after high-risk PK, with 45.8% of the CsA eyes experiencing rejection compared to 16% of the topical tacrolimus group [61••]. Another recent study similarly demonstrated lower rejection rates and higher graft survival for 0.1% topical tacrolimus compared to topical 1% CsA [62••]. Furthermore, topical tacrolimus has been shown to be as efficacious as MMF with less adverse events [63••].

While the ointment is available commercially in the 0.03% and 0.1% strengths, obtaining compounded topical drop formulations can be a barrier.

Systemic Tacrolimus

Systemic tacrolimus has been utilized in multiple studies with high-risk grafts and has yielded over 70–80% graft success rates [64, 65]. Additionally, 2 studies demonstrated improved graft survival and fewer graft rejections with tacrolimus compared to previous treatment with CsA. Only 2 eyes developed mild, reversible rejection on tacrolimus, and there was a lower incidence of adverse reactions than CsA [54, 64, 66]. Tacrolimus has demonstrated favorable long-term (5-year) PK graft survival. In a retrospective review of 35 high-risk PKs with the majority (52%) receiving tacrolimus and the rest using combined therapies or MMF, there was an overall graft survival of 73.5% with only 40% having rejected over the follow-up, and most were reversible [66]. A retrospective study on 24 pediatric PKs demonstrated the efficacy and safety of tacrolimus. One-year graft survival was 73%, 2-year survival was 67%, and there were no associated adverse reactions. Infection was the main cause with graft failure in this study, not immunological rejection [67•].

Adverse side effects with tacrolimus most commonly included hypertension, headaches, malaise, paresthesias, tremors, and increased serum creatine which were typically reversible [64]. Similar to CsA, tacrolimus requires serum level monitoring, and duration of therapy is often individualized for this patient population.

Rapamycin

Rapamycin is a bacterial macrolide that has immunosuppressive and antifungal properties. It inhibits T cell proliferation, expands regulatory T cells, and prevents both neovascular proliferation and organ transplant rejection [68]. In a pilot study comparing rapamycin and MMF, no immune reactions during 6 months of treatment were noted. However, within about 2 years, 2/10 rapamycin patients and 5/24 MMF patients experienced reversible immune reactions, with the rapamycin patients sustaining various adverse effects including hypercholesterolemia, furunculosis, exanthema, hypertriglyceridemia, lactate dehydrogenase elevation, and gastrointestinal disturbances [69]. Other reported adverse events are headaches, epistaxis, diarrhea, thrombocytopenia, and leukopenia. Rapamycin in conjunction with a drug delivery vehicle has demonstrated immunosuppressive efficacy with minimal systemic side effects [68].

Lifitegrast

Topical lifitegrast was approved for the treatment of dry eye disease in 2016 and is a lymphocyte function-associated antigen-1 (LFA-1) antagonist, where its binding to LFA-1 prevents it from binding with intracellular adhesion molecule 1 (ICAM-1) [70•]. While there have yet to be formal studies of its use in high-risk keratoplasty, we have clinically used lifitegrast 5% in a similar manner to topical CsA in this population 2–4 times daily in addition to topical corticosteroids.

Antimetabolites

Mycophenolate Mofetil

Affecting guanosine synthesis, MMF inhibits the immunological responses of T cells and B cells. In a prospective study using MMF as an adjunct to systemic and topical corticosteroids in high-risk PKs, MMF was found to reduce the risk of graft rejection by 11-fold compared to systemic and topical corticosteroids alone [71]. This confirmed earlier short- and intermediate-term results demonstrating 83% of high-risk grafts without immune reactions with MMF compared to 64.5% (control) [51, 72]. While early prospective randomized studies comparing 6 months of either systemic cyclosporin A (CsA) or MMF (with adjunct systemic corticosteroids) found no significant difference in efficacy [73, 74], a larger retrospective study found a stronger effect for MMF compared to CsA in preventing immune reactions after high-risk keratoplasty, even with a shorter course of MMF [75]. A systematic review of 4 studies using MMF found a rejection-free graft survival rate of 89.05% at 1 year (4 studies) and 76.5% at 3 years (2 studies); reversibility of rejection episodes was 91.7% [52]. More recently, systemic MMF used as adjunct therapy with systemic corticosteroids for high risk PKs demonstrated a high success rate with only 15.6% developing endothelial rejection and 9.4% failing; however, when compared to topical tacrolimus (with adjunct systemic corticosteroids), there was no significant difference [63••]. MMF can be used as a single agent or in combination with other agents such as tacrolimus, CsA, or sirolimus [76].

