




# A comprehensive review of intravitreal immunosuppressants and biologicals used in ophthalmology

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**Abstract:** Systemic immunosuppressants and biologicals have been a valuable tool in the treatment of inflammatory diseases and malignancies. The safety profile of these drugs has been debatable, especially in localized systems, such as the eye. This has led to the search for fairly local approaches, such as intravitreal, subconjunctival, and topical route of administration. Immunosuppressants have been used as a second-line drug in patients intolerant to corticosteroids or those who develop multiple recurrences on weaning corticosteroids. Similarly, biologicals have also been used as the next line of therapy, when adequate control of inflammation could not be attained or immunosuppressants were contraindicated to patients. Intravitreal immunosuppressants, such as methotrexate and sirolimus, have been extensively studied in noninfectious posterior uveitis, whereas limited studies have established the efficacy of intravitreal biologicals, such as infliximab and adalimumab. Most of these drugs have shown good safety profile and tolerability in animal studies alone and have not been studied further in human subjects. However, most of the studies in literature are single-case reports or case series which limits the level of evidence. In this comprehensive review, we discuss the mechanism of action, pharmacodynamics, pharmacokinetics, indications, efficacy, and side effects of different intravitreal immunosuppressants and biologicals that have been studied in literature.

**Keywords:** adalimumab, cyclosporine, etanercept, golimumab, infliximab, intravitreal, methotrexate, natalizumab, rituximab, sirolimus, tacrolimus

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## Introduction

In the last two decades, a large number of immunosuppressants and biologics have been studied for various indications, such as intraocular lymphoma, uveitis, age-related macular degeneration, diabetic macular edema, and proliferative vitreoretinopathy (PVR). Quite a lot of pre-clinical animal studies have provided initial data on the pharmacokinetics and safety of these molecules. Following which many of these molecules have also been tested in clinical trials. In this review, we aim to summarize the observations of these studies.

## Method of literature search

Literature search for this review was done extensively using PubMed and Cochrane database.

The following keywords and their iterations were used for the search; ‘intravitreal immunosuppressant’ and ‘intravitreal biologicals’. Out of the 9584 related articles, we excluded intravitreal steroids and systemic drugs and found studies and case reports on four immunosuppressants and six biologicals, namely ‘intravitreal methotrexate’, ‘intravitreal sirolimus’, ‘intravitreal tacrolimus’, ‘intravitreal cyclosporine’, ‘intravitreal infliximab’, ‘intravitreal adalimumab’, ‘intravitreal etanercept’, ‘intravitreal golimumab’, ‘intravitreal natalizumab’, and ‘intravitreal rituximab’. Out of 524 articles, we excluded duplications, studies with only systemic use of the drug, and narrative reviews. Articles were carefully read, and those that included animal studies, pharmacokinetic and pharmacodynamics (PKPD)

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studies, human studies, and case reports and adverse effects were considered for the review.

### Immunosuppressants

The following immunosuppressants have been used intravitreally either in human patients/subjects or in animal models (Table 1):

- Methotrexate.
- Sirolimus.
- Cyclosporine.
- Tacrolimus.

#### Methotrexate

Methotrexate (MTX) works by inhibiting the dihydrofolate reductase (DHFR) enzyme which is needed for folate synthesis, thereby inhibiting DNA synthesis and cell proliferation. MTX polyglutamates inhibit aminoimidazole-4-carboxamide ribonucleotide (AICAR) transformylase (ATIC) which leads to the release of adenosine into the extracellular matrix. This further leads to the inhibition of leukocytes, chemotaxis, and secretion of TNF-alpha, interleukin (IL)-8, and IL 6 by monocytes.<sup>1</sup> MTX was first approved by United States Food and Drug Administration (US FDA) in 1953 as a subcutaneous injection for patients with rheumatoid arthritis, polyarticular juvenile idiopathic arthritis (JIA), and recalcitrant psoriasis.<sup>2</sup> However, it has started being widely used as an oral and an intravenous agent as chemotherapy for malignant tumors, immunosuppressant for autoimmune diseases, and as an

abortifacient to terminate pregnancies.<sup>3</sup> In ophthalmology, its intravitreal use was initially restricted to the cases of intraocular lymphoma; however, currently investigators have explored its utility in many other ocular pathologies as well.

*Pharmacokinetics.* In a study in rabbits in which intravitreal injection of 800 µg/0.1 mL of MTX was administered, the volume of distribution was 1.33 mL and the drug remained over the effective dose for almost 81 h with a half-life of 5.9 h.<sup>4</sup> In another study, therapeutic levels of MTX were observed in the vitreous at 48–72 h of intravitreal injection of 400 µg/0.1 mL of the drug.<sup>5</sup>

*Animal studies.* Short-term and long-term ultrastructural changes were studied in rabbit models after injecting high-dose (800 µg/0.1 mL) MTX intravitreally at serial intervals. Early changes included retinal edema, vacuolization, and disintegration of retinal mitochondrial cells, and long-term effects included cellular disorganization in different retinal layers.<sup>6</sup> Eman *et al.* showed a significant reduction in a-wave and b-wave on electroretinogram (ERG), raised relative caspase-3 activity and typical ladder pattern of internucleosomal fragmentation on gel electrophoresis (characteristic of apoptosis) on injecting 800 µg/0.1 mL MTX into the vitreous cavity.<sup>7</sup>

Single intravitreal injection of 400 and 800 µg of MTX did not show significant anti-inflammatory effects in experimentally induced uveitis in rabbits.<sup>8</sup> However, Deng *et al.*<sup>9</sup> found intravitreal MTX to be better than dexamethasone in

**Table 1.** Properties of intravitreal immunosuppressants.

Drug	Mechanism of action	First FDA approval	Route of administration	Systemic dose	Intravitreal dose	Vitreous half-life (days)
Methotrexate	DHFR enzyme inhibitor	1953 for RA and JIA	Intravenous, intramuscular, subcutaneous, oral, and intrathecal	Dose varies widely	400 µg/0.1 mL	5.9 h
Sirolimus	mTOR inhibition	2015 for LAM	Oral	Dose varies widely	352 µg	8–9 days
Cyclosporine	Calcineurin inhibitor	1983 for organ transplantation	Oral	Dose varies widely	–	NK
Tacrolimus	Calcineurin inhibitor	1994 for organ transplantation	Oral	Dose varies widely	–	NK

DHFR, dihydrofolate reductase; FDA, Food and Drug Administration; JIA, juvenile idiopathic arthritis; LAM, lymphangioidiomyomatosis; mTOR, mammalian target of rapamycin; NK, not known; RA, rheumatoid arthritis.