Side effect profiles for MMF include gastrointestinal issues, hematological issues (e.g., anemia, leukopenia), and neurovegetative disorders (e.g., tremor, vertigo) [71, 73]. Side effects are uncommon and reversible with cessation, but when they arise, they are much less severe compared to other immunosuppressive agents [71]. MMF is contraindicated in women of child bearing age because of the teratogenic propensity [77].

Methotrexate

Methotrexate is an antimetabolite drug, commonly used for ocular inflammation and autoimmune diseases, that inhibits dihydrofolate reductase leading to reduced cell proliferation and an increased rate of T cell apoptosis, ultimately altering the production of cytokines and the humoral response. Amongst a cohort of 16 high-risk keratoplasty patients administered methotrexate, graft survival was high (83.3%) at 1 year post-surgery, with a mean graft survival time of 13.25 months [78]. In a larger retrospective study conducted by the same group, the use of postoperative systemic methotrexate was compared to standard topical reagents. Here, 61.9% of the patients undergoing postoperative methotrexate therapy and 33.33% of the control group had grade 3/4 (i.e., high) clarity grafts. Those on systemic methotrexate were found to have a statistically significant chance of having a grade 4 clear graft compared to the control group (p-value 0.0374) [79••].

Adverse events included dyspepsia, bone marrow suppression, elevated aminotransferases, and stomatitis, although these can be minimized with daily folic acid supplementation [78, 79••]. Despite a paucity of literature regarding methotrexate use for keratoplasty systemic immunosuppression, this was noted to be the 3rd most frequently used agent in a 2003 German survey of cornea specialists behind CsA and MMF [80].

Azathioprine

Azathioprine is a purine analogue used for its immunosuppressive impact by affecting DNA and RNA [81]. In general, the agent is not typically used as monotherapy currently, but instead used in conjunction with prednisone or other systemic immunosuppression agents (e.g., CsA) in high-risk graft patients [35]. When used for rejection in high-risk grafts, Barraquer noted effectiveness with azathioprine in inhibiting vascular invasion of the grafts, need for less corticosteroid to treat rejection episodes, and improved visual results following allograft rejection [82]. Nguyen et al. reported its use with concomitant prednisone and CsA for a cohort of 6 repeat grafts with maintenance of clear grafts with 1–3-year follow-up. The same group published extended follow-up (2–4 years) with this regimen on 3 eyes with success on preventing and reversing graft rejection (only 1 rejection episode in 1 eye) in high-risk grafts [83, 84]. Fu et al. reported a tapering dose of azathioprine and prednisone following 6 months of intravenous cyclophosphamide for disease control of a large diameter (limbus-to-limbus) PK for peripheral ulcerative keratitis related perforation. The graft remained clear for over a decade at last follow-up [85•].

While the main reported side effect is dose-dependent bone marrow suppression, bilateral macular hemorrhage in the setting of aplastic anemia has also been reported in a keratoplasty patient on azathioprine [86, 87]. Of note, the teratogenicity of MMF has often been cited as a reason to switch to azathioprine in the context of non-ocular organ transplants and would likely be applicable for keratoplasties [77].