reducing inflammation in experimentally induced bacterial endophthalmitis with *Staphylococcus epidermidis* in rabbits. Drug-loaded hydrogels in a portable iontophoretic device,<sup>10</sup> chitosan, and polylactic acid-based MTX intravitreal microimplants,<sup>11</sup> along with lipophilic surface modification using Poly(lactic-co-glycolic acid) (PLGA)<sup>12</sup> and biodegradable microneedle implants were studied and all of them showed increased duration and safe release of the drug when injected into the vitreous cavity.<sup>13,14</sup>

*Vitreoretinal lymphoma.* Intravitreal MTX has been the drug of choice in vitreoretinal lymphomas (especially unilateral cases) and is usually combined with systemic chemotherapy and radiotherapy in bilateral cases and those associated with central nervous system [primary central nervous system lymphoma (PCNSL)].<sup>15</sup> There is a single case report describing complete remission with bilateral intravitreal MTX alone in bilateral primary intraocular lymphoma (PIOL) without CNS involvement.<sup>16</sup> The patient did not show any signs of recurrence or CNS disease until 15 months follow-up. Intravitreal MTX does not protect against CNS involvement and systemic therapy is required to treat concurrent CNS lymphoma. The preferred dose of the intravitreal regimen, described by Fishburne *et al.*,<sup>17</sup> is 400 µg/0.1 mL. Twice weekly injections are recommended in the first 4 weeks, followed by once weekly for 8 weeks, and once monthly for the next 9 months. A single dose of intravitreal MTX (400 µg/0.1 mL) was effective for 5 days.<sup>18</sup> The safety and efficacy of this regimen was evaluated by Smith *et al.*,<sup>19</sup> and they found that all patients were clinically cleared of the malignant cells within a maximum of 12 injections. Another study by Ma *et al.*<sup>20</sup> showed complete remission of PIOL in cases treated with a combination of intravitreal MTX and intravenous high-dose MTX. Many other studies have shown similar remission rates with the same regimen.<sup>21–23</sup> Intravitreal MTX has also been combined with intravitreal rituximab to reduce the toxicity of MTX.<sup>24</sup> Systemic high-dose MTX following intravitreal regimen showed a better duration of CNS lymphoma-free survival than those treated with intravitreal regimen alone.<sup>25,26</sup> A study also showed that the combination of treatment modalities (intravitreal MTX injections, MTX-based systemic induction chemotherapy and consolidation high-dose cytarabine and reduced-dose whole brain radiation therapy) for PIOL showed reduction in CNS relapse and increase in

progression-free survival and overall survival without change in cognitive dysfunction.<sup>25</sup> Patients who underwent combined systemic and intraocular chemotherapy had better CNS relapse-free survival, failure-free survival, and overall survival rates according to a single-center experience of 28 years which studied primary vitreoretinal lymphoma (PVRL), concurrent VRL and CNSL, and secondary VRL. Patients with PVRL had significantly better overall survival compared to the concurrent disease.<sup>26</sup> A single case report showed complete regression of PIOL lesion with repeated cycles of intravitreal MTX (three doses at Days 1, 5, and 8) followed by subtenon dexamethasone (on Day 9) at 4- to 6-week intervals. This regimen can serve as a palliative therapy for certain patients.<sup>27</sup> Intravitreal MTX has been effective as a rescue treatment in relapse of PIOL following systemic chemotherapy and radiotherapy.<sup>18,28,29</sup> A case of massive subretinal lymphoma was treated with monthly injection of MTX and rituximab for 3 months and showed massive reduction in the size of the lesion and significant improvement of vision without any CNS or ocular relapse.<sup>30</sup> Another case of diffuse large B-cell lymphoma (DLBCL) with bilateral iris involvement also showed significant reduction of tumor size with six doses of intravitreal MTX.<sup>31</sup>

*Mantle cell lymphoma.* Combination of chemotherapy, systemic ibrutinib, intravitreal MTX, and rituximab has shown marked improvement in a patient with anterior segment metastasis of mantle cell lymphoma.<sup>32</sup> A diagnosed case of mantle cell lymphoma on systemic chemotherapy (CHOP regimen) was presented with bilateral ciliary body mass and anterior uveitis with hypopyon in the left eye. The patient underwent 40 Gy external beam irradiation in 20 fractions over a period of 1 month followed by multiple doses of intravitreal MTX (400 µg/0.1 mL) in the left eye over 12 months.<sup>33</sup> Complete regression of the ciliary body tumor was achieved with improvement of best-corrected visual acuity (BCVA) following cataract surgery. A case of immunohistopathological biopsy proven that bilateral optic nerve infiltration of mantle cell lymphoma was treated successfully with a series of bilateral intravitreal MTX and systemic ibrutinib.<sup>34</sup>

*Intraocular T cell lymphoma.* Biopsy-proven primary vitreoretinal natural killer/T cell lymphoma with breast involvement resolved with chemotherapy, intravitreal MTX, and ocular radiotherapy.<sup>35</sup> Two patients with ocular involvement following

systemic natural killer/T cell lymphoma achieved complete remission after single injection of intravitreal MTX.<sup>36</sup> Another patient who presented with intraocular T cell lymphoma as a manifestation of mycosis fungoides was also successfully treated with intravitreal MTX.<sup>37</sup> A patient who was diagnosed to have NK/T cell lymphoma of the testes, developed nasopharyngeal (underwent surgical resection) and ocular metastasis post orchiectomy. The patient achieved complete remission following serial injections of MTX in both eyes, intravenous MTX, and whole-body radiation therapy.<sup>38</sup> The intravitreal dose may be effective as a palliative, vision-restoring measure in patients with systemic T cell lymphoma with ocular involvement.

*Ocular leukemic infiltrates.* A retrospective study of 11 eyes of leukemia with ocular tumor infiltration undergoing serial intravitreal MTX injections showed clinical resolution of the inflammatory response with regression of tumor cell infiltrates.<sup>39</sup> In another report, a case of unilateral iris and ciliary relapse of acute lymphoblastic leukemia was successfully treated with intravitreal MTX with complete regression.<sup>40</sup>

*Acute exudative polymorphous vitelliform maculopathy.* Acute exudative polymorphous vitelliform maculopathy (AEPVM) following cutaneous melanoma which was refractory to oral prednisolone, intravitreal triamcinolone, and bevacizumab showed complete resolution of subretinal fluid with significant gain of vision with a single dose of intravitreal MTX.<sup>41</sup>

*Monitoring of therapy.* Serial optical coherence tomography (OCT) scans can be used to monitor patients treated with intravitreal MTX for PVRL. OCT features at initial visit (vitreous cells, intraretinal infiltration, subretinal hyperreflective infiltration, outer retina fuzzy borders, pigment epithelium detachments, and subretinal fluid) showed resolution on serial OCT (except vitreous cells) with serial injections of intravitreal MTX.<sup>42</sup>

A high IL10/IL6 ratio from the aqueous humor has been used for the diagnosis of intraocular lymphoma. Serial decrease in IL-10 and IL10/IL6 ratio has been observed with serial injections of MTX and thus can aid in monitoring therapy.<sup>43-45</sup>

A recent case report showed disappearance of MYD88 L265 P mutation from the aqueous

samples of both the eyes of a case of PVRL on serial injections of intravitreal MTX and this could also be a possible tool for monitoring disease course in the future.<sup>46</sup>

Until 2006, the only indication of use of intravitreal MTX was intraocular lymphoma. Hardwig *et al.*<sup>47</sup> subsequently evaluated its efficacy in ocular diseases other than ocular lymphoma. In this retrospective series, seven eyes with posterior uveitis, five eyes with proliferative diabetic retinopathy and tractional retinal detachment (TRD), and one patient each of epithelial downgrowth and idiopathic fibrovascular proliferation were studied. Improvement in visual acuity was seen in most patients. Visual decline in four patients was attributed to the natural history of the disease.

*Uveitis.* Systemic MTX has been widely used in different ocular inflammatory conditions. Local therapy has also been found to be effective in cases of posterior noninfectious uveitis. In a pilot study by Taylor *et al.* on cases of unilateral noninfectious uveitis with raised intraocular pressure (IOP), long periods of remission were seen with a single dose of intravitreal MTX (400 µg/0.1 mL). Relapse was seen in one-third of the patients at a mean of 4 months and were reinjected with the same dose and showed similar remission.<sup>48</sup> A similar multicentric study by the same author using intravitreal MTX as a first-line therapy for noninfectious uveitis found a good response in 30/38 eyes. There was a relapse in eight eyes at a median of 3 months and they were reinjected with same dose. Overall, an extended period of remission of over 17 months was observed. Out of 14 patients also taking systemic therapy, dose reduction could be achieved in eight patients.<sup>49</sup> However, lack of control groups or comparison with standard treatment limits the utility of this study. Macular Edema Ranibizumab v. Intravitreal Anti-inflammatory Therapy (MERIT) trial is a multicentric, randomized, interventional study which compares the efficacy of intravitreal ranibizumab (0.5 mg), MTX (0.4 mg), and dexamethasone implant (0.7 mg), and the results are expected in the end of 2021.<sup>50</sup> The results of this randomized study could help us better in knowing the efficacy of MTX in uveitis

*Choroidal tuberculoma.* Two case reports show gross reduction in the size of the tuberculoma and improvement of visual acuity following intravitreal MTX.<sup>51</sup> Another case which developed paradoxical worsening of granuloma, exudative retinal

detachment, and new yellowish white subretinal lesions on antitubercular therapy was managed with high-dose steroids, intravitreal MTX, and ranibizumab.<sup>52</sup>

*Tubercular serpiginous-like choroiditis.* A case of presumed tubercular serpiginous-like choroiditis unresponsive to anti-tubercular therapy (ATT), corticosteroids, and azathioprine was reported to develop an extended remission of over 24 months with two intravitreal MTX injections 1 month apart.<sup>53</sup> In another case series, choroidal lesion healed with a single dose of intravitreal MTX in all the three eyes with improvement in visual acuity in two.<sup>54</sup> Two patients of tuberculosis-related uveitis underwent intravitreal MTX in one eye at Week 8 of antitubercular therapy. Both patients showed resolution of leakage on fundus fluorescein angiography (FFA) from optic disk, reduction of vitritis, and slight reduction of macular edema.<sup>55</sup> Thus, intravitreal MTX may prove to be effective in reducing inflammation even in cases of infective uveitis provided administered after adequate antimicrobial therapy.

*Behcet's disease.* The role of intravitreal MTX in refractory Behcet's disease was studied in seven patients who failed to respond to conventional treatment and had raised IOP with steroid injections. After a mean of 4.3 injections (400 µg/0.1 mL every month), six patients showed significant improvement in visual acuity and four patients had considerable decrease in fluorescein leakage. Intravitreal MTX was also associated with a significant reduction of IL-6 and IL-8 levels.<sup>56</sup>

A nonrandomized comparative study compared the effectiveness of intravitreal MTX (400 µg/0.1 mL) and retrobulbar triamcinolone acetonide (40 mg/mL) in 31 adult patients with bilateral disease. Intravitreal MTX was injected into the right eye, and retrobulbar triamcinolone acetonide was injected into the left. Pre-injection data, such as anterior chamber reaction, vitreous cells, IOP, and BCVA, were comparable between the groups. Patients were examined 1 week after the injection and monthly for a period of 6 months. There was no statistically significant difference between both the groups in terms of visual acuity, anterior chamber reaction, or vitreous activity; however, post-injection IOP was elevated in two eyes in the triamcinolone group which was adequately managed with topical glaucoma medications.<sup>57</sup>

*VKH.* There is a single case report which shows the efficacy of a single dose of intravitreal MTX (400 µg/0.1 mL) in a case of Vogt-Koyanagi-Harada disease (VKH) which was previously treated with oral prednisolone and mycophenolate mofetil, subcutaneous triamcinolone, and subcutaneous adalimumab.<sup>58</sup> The patient suffered from bilateral panuveitis and was treated with oral prednisolone, mycophenolate mofetil. Two doses of subtenon triamcinolone and subcutaneous adalimumab were also administered earlier. The patient was intolerable to steroids and developed hepatotoxicity to mycophenolate. Intravitreal MTX was injected in both eyes which led to the significant improvement of vision, and this was followed by cataract surgery and intravitreal fluocinolone implant. In this case, intravitreal MTX reduced inflammation; however, as it is a single-case study, we cannot fully comment on the efficacy of this approach and the duration of action of the intravitreally injected drug.

*MFC with CNV.* Single dose of intravitreal MTX in a patient of multifocal choroiditis (MFC) with recurrent inflammatory choroidal neovascular membran (CNV) following multiple doses of intravitreal ranibizumab showed significant improvement of vision and no recurrence for over 20 months.<sup>59</sup>

*Epstein-Barr virus necrotizing retinitis.* Intravitreal MTX was found to be effective in a case of necrotizing retinitis with Epstein-Barr virus (EBV)-positive ocular fluid which was on oral methylprednisolone for 20 years for interstitial pneumonia. The disease did not respond to systemic ganciclovir or acyclovir; however, a single dose of intravitreal MTX showed significant clinical improvement with decrease in copy number of EBV-DNA.<sup>60</sup>

*CMV retinitis.* Intravitreal MTX with oral valganciclovir showed profound improvement in a patient with bilateral CMV retinitis and cystoid macular edema (CME) along with disk edema of the right eye following bone marrow transplant for acute myeloid leukemia (AML). BCVA was counting fingers in the right eye and 20/100 in the left. Serial intravitreal MTX was administered only in the right eye (worse eye). BCVA improved in both eyes (20/200 right eye (OD) and 20/63 left eye (OS)); however, CME resolution was apparent only in the right eye along with resolving disk edema, whereas it was persistent in the left eye on oral valganciclovir treatment alone.<sup>61</sup>