Biological Response Modifiers

Basiliximab

Basiliximab is a chimeric monoclonal antibody directed against the IL-2 receptor antibody, inhibiting T cell proliferation [87]. An advantage of monoclonal antibodies is their specificity toward the target antigen leading to a better safety profile. While it has been primarily used for prophylaxis of acute rejection in renal transplants, it has been used in a couple studies for treatment with penetrating keratoplasties [88]. Schmitz et al., studied combination basiliximab and CsA for 7 high-risk keratoplasties and reported only 1 graft with reversible endothelial rejection during the mean 18 months of follow-up [89]. Basiliximab has also demonstrated similar efficacy to CsA with 4/10 taking basiliximab developing immune reactions and 2 in the CsA group. However, no patients taking basiliximab demonstrated side effects while 2 taking CsA had to discontinue due to its side effects [88]. Basiliximab has also been used for induction therapy for high-risk patients [3••].

Immunomodulatory Agents

Certain immunomodulatory agents that specifically target angiogenic growth factors have been used for high-risk keratoplasty. Aflibercept is a recombinant fusion protein that exhibits a high affinity for VEGF-A factor, an angiogenic factor, and other growth factors that contribute to corneal neovascularization [90•]. In a study of high-risk keratoplasties, 2 mg of subconjunctival Aflibercept was administered pre-operatively as a monotherapy and in conjunction with laser coagulation. Over 24.5 months (mean) follow-up, rejection occurred in 23% of the monotherapy group, 35% of the combined therapy group, and 60% of a control group (no antiangiogenic therapy) [91••]. Bevacizumab is a recombinant humanized monoclonal antibody that prevents angiogenesis by blocking VEGF adhering to its receptors. Hos et al. injected bevacizumab preoperatively into 31 high-risk patient eyes along with vessel cauterization on the cornea. Over the mean 1.5-year follow-up, there were 4 graft rejections, and estimated probabilities of graft survival rate were

Table 1 Summary of topical immunosuppressive agents used for high-risk keratoplasty

Reagent	Dosage	Duration	Follow-up (mean)	Efficacy/Outcomes	Side effects
Topical corticosteroid	Prednisolone Acetate — 1% [60•] Postop: every 2, 3, and 4 h and then taper off during the 6–12-month period [38•, 60•] Rejection treatment: every hour (+ systemic) while awake with decreasing night frequency × 2 weeks; after response seen, halve frequency every week until maintenance dose [60•]	6–12 months [38•, 60•]	19 months [60•] 12 months [96•] Recommended to maintain indefinitely [38•]	Graft rejection rate of 17.1% over 1 year; high-risk keratoplasty only 15% of cohort [96•] 85.7% rejection resolution rate for rejection in high-risk keratoplasties [60•]	Ocular hypertension and cataract formation [38•, 97•]
	Dexamethasone 0.7 mg implant [39]	6 months (does not need surgical removal) [39]	6 months [39]	Successful treatment of 4 cases with rejection refractory to topical, periocular, and systemic corticosteroids [39]	Cataract formation [39]
	Difluprednate — 0.05% Every 1–3 h while awake initially and tapered based on clinical response [37••]	Tapered based on clinical response [96•]	3 months [37••]	41.7% rejection resolution rate in high-risk compared to 100% in non-high-risk eyes [37••]	Increase intraocular pressure [37••, 38•]
Topical cyclosporine	0.05% QID [43, 44]	12 months [45••]	20.2 months	No better efficacy with topical corticosteroids compared to corticosteroids alone	No systemic or local reaction
	1.00% [61••]	18 months [61••]	24 months [61••]	45.8% of the CsA eyes experienced rejection compared to 16% of the topical 0.1% tacrolimus [61••]	Not indicated [61••]
	2% QID → BID [49]	Administered 24–48 h pre-operative and tapered off in 3–6 months [49]	16 months [49]	91% of the patients grafts remained clear [49]	Not known in the study [49]
	2% (adjunct to topical corticosteroids) QID [47]	Tapered to daily over 3 months [47, 48]	Range 3 months to 3 years for pediatric patients [47]	Amongst pediatric patients: 88.9% of the CsA group and 38.5% in the control group demonstrated rejection free survival ($p = 0.0465$). The graft survival rate was 88.9% in the CsA group and 46.2% in the control group ($p = 0.6$) [47]	Moderate irritation [47]
	2% (adjunct to topical corticosteroids) QID, [48]	13 months (mean) for adult patients [48]	10 months for adult patients [48]	Amongst adult patients: 69.7% of the CsA group and 45.4% of the control group demonstrated rejection free survival ($p = 0.03$), with no statistical significance for graft survival rate ($p = 0.227$) [48]	Intraocular pressure elevation, complicated cataracts, and delayed wound healing [48]
Topical tacrolimus	0.03% qid [56, 63••]	12 months [63••]	12 months [63••]	0.03% Tacrolimus had a graft free rejection rate of 20% [63••] 0.03% Tacrolimus had an irreversible graft rejection rate of 19.4% [56]	Conjunctival injection, superficial punctate keratitis, and burning sensation [98]
	0.1% qid [65]	Clinical decision for duration [65]	12 months [65]	0.1% Tacrolimus had a 4% rejection rate and another study was 16% [65]	Conjunctival injection, superficial punctate keratitis, and burning sensation [98]

mg milligram, *QID* four times daily, *BID* twice a day, *CsA* cyclosporine A

Table 2 Summary of systemic immunosuppressive agents used for high-risk keratoplasty

Reagent	Dosage	Duration	Follow-up	Efficacy/Outcomes	Side effects
Systemic corticosteroids	Prednisone, oral — 1 mg/kg daily [41]	6–8 weeks or 1–3 months [41]	Not indicated [41]	Oral prednisone monotherapy had a graft survival rate of 62.5%	Musculoskeletal, cardiovascular, and metabolic complications. Infections [99•]
	Methylprednisolone pulse therapy — 500 mg per pulse [40]	Single pulse or 2 pulse, either 24 or 48 h later [40]	Not indicated	Rate of rejection reversal single pulse 83.3%; second pulse 24 h later 74.2%, and second pulse 48 h later 79.3% [40]	
Systemic cyclosporine	3 to 4 mg/kg daily. Goal trough range (130–170) [50]	Short-term group 6 months; long-term group 12 months [50]	Short-term group 26.7 months; long-term group 52.8 months [50]	CsA reduces failure from rejection; longer treatment was more efficacious [50]	Rash, tuberculosis abscess, hypertension, acute kidney injury, hirsutism [50]
	Loading dose 5 mg/kg/day for 3–7 days, followed by 2.5–3.5 mg/kg/day Trough levels: 120 to 150 ng/mL [41]	6 months to 1 year [41]	2.1 years [41]	The mean survival time of the grafts was 33.6 months. At last follow-up, 34.0% of grafts remained clear. Adverse effects occurred in 81.8% of subjects [41]	Hypertension, HSV keratitis, elevated liver enzymes, acute kidney injury, neutropenia, weakness, hypertrichosis, GI effects [41]
	3 mg/kg/day (IV) from day 0 to 6. PO 5 mg/kg/day. The CsA level 2 h after administration was maintained at 800 to 1000 ng/mL for the first 3 months 600 to 800 ng/mL [54]	6 months and another 6 months if tolerated well [54]	42.7 months [54]	No differences in endothelial graft rejection compared to control after 3 years of administration with monitoring [54]	Nausea, eczema, fungal keratitis, chest pain, back pain [54]
	3–5 mg/kg/day; trough levels: 75 and 180 µg/l [100]	Minimum 12 months unless limited by adverse events [100]	Median follow-up 22 months in CsA group and 27 months in control [83]	CsA resulted in 36.7% rejection as compared to 53.1% in the control group. Similar graft failure rates [100]	Uremia, acute kidney injury, hypertension, gum hyperplasia, increased sweating, backache, nausea, oral candidiasis, cramps, and peripheral paresthesias [100]
	5 mg/kg/day × 2 weeks by 3 mg/kg. Trough level: 100–150 ng/mL [48]	Mean 5.4 months [48]	60 months [48]	CsA for several months did not decrease rejection or improve the rate of graft clarity long-term when compared to control [48]	Hypertension, transient renal and liver abnormalities [48]
	Trough levels: 120–150 ng/mL [88]	6 months [88]	16 months [88]	Higher efficacy in preventing immune reactions after high-risk keratoplasty than Basilixi-mab, but higher adverse effects [88]	20% in CsA group stopped due to adverse events [88]