**Proliferative vitreoretinopathy.** MTX has shown to have a promising role in treatment and prevention of proliferative retinopathy due to its anti-inflammatory and antifibrotic activity. Elliot *et al.* studied the efficacy of serial intravitreal MTX (400 µg/0.1 mL) in cases with pre-existing PVR (PVR C or higher) and those at high risk to develop PVR following vitrectomy with silicon oil tamponade. The first dose was given intraoperatively followed by weekly doses for 2 months and one at third month (10 doses). However, 80% of the patients had attached retina at the end of 39 months and median visual acuity of 20/200.<sup>62</sup> Another retrospective study showed 100% reattachment in five patients with severe PVR and recurrent detachment who underwent post vitrectomy serial injections of 200 µg/0.05 mL intravitreal MTX for 10 weeks.<sup>63</sup> Denstedt *et al.* report a patient who suffered inadvertent needle injury during acupuncture and developed retinal detachment, vitreous hemorrhage, and a large area of bare sclera nasal to the disk. Following vitreoretinal surgery, the patient developed PVR proliferation under oil and underwent nine doses of intravitreal MTX (200 µg/0.05 mL, 2–3 weekly). This stabilized the pre-retinal fibrosis, and the patient attained a visual acuity of 20/40 at final follow-up.<sup>64</sup>

Sadaka *et al.* published their retrospective study of retinal detachment complicated with PVR who underwent vitrectomy with oil tamponade. MTX infusion (40 mg of drug in 500 mL balanced saline solution) was administered during vitrectomy. Retinal reattachment rate was 90% with 66% gaining vision better than 20/200.<sup>65</sup> Gain Understanding Against Retinal Detachment (GUARD) trial, which is a multicentric clinical trial on the efficacy of serial MTX injection (0.8 mg), has been initiated and the results are expected next year.<sup>66</sup> Intravitreal MTX in a cumulative dose of up to 1200 µg was found to be safe in silicone-filled eyes.<sup>67</sup>

**Recurrent epithelial downgrowth.** Two case reports demonstrate complete resolution with no recurrence in recurrent epithelial downgrowth. In one report, 12 weekly doses were used,<sup>68</sup> whereas the other report showed complete disappearance after 6 weekly doses.<sup>69</sup>

**Diabetic macular edema.** A prospective pilot study, which compared three monthly injections of a combination of intravitreal, bevacizumab, and MTX with intravitreal bevacizumab alone, demonstrated no significant additional benefit of

adding MTX.<sup>70</sup> In another prospective study, the use of three or more intravitreal MTX injections in eyes with persistent diabetic macular edema (DME) resulted in reduction of central macular thickness (CMT) in a significant proportion of eyes. Significant visual improvement was found only in 16.6% of eyes.<sup>71</sup>

**Exudative age related macular degeneration (AMD).** Two patients with refractory CNV secondary to age related macular degeneration (AMD) after treatment with a single dose of intravitreal MTX showed resolution of subretinal fluid with improvement in vision.<sup>72</sup>

A pilot study on seven eyes evaluating the combination of a single dose of intravitreal MTX with multiple intravitreal bevacizumab showed significant visual improvement but insignificant reduction of CMT from baseline. The authors propose that the addition of intravitreal MTX in neovascular AMD may enhance the therapeutic effect of bevacizumab and may reduce the development of a fibrous component and disciform scar.<sup>73</sup>

**Retinoblastoma.** There is a report of using serial intravitreal MTX (according to lymphoma protocol) in five eyes with retinoblastoma. Most of these eyes showed either partial or complete remission of vitreous seeds, subretinal seeds, and small retinal foci.<sup>74</sup>

**Side effects.** Corneal epithelial toxicity is a common side effect in patients receiving repeated intravitreal MTX. Other side effects that have been described are cataract, glaucoma, maculopathy, vitreous hemorrhage, optic atrophy, sterile endophthalmitis, and epiretinal membrane formation.<sup>19–21</sup>

**Corneal epithelial toxicity.** Jeong *et al.* have published a retrospective study to determine incidence, risk factors, and treatment of corneal epithelial toxicity following intravitreal MTX. *In vitro* analysis of cytotoxic effect of MTX on corneal epithelial cells was also performed. The incidence of epitheliopathy was found to be 22.7% after an average number of 5.7 injections. The average time to the development of epitheliopathy was 5.7 weeks from the first injection. The most common findings were superficial punctate erosions, central epithelial defects, and epithelial sheet-like opacity. Patients who used concurrent antiglaucoma medications were found to be at a higher risk of developing epitheliopathy. Epitheliopathy resolved in all cases in which either the MTX was stopped, or patients

were started on topical lubricants, autologous serum, topical antibiotics, topical steroids, therapeutic contact lens, oral folic acid supplementation, or topical antiviral agents. *In vitro* study revealed that MTX could suppress proliferation, induce apoptosis, and generate oxidative stress in corneal epithelial cells. Folinic acid supplementation *in vitro* showed the preservation of corneal epithelial cell viability and inhibited apoptosis.<sup>75</sup> Zhou *et al.*<sup>76</sup> found a reduction in incidence of keratopathy on changing to monthly intravitreal injections after remission of disease.

A case report mentions significant improvement in corneal toxicity within 1 week of commencing oral folic acid (1 mg daily).<sup>77</sup> Pranita *et al.* in a report successfully treated the epithelial toxicity with topical chloramphenicol, loteprednol, cyclosporine, folinic acid (5 mg/mL), and oral folic acid 5 mg once a day. This is the only case report that has used topical folinic acid drops for MTX-induced corneal toxicity.<sup>78</sup> Ghasemi Falavarjani *et al.*<sup>79</sup> found that there were no significant effects on the endothelial cell density, coefficient of variation, average cell area, hexagonality, and central corneal thickness measurements before and after injections.

**Resistance to MTX.** Resistance to multiple intravitreal doses of MTX in PVRL has also been seen. The mechanism of resistance is believed to be due to decreased expression of reduced folate carrier (RFC) and folate binding protein (FBP) and increased expression of multidrug resistance protein (MRP).<sup>80</sup> A large sample size is required to hypothesize the mechanism of resistance to MTX. Such single reports could be coincidental without any relationship. In a case report where resistance to MTX was suspected, vitrectomy was undertaken which led to the resolution of disease for more than 2 years.<sup>81</sup>

**Limitations.** Intravitreal and systemic MTX is the drug of choice in PIOL and PCNSL. However, randomized controlled trials and prospective studies are very limited in literature and most studies are single-case reports, case series, or retrospective studies without a systematic methodology. Studies with larger sample size are usually cases of PIOL/PCNSL with different treatment regimens which are not comparable.