Table 2 (continued)

Reagent	Dosage	Duration	Follow-up	Efficacy/Outcomes	Side effects
Systemic tacrolimus	Pediatrics: 0.1 mg/kg/day (trough levels 2–4 ng/ml) × 2 years tacrolimus 0.03% ointment [67•]	24 months [67•]	77 month [67•]	15/24 of the patients had graft failures (62.5%). Only 2/15 had graft failures related to rejection. Tacrolimus benefited 1-year graft survival [67•]	Negligible immunosuppressive adverse events in any of the children [67•]
	Adults: 0.05 to 0.1 mg/kg/day (target trough levels 8 to 10 ng/mL × 2 months 5 to 6 ng/mL [54]	Minimum 6 months [54]	35 months [54]	After rejection while on CsA, 9/11 (81%) of the patients had corneal graft clarity with no signs of rejection on tacrolimus [54] The 2 patients that had reported rejection episodes were mild and reversible	Negligible irreversible side effects in this study [54]
	Adults: 2.5 mg PO qd Target trough (1–12 ug/L) [64]	18–24 months [64]	33 months [64]	43 patients total with 8 experiencing a rejection episode and 5 experiencing graft failure [64]	Common events: Exacerbation of elevated blood pressure and hypertension Rare events: GI symptoms, nephrotoxicity, paresthesias [64]
	Adults: 0.03 mg/kg/d 0.08 mg/kg/d (target trough 5–10) [66]	Clinical decision [66]	5 years [66]	Graft survival after the 5-year follow-up was 73.5% Rejection happened in 14 grafts (40%) with 10/14 grafts having reversible rejection episodes [66]	3 patients (10% of the total) had severe adverse reactions such as deranged liver function tests ($n=2$) and alopecia ($n=1$) [66]
	Adults: 4.4 mg qd (2–12 mg) [65] Target trough (1–12 ug/L) [65]	Clinical decision [65]	24 months [65]	17 patients total with 13 patients having clear grafts (76.5%). No irreversible graft rejection while on tacrolimus [65]	Hypertension and renal toxicity [65]
	0.05 to 0.1 mg/kg/day. Target trough 8 to 10 ng/mL × 2 months 5 to 6 ng/mL [101]	At least 6 months; mean treatment 18.1 months [101]	48.9 months [101]	Improved preventive effects of tacrolimus on graft rejection compared with CsA (previously failed). Reversible rejection observed in 2 eyes [101]	Nephrotoxicity, muscle pain [101]

Table 2 (continued)