### *Sirolimus*

Sirolimus (rapamycin) is a macrolide antibiotic produced by a strain of *Streptomyces hygroscopicus*.<sup>82</sup>

It acts by inhibiting the mammalian target of rapamycin (mTOR) which interrupts  $T_H$  activation and clonal expansion. Sirolimus also inhibits IL-2, IL-4, and IL-5 by both calcium-dependent and -independent pathways.<sup>83</sup> Sirolimus was first approved by FDA for the treatment of lymphangioleiomyomatosis. Sirolimus has also been used to coat coronary stents to prevent restenosis due to its antiproliferative effect.<sup>84</sup> Its ocular use has been mostly limited to intravitreal or subconjunctival injections for the treatment of noninfectious posterior uveitis.<sup>85</sup>

**Animal studies.** Intravitreal sirolimus (10 mg) was found to be present in vitreous even 21 days after injection in horse eyes. No ocular toxicity was seen on histopathological evaluation after 21 days.<sup>86</sup> Different doses of intravitreal sirolimus (20–1000  $\mu$ g) were injected in rabbit eyes, and no histopathological toxic effects were seen. Doses of 50, 200, and 1000  $\mu$ g showed slight decrease in amplitude on ERG but this was not dose-related.<sup>87</sup> Doses up to 220  $\mu$ g in rabbits and 352  $\mu$ g in humans were well tolerated without any adverse effects in a study by Mudumba *et al.* The drug persisted for around 90 days in the vitreous of rabbits, and very trace amounts (1.99 ng/mL) were seen in the blood sample of humans at Day 7 after the 352  $\mu$ g dose. NMDA-induced retinal damage in rats was successfully attenuated by intravitreal sirolimus by the activation of the ERK pathway in retinal Müller cells, demonstrating its neuroprotective property.<sup>88</sup> Intravitreal sirolimus also reduced the NMDA-induced inflammation and capillary degeneration in rat eyes.<sup>89</sup>

**Drug delivery systems.** PLGA sirolimus implants showed decreased inflammatory scores and significant reduction of proteins even after 35 days in treated eyes with no toxic effects in rabbit eyes.<sup>88,90</sup> Abud *et al.*<sup>91</sup> evaluated the *in vivo* and *in vitro* toxicity (rabbit eyes) of liposomal-encapsulated sirolimus (LES) and found them to be stable even after 3 months. ARPE-19 cell viability and terminal deoxynucleotidyl transferase nick-end labeling (TUNEL) assay were comparable between LES, empty liposomes, and balanced salt solution (BSS) groups, and glial fibrillary acid protein (GFAP) immunohistochemistry did not show activation of Müller cells. This shows that liposome-encapsulated sirolimus is a safe alternate drug delivery system for sustained effect. Sirolimus-loaded polymeric micelles also showed promising results in experimental autoimmune-induced uveitis (EAU) in rat models.<sup>92</sup> Engineered porous

silicon crystals enable sustained drug delivery; additionally, these allow real-time monitoring of drug release in the form of color change which can help note the real-time drug concentration in the vitreous.<sup>93,94</sup>

*Noninfectious posterior uveitis.* Sirolimus as a therapeutic approach for Uveitis (SAVE), SAVE-2, and Sirolimus Study Assessing Double-Masked Uveitis Treatment (SAKURA) were landmark trials that studied the use of intravitreal sirolimus in noninfectious uveitis patients. SAVE compared the efficacy and ocular tolerability of subconjunctival (1320 µg) and intravitreal (352 µg) injection of sirolimus at 6 months.<sup>95</sup> The injection was administered at Days 0, 60, and 120 in each group. There was significant reduction of vitreous haze from baseline in both groups with 60% showing a reduction of two steps or more and 40% showing a one-step reduction; however, on comparing the two groups, the outcome was not significant. The dose of systemic corticosteroid could be reduced to less than 10 mg/day in most patients of both the groups. One-third of the patients showed a visual gain at the end of 6 months, 50% had stable vision, and 20% had lost one more line of visual acuity. However, this outcome was not statistically significant when compared to baseline and between the two groups. Reduction in CMT was noticed at 3 months in most patients. This reduction was not maintained at sixth month with 30% having worsening of macular edema. Both the doses were well tolerated with local injection site hyperemia and chemosis in patients taking subconjunctival injections. Vitreous floaters were the most common adverse event (AE) of the intravitreal dose. Following the 6-month outcome study of SAVE trial, 12-month outcome was also studied in which the patients of the respective groups were administered injections as and when needed with a minimum gap of 2 months between two doses till 12 months.<sup>96</sup> The overall treatment frequency was two injections/eye in the intravitreal group and 2.36 injections/eye in the subconjunctival group. The results were similar to the 6-month study with 70% showing two or more step reduction of vitreous haze and 36% gaining greater than one line of visual acuity. Corticosteroid-sparing effect was seen in most patients with non-significant change in CMT.<sup>97</sup>

SAVE-2 studied the effect of two different doses of intravitreal sirolimus (monthly 440 µg and two monthly 880 µg).<sup>98</sup> The reduction of vitreous haze was significant from baseline but not

significant between the groups. Both groups did not show significant change in BCVA or CMT from baseline. There was a significant decline in the number of patients continuing corticosteroid or immunomodulators from baseline.

SAKURA study was a double-masked 6-month study which compared different doses (44, 440, and 880 µg) of a proprietary formulation of sirolimus injected intravitreally at 2-month intervals (Days 0, 60, and 120).<sup>99</sup> A significantly higher proportion of patients in the 440 µg group achieved nil vitreous haze at fifth month compared to the other two groups. Visual acuity was preserved, and corticosteroid dose was successfully tapered off in all three groups.<sup>100</sup> Another study which combined the results of SAKURA 1 and SAKURA 2 studies was carried out by Merrill *et al.*<sup>101</sup> In this study, the 880 µg dose group was removed and the other two doses were compared. The vitreous haze 0 response rate was significantly higher in the 440 µg dose group. BCVA was stable in both the groups, and corticosteroid was tapered successfully and equally in both groups.

*Geographic atrophy.* Petrou *et al.*<sup>102</sup> studied the safety and efficacy of intravitreal sirolimus (440 µg) in geographic atrophy and found new perilesional changes that were associated with accelerated retinal thinning around the geographic atrophy with no benefit in relation to change in central retinal thickness (CRT) or BCVA. Another similar study with a larger sample size found no change in the progression rate of GA area or visual acuity. Three patients developed sterile endophthalmitis which prompted early stoppage of the study due to lack of benefit.<sup>103</sup>

*Wet AMD.* A pilot study on the efficacy of intravitreal sirolimus compared the 440 µg (two monthly injections) with bevacizumab (1.25 mg) or aflibercept (2 mg).<sup>104</sup> Visual acuity outcome was similar in both groups despite having a significantly better reduction in central macular thickness in the anti-vascular endothelial growth factor (anti-VEGF) group. Serious ocular AEs, such as central retinal artery occlusion, larger subretinal hemorrhage, and anterior uveitis, with elevated IOP in the sirolimus group merit further evaluation of the drug. A report that compared two monthly intravitreal sirolimus (440 µg) with monthly anti-VEGF injections in patients with chronic neovascular AMD and persistent retinal fluid showed significant reduction in central subfield thickness and subretinal fluid. However, the



change in visual acuity and intraretinal fluid remained the same.<sup>105</sup> Another study showed that a combination of sirolimus and aflibercept showed better resolution of intraretinal and subretinal fluid compared to aflibercept monotherapy, but the reduction of CMT was insignificant.<sup>106</sup>

*Diabetic macular edema.* The safety and tolerability of local sirolimus injections was studied by injecting a single dose of different concentrations of intravitreal (44, 110, 176, 264, or 352 µg) and subconjunctival (220, 440, 880, 1320, or 1760 µg) sirolimus in patients with diabetic macular edema, and outcomes were observed at 90 days.<sup>107</sup> Serious AEs were not seen in both groups. There was improvement in visual acuity and central macular thickness in both the groups; however, since this was a phase I study, the significance was not evaluated.

### Cyclosporine

Cyclosporine is a calcineurin inhibitor and a potent immunomodulatory agent which acts mainly by inhibiting the production of cytokines (mainly IL-2) involved in the regulation of T cell activation.<sup>108</sup> Oral cyclosporine was first FDA-approved in 1983 for the treatment of organ rejection in solid organ transplantation. Topical formulation of cyclosporine was FDA-approved in 2003 under the brand name Restasis (0.05%) for chronic dry eye.<sup>109</sup> Many animal studies have been conducted to study the efficacy, toxicity, and different modes of drug delivery. However, there are no human studies or case reports till date.

*Animal studies.* Several animal studies provide evidence of intravitreal administration showing good immunosuppressive action in experimentally induced uveitis. A study by Liversidge *et al.*<sup>110</sup> showed that intravitreal cyclosporine A (CsA) suppressed the production of major histocompatibility complex (MHC) class 2 (I-a) antigen-inducing lymphokines thereby inhibiting intraocular inflammation. A study by Jaffe *et al.*<sup>111</sup> using intravitreal sustained release CsA in rats sensitized by intraocular inoculation of mycobacterium tuberculosis showed significant decrease in inflammation, reduced b-wave suppression, and reduced inflammatory changes on histopathological examination of cyclosporine-treated eyes compared to untreated eyes. High concentration of the drug was measured in the vitreous cavity even at 6.5 months, and trace amounts were present in the peripheral blood.

In a study by Wang *et al.*, intravitreal injection of CsA (42 ng/2 µL) in streptozocin-induced diabetic rats showed attenuation of retinal expression of inflammatory mediators [IL-1 beta and transforming growth factor (TGF)] and reduced edema and disorganization of the retinal layers. In a subgroup of this study, where recombinant home mobility group box 1 (HMGB 1) was injected along with CsA, suppression of HMGB 1 (proinflammatory mediator) was also noted thereby proving a protective effect on the retina.<sup>112</sup> In another study by Zong *et al.*, the a-wave and b-wave amplitudes of diabetic rats treated with cyclosporine were significantly higher than untreated diabetic rats. The ultrastructural changes seen on electron microscopy were also less severe.<sup>113</sup> A study by Karaküçük *et al.*<sup>114</sup> showed that intravitreal cyclosporine could also be used as an adjuvant to radiotherapy to inhibit intraocular proliferation following penetrating ocular injury in rabbits. Jong *et al.* studied the effect of intramuscular (25 mg/kg) and intravitreal CsA (5 mg) administered before and after the induction of herpes simplex (HSV) uveitis and found that a combination of intramuscular and intravitreal CsA before the induction of uveitis significantly reduced the uveitis.<sup>115</sup>

*Retinal toxicity.* Grisolano and Peyman<sup>116</sup> on comparing two different concentrations of cyclosporine found that 100 µg of intravitreal cyclosporine did not show any toxicity; however, the 200 µg showed adverse histologic changes in the retina in the form of patchy loss of outer segments of the retina. Alghadyan *et al.*<sup>119</sup> studied the toxicity of liposomal-bound cyclosporine in albino rats and found no adverse histological changes in doses up to 500 µg.

*Immunosuppressive agent in animal studies.* Intravitreal cyclosporine has also been used to make *in vivo* retinoblastoma models using Y79 cells injected in rats as this induced partial immunosuppression which allowed better growth of the tumor cells in the rat model.<sup>117</sup> The same was observed in another study in which human retinal pigment epithelial (RPE) xenografts injected intravitreally in rabbits survived longer in eyes with local immunosuppression using intravitreal cyclosporine implying that T lymphocytes were responsible for rejection and immunosuppression and a specific T cell inhibitor-like cyclosporine could prolong the survival of these xenografts.<sup>118</sup>

**Drug delivery.** Various sustained release drug delivery systems, such as liposome-bound drug embedded in copolymers (PLGA), biodegradable copolymer microspheres, or oleogel rods with biocompatible gelators, have been studied. Half-life of free cyclosporine was found to be about 6 h, and liposomal-bound cyclosporine was around 3 days.<sup>119</sup> Sustained release intravitreal implants can maintain therapeutic levels from 10 weeks to 3 years as shown by various studies.<sup>111,120–122</sup> Intravitreal implants can maintain a concentration of 500 ng/mL with an implant containing 10 µg over a period of 6 months<sup>121</sup> or  $0.06 \pm 0.02$  µg/mL with an implant containing 100 µg over a period of 10 weeks.<sup>122</sup> Intravitreal biodegradable implant of cyclosporine in experimental uveitis showed that the device can maintain approximately 102 ng/mL at 1–3 weeks, 491 ng/mL at 4–10 weeks, and 257 ng/mL at 14 weeks and effectively reduce intraocular inflammation with no toxicity.<sup>123</sup>

In 2006, He *et al.*<sup>124</sup> showed that intravitreal injection of biodegradable (PLGA) copolymer microspheres containing CsA in experimental uveitis in rabbits decreased intraocular inflammation significantly and could maintain a therapeutic concentration for at least 65 days in disease-related tissues, such as choroid, retina, iris, and ciliary body. However, there was reversible depression of b-wave amplitude on ERG. Pearson *et al.*<sup>122</sup> also showed reversible decrease in b-wave amplitude and lens opacification in rabbits which were not seen in Cynomolgus monkeys. Similar reversible depression of b-wave amplitude was noted in another study of sustained release of 100 µg CsA with 2 mg dexamethasone using an intravitreal device in contrast to plain dexamethasone-releasing device. No changes were seen on histological or clinical examination in both groups.<sup>122</sup> Dong *et al.*<sup>123</sup> studied the effects of oral CsA (15 mg/kg), intravitreal biodegradable cyclosporine implant (2 mg), non-medicated implant, and controls in experimental uveitis. The biodegradable implant showed better control of inflammation, less systemic toxicity, and less depression of b-wave amplitudes compared to the other three groups.