Reagent	Dosage	Duration	Follow-up	Efficacy/Outcomes	Side effects
Rapamycin	Daily administration for target trough levels 4–10 ng/mL × 6 months tapered over 2 weeks [69]	6 months [69]	2 years [69]	There were no immune reactions during 6 months of treatment, but within about 2 years, 2/10 rapamycin patients vs. 5/24 MMF patients experienced reversible immune reactions [69]	Hypercholesterolemia, furunculosis, exanthema, hypertriglyceridemia, LDH elevation, GI disturbance [69]
	2 to 6 mg daily for trough level 12 and 20 ng/mL; combined with MMF 2 g daily [76]	Rapamycin 3 years, MMF 1 year [76]	Minimum 13 months [76]	5 of 6 grafts survived > 1 year with 1 developing irreversible rejection [76]	MMF: hepatotoxicity; Rapamycin: hypercholesterolemia, furunculosis, measles [76]
Mycophenolate Mofetil	1,000 mg BID × 1 month 500 mg BID × 5 month 250 mg BID × 6 months [71]	12 months [71]	24 months [71]	Reduced graft rejection by 11-fold [71]	GI, hematological, neurovegatative, teratogenic [71, 73] Minor events more common[73]
	1000 mg BID × 6 months 1000 mg daily × 6 months [63••, 71]	12 months [63••, 71, 102•]	12 months [63••]	Rejection-free graft survival of 84.4% [63••]	GI disturbances [63••]
	1000 mg BID × 6 months 500 mg BID × 2 weeks [51, 72–74]	6 months [72]	9.2–34.0 months [72]	Intermediate-term results: 83% grafts without immune reactions compared to 64.5% (control) [72]	
Methotrexate	10 mg/week starting a month before surgery, 15 mg the day of surgery, and 20 mg/week a month after surgery [78]		~ 13 months [78]	Graft survival was high at one year post surgery at 83.3%, with the mean graft survival time being 13.25 months [78]	12.5% of patients experienced dyspepsia [78]
	Postoperatively 7.5 mg/week with 10 mg folic acid given 5x/week [79••]	One year [79••]	71–73 weeks [79••]	61.9% of the patients undergoing postoperative methotrexate therapy and 33.33% of the control group had grade 3/4 (i.e. high) clarity grafts [79••]	Bone marrow suppression, elevated aminotransferases, and stomatitis; minimized by folate supplementation [79••]
Azathioprine	Minimum: 1 mg/kg of body weight daily [82]	1–3 months and then tapered off [82]	Not indicated	Aided in treatment of acute endothelial rejection of large diameter, high risk PKs, decreased need for corticosteroids [82]	Platelet depression, leukopenia, and altered liver function [82]
	Systemic triple immunosuppressive regimen: prednisone 1 mg/kg/day, cyclosporine 2–3 mg/kg/day, azathioprine 1.5 mg/kg/day [83, 84]	9–17 months, then tapered off [83, 84]	Not indicated	3 high-risk PKs remained clear with 1–3 years follow-up [83, 84]	Alopecia, elevated renal function [83, 84]

Table 2 (continued)

Reagent	Dosage	Duration	Follow-up	Efficacy/Outcomes	Side effects
Basiliximab	Group 1:20 mg (basiliximab, preoperatively and 4 days postoperatively) Group 2: CsA (target level 120–150 ng/ml) [88, 89]	6 months in CsA group	1.3 years [88]	4/10 that received basiliximab showed corneal immune reactions and 2/10 in the CsA groups. However, no basiliximab patients demonstrated side effects while two treated with CsA had to discontinue due to its side effects [88]	No adverse effects for basiliximab; CsA atypical pneumonia, cephalgia, tremors, fatigue, dizziness, leg cramps [88]
	20 mg (basiliximab, preoperatively and 4 days postoperatively) + CsA (target level 150–180 ng/ml) [89]	12–18 months of CsA [89]	18.1 months [89]	During this pilot study, only 1 reversible endothelial rejection occurred CsA had to be lowered; otherwise, no immune reactions were observed [89]	Nephrotoxicity and hypertension due to CsA [89]
Aflibercept	2 mg subconjunctival injection (alone) 2 mg subconjunctival injection (laser coagulation) [91••]	Repeated injections at 1 month intervals until graft had stable neovascularization [91••]	4–30 months [91••]	77% rejection free graft survival with injection alone 65% rejection free graft with laser coagulation [91••]	No clinical side effects observed [91••]
Bevacizumab	2.5 mg in 0.1 mL subconjunctival injection [92••]	Repeated if reperfusion of occluded blood vessels [92••]	18 months [92••]	4 graft rejections, and estimated probabilities of graft survival rate were 92.9%, 78.4%, and 78.4% for 1, 2, and 3 years, respectively [92••]	No clinical side effects observed [92••]
	Subconjunctival bevacizumab (2.5 mg/0.1 ml) + topical bevacizumab (10 mg/ml) [93••]	4 weeks [93••]	52 weeks [93••]	No significant difference in rates of endothelial rejection at 1 year. As post hoc analysis with extended follow-up demonstrated a significant decrease in rejection with bevacizumab, the study may have been underpowered [93••]	