Intravitreal cyclosporine has been studied as a treatment modality in recurrent uveitis in horses.<sup>125–128</sup> Gilger *et al.*<sup>126</sup> showed that CsA implants did not completely eliminate the development of a recurrent experimental inflammatory episode in horses, but the duration and severity of

inflammation, cellular infiltration, tissue destruction, and proinflammatory cytokines levels were significantly less in the eyes implanted with the CsA device.

### Tacrolimus

**Introduction.** Tacrolimus (FK506) is an antibiotic macrolide isolated from *Streptomyces tsukubensis* and was used to prevent allograft rejection in the liver, kidney, lung, and heart transplantations. Tacrolimus forms a complex with a specific FK506-binding protein called immunophilin and inhibits the activation of calcineurin, a Ca<sup>2+</sup>-dependent enzyme, and exhibits various pharmacological actions.<sup>129</sup> The immunosuppressive action is manifested by inhibiting the nuclear factor of the activated T cell to suppress the transcription of IL-2.<sup>130</sup> Other off-label indications of oral tacrolimus include Crohn disease, graft versus host disease (GVHD), myasthenia gravis, and rheumatoid arthritis.<sup>131</sup>

**Animal studies.** A study by Passos *et al.*<sup>132</sup> showed that doses of 10 and 50 µg are non-toxic to rabbit eyes on the basis of clinical, ERG, and light microscopy findings, whereas doses of 100 and 250 µg led to transient vitreous opacities and 500 and 1000 µg of tacrolimus were toxic to retina. The group which received 500 and above showed decreased amplitude of b-wave in ERG and mild disorganization of retina on light microscopy.<sup>132</sup> Another study showed that 50 µg is toxic to the retina as swelling of the mitochondria of photoreceptors could be seen on electron microscopy in rabbit eyes, whereas 25 µg or less was safe.<sup>133</sup>

**Neuroprotection.** Tacrolimus may also have a neuroprotective effect and improve axonal regeneration following damage to peripheral nerve fibers. In a study by Rosenstiel *et al.*,<sup>134</sup> application of tacrolimus intravitreally immediately after optic nerve crush (both *in vivo* and *in vitro*) induced elongation of regenerating fibers for about 1.2 mm around the lesion. Intravitreal tacrolimus also reduces Akt and Bad dephosphorylation which are proapoptotic factors seen during ischemic damage of rat retina.<sup>135</sup>

**Experimental autoimmune-induced uveitis.** In tacrolimus-treated eyes, interferon gamma and tumor necrosis factor (TNF) alpha were inhibited.<sup>129</sup> Another study showed that intravitreal injection of 10 µg of tacrolimus was effective in suppressing ongoing EAU in rats.<sup>136</sup> They also

found that genes for molecules associated with neuroprotection (estrogen receptor, erythropoietin receptor, gamma-aminobutyric acid receptor, protein kinase C, glial cell-line-derived neurotrophic factor receptor, fibroblast growth factor, and neuropeptide Y receptor) were upregulated in the retina with intravitreal tacrolimus.

Turgut *et al.*<sup>137</sup> evaluated the efficacy of intravitreal tacrolimus in the treatment of experimental PVR and demonstrated significantly decreased levels of TGF- $\beta$ , platelet-derived growth factor, and fibroblast growth factor which are involved in PVR development.

**Drug delivery.** A study evaluated the efficacy of a biodegradable polymeric scleral plug containing tacrolimus in a rabbit model for chronic uveitis. The device maintained constant levels of 480–350 ng/g of tacrolimus in the vitreous for 4 weeks and it was effective in suppressing inflammation for at least 6 weeks.<sup>138</sup> Another study showed that treatment of EAU with intravitreal injection of liposomal tacrolimus was able to deliver 200 ng/day of tacrolimus for 14 days and significantly reduced intraocular inflammation. No toxic effects could be seen on ERG.<sup>139</sup> Tacrolimus-loaded PLGA implants and tacrolimus in functionalized silica nanoparticles with 3-aminopropyltriethoxysilane (MSNAPTES) are other safe and effective intraocular drug delivery systems that can provide a sustained intraocular concentration of tacrolimus.<sup>140,141</sup>

## Biologicals

The following biological agents have been used intravitreally either in human patients/subjects or in animal models (Table 2):

- Infliximab.
- Adalimumab.
- Rituximab.
- Etanercept.
- Natalizumab.
- Golimumab.

### *Infliximab*

Infliximab is a chimeric monoclonal IgG1 antibody made up of human constant and murine variable regions and inhibits TNF (TNF-alpha blocker). It has used as an intravenous agent since 1994 for diseases, such as rheumatoid arthritis,<sup>142</sup> inflammatory bowel disease,<sup>143</sup> ankylosing

**Table 2.** Properties of intravitreal biologicals.

Drug	Mechanism of action	Type of protein	MW (kDA)	First FDA approval	Brand name	Route of administration	Systemic dose	Intravitreal dose	Vitreous half-life (days)
Infliximab	AntiTNF(alpha)	Chimeric monoclonal IgG1 (human constant and murine variable)	149	1998 for Crohn's disease	Remicade	Intravenous	5 mg/kg	0.5–2 mg	6.5–8.5
Adalimumab	AntiTNF(alpha)	Fully human monoclonal IgG1	148	2002 for RA	Humira	Subcutaneous	40 mg	1–2 mg	NK
Etanercept	AntiTNF(alpha)	Fusion protein (Fuses TNF receptor 2 and constant end of IgG1)	150	1998 for RA	Enbrel	Subcutaneous	50 mg	2.5 mg	NK
Golimumab	AntiTNF(alpha)	Fully human monoclonal IgG1	150	2009 for RA <sup>+</sup> , PsA, and AS	Simponi	Subcutaneous Intravenous	50 mg 2 mg/kg IV	–	NK
Natalizumab	A4B1 integrin inhibitor	Human monoclonal IgG4	149	2004 for MS	Tysabri	Intravenous	300 mg	–	NK
Rituximab	Anti-CD20	Chimeric monoclonal IgG1 (human constant and murine variable)	145	1997 for NHL	Rituxan	Intravenous	375 mg/m <sup>2</sup>	1 mg	4.7