mg milligram, *kg* kilogram, *QID* four times daily, *BID* twice a day, *HSV* herpes simplex virus, *ng* nanogram, *mL* milliliter, *μg* microgram, l liter, *PO* orally, *g* gram, *GI* gastrointestinal, *CsA* cyclosporine A, *MMF* Mycophenolate mofetil

92.9%, 78.4%, and 78.4% for 1, 2, and 3 years, respectively [92••]. A recent prospective multicenter randomized control trial comparing subconjunctival bevacizumab followed by topical bevacizumab to placebo found no significant difference in rates of endothelial rejection at 1 year. As post hoc analysis with extended follow-up demonstrated a significant effect, the study may have been underpowered [93••]. No serious adverse events were noted with these agents.

Conclusion

Here, we have presented a literature review on a variety of immunosuppressive agents used for high-risk keratoplasty patients. As these keratoplasties are at increased risk for rejection, we should make every effort to minimize rejection and failure risk as we already know each subsequent keratoplasty has a potentially shorter and lower survival rate. We have learned from our work with ocular surface stem cell transplantation that long-term allograft survival requires adequate immunosuppression (3-agent immunosuppression with MMF, tacrolimus, and tapered prednisone), with the greatest success coming from protocols based on work from our solid organ transplantation colleagues [94, 95]. As high-risk keratoplasties no longer possess the inherent factors that decrease rejection and subsequent failure, we should treat them similarly.

In general, most of the reviewed studies (see Tables 1 and 2) only had short-term and intermediate-term follow-up, and many used systemic immunosuppression for less than 1 year. Quite a few studies also noted immune reactions after immunosuppression was discontinued. Hill demonstrated that long-term therapy significantly improved graft survival [52]. Chow et al. utilized multiple regimens [66], even with 2- and 3-agent regimens, to produce high long-term survival rates.

Recommended Approach

1. No prior rejection, but high-risk keratoplasty:
    a. Potent and frequent topical corticosteroids—continued indefinitely on a tapering dose
    b. Topical tacrolimus (or CsA) as adjunctive therapy—continued indefinitely
    c. Consider systemic/periocular corticosteroids perioperatively

2. Prior rejection in high-risk keratoplasty:
    a. Above treatment
    b. Systemic MMF ± tacrolimus (consider single agent if underlying co-morbidities)
    c. Alternative systemic immunosuppressants (azathioprine, cyclosporine, methotrexate)

Due to potential adverse effects from systemic immunosuppression, it is reasonable to maintain initial high-risk grafts on combined topical non-corticosteroid immunosuppressants (i.e., cyclosporine or tacrolimus) in addition to the maintenance corticosteroid with or without peri-operative systemic/periocular corticosteroids. If there has been rejection in the past, we believe the patient needs systemic immunosuppression for long-term graft survival. MMF and tacrolimus are typically considered first with peri-operative systemic/periocular corticosteroids and maintenance topical corticosteroids and topical immunosuppressant. Unless there are underlying co-morbidities that would preclude a 3-agent immunosuppression regimen, we believe this offers the best chance to attain long-term graft survival. We have found most keratoplasty patients will be healthier and more tolerant of immunosuppression than solid organ transplant patients. Additionally, it has been helpful to involve a rheumatologist or organ transplant specialist to monitor for adverse events and laboratory abnormalities.

Declarations

Conflict of Interest AYC has consulted for LayerBio and Sight Sciences. EJH has consulted for Alcon Laboratories, Allergan, Bausch and Lomb, Kala Pharmaceuticals, Mati Pharmaceuticals, and Senju Pharmaceuticals. AMA and CBR do not have any financial disclosures.

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