AS, ankylosing spondylitis; FDA, Food and Drug Administration; MS, multiple sclerosis; NHL, non-Hodgkin's lymphoma; NK, not known; PsA, psoriatic arthropathy; RA, rheumatoid arthritis; TNF, tumor necrosis factor; MW, molecular weight.

spondylitis,<sup>144</sup> and other systemic inflammatory diseases. However, the first report of its use as an intravitreal agent came in 2009 when it was used in the cases of neovascular AMD not responding to antiVEGF.<sup>145</sup> It was FDA-approved first as an intravenous agent for Crohn's disease in 1998 and later for diseases, such as rheumatoid arthritis, ulcerative colitis, psoriatic arthritis, ankylosing spondylitis, and plaque psoriasis.<sup>146</sup> Infliximab acts by binding to TNF receptors with high affinity and disrupts the proinflammatory cascade signaling which leads to downregulation of proinflammatory cytokines, such as IL-1, IL6, and so on, reduction of lymphocyte and leukocyte migration, apoptosis of TNF producing cells (activated monocytes), and reduction of endothelial adhesion molecules and acute phase proteins.<sup>147</sup> The molecular weight of infliximab is 149 kD, and its vitreous half-life following an intravitreal injection was found to be 6.5–8.5 days in animal models.<sup>148</sup>

*Animal studies.* Many studies have shown that intravitreal doses of upto 2 mg/0.1 mL were well tolerated in rabbit eyes.<sup>149–153</sup> Giansanti *et al.* showed significant edema of the nerve fiber layer at a dose of 3.3 mg which was not seen at lower doses. However, there was no electrophysiological change at this dose.<sup>149</sup> Another study by Theodossiadis *et al.*<sup>151</sup> observed perilimbal hyperemia and vitreous flare on Day 5 after injection of 8 mg or higher. Significant reduction in amplitude of b-wave was seen in doses of 5 mg and higher and ERG responses to 30 Hz flicker stimulus were found to be the most sensitive tool to detect retinal toxicity. Histopathological changes, such as edema of the ganglion cell layer and nerve fiber layer, microcysts in the inner layers and thickening of the outer layers, were also evident in 5 mg and higher doses. A recent study to look for dose-dependent retinal safety in albino rates showed no significant histological or electrophysiological changes at a dose as high as 7.5 mg/0.1 mL. The only significant effect at this dose was increased GFAP immunoreactivity in the Müller cells of peripheral retina which was attributed to retinal stress response.<sup>150</sup> Serial injections (2–3) of 2 mg intravitreal infliximab also did not show any significant adverse changes in the histopathology or electrophysiology of the retina even after 90 days.<sup>154</sup> The immunoreactivity of infliximab can be maintained over 6 weeks when stored at 4°C and is also suitable for compounding which could be a cost-effective alternative.<sup>155</sup>

Two studies provided evidence of suppression of ocular inflammation in experimental endotoxin-induced uveitis.<sup>156,157</sup> A study on experimental endophthalmitis using *S. epidermidis* showed that infliximab was as effective as dexamethasone in controlling inflammation when injected intravitreally along with vancomycin.<sup>158</sup> In contrast to this, two studies which compared intravenous and intravitreal infliximab showed significantly better control of inflammation with the intravenous regimen. One of these studies showed no change with intravitreal dose<sup>159</sup> while the other showed exacerbation of inflammation with intravitreal injection.<sup>160</sup> Studies on experimental laser-induced choroidal neovascularization showed dose-dependent decrease in CNV activity with the injection (1.5 mg – 11% reduction and 15 mg – 68%).<sup>161</sup> However, another study in which doses from 10 to 320 µg were given, response was seen in lower doses (up to 40 µg), and higher doses did not have any effect (80 and 320 µg). Expression of markers, such as VEGF, TGF-β, vWF, syndecan-4, and sulfated glycosaminoglycans, were also reduced in doses up to 80 µg. High-dose infliximab also showed a 20% decrease in the viability of endothelial cells.<sup>162</sup> An animal study on the use of intravitreal infliximab was done to assess the cytokine levels associated with PVR (TNF-A, IL-1, IL-6, PDGF, and TNF-B). Significant reduction of all cytokines except TGF-B was seen in the enucleated vitreous.<sup>163</sup>

*Human studies.* TNF-alpha inhibitors have been widely used in the treatment of uveitis and is currently being considered as the third step after steroids and immunosuppressants in the step ladder management of uveitis. Intravenous infusion is the most common mode of delivery. Since this can potentially have systemic side effects, intravitreal drug delivery has also been explored. Doses between 1 and 1.5 mg/0.1 mL have been used in different studies with equivocal results. Farvardin *et al.* studied the response of a single intravitreal injection of 1.5 mg/0.1 mL in eyes of chronic persistent noninfectious uveitis and found significant improvement in visual acuity, reduction in central macular thickness, and vitreous haze. However, this effect was temporary (lasted about 4 weeks) with relapse of the disease and visual acuity and central macular thickness returning to the preinjection status at 6 months.<sup>164,165</sup>

*Behcet's disease.* Hamza *et al.* had studied the effect of intravitreal infliximab in 20 eyes of refractory uveitis in Behcet's disease. Three injections

of 1 mg/0.1 mL 6 weeks apart were administered. Significant improvement in BCVA, central foveal thickness (CFT), and vitreous haze was seen in these patients, and patients with retinitis, vasculitis, and papillitis drastically improved during the course of the disease. No significant electrophysiological or ocular AEs were noted.<sup>166</sup> Another study by Markomichelakis *et al.* using a single intravitreal dose (1 mg/0.05 mL) showed significant improvement in BCVA, anterior chamber cells, vitreous haze, and CRT. Retinitis and vitritis cleared after 14 days of injection in most eyes. CME was the most resistant of all and resolved in only 2 out of 11 patients. The maximum follow-up period was only 30 days and hence long-term outcomes could not be deduced.<sup>167</sup> A recent long-term prospective study on the use of monthly intravitreal infliximab in Behcet's disease with active posterior uveitis was analyzed. Nine monthly injections of intravitreal infliximab (1 mg/0.05 mL) was administered in patients with vitritis, vasculitis, retinitis, and papillitis with proven Behcet's disease and on systemic steroids and immunosuppressants. Consecutive injections were administered only when there was clinical improvement or visual gain following each injection, and there was no evidence of adverse effects to the drug. Patients who did not improve with any dose were not given the next monthly dose of injection and were considered treatment failure. Of the 20 eyes of 14 patients who had participated in the study, 7 (35%) eyes had successful treatment of all nine doses, whereas 13 (65%) eyes were considered treatment failure. Four of these eyes had an exacerbation of the disease of which one patient had to undergo vitrectomy to control the disease. Nine eyes failed to be controlled over 6 months, and the estimated time of failure of these nine eyes was  $3.3 \pm 0.2$  months. Of the eyes that underwent successful treatment with nine doses, there was significant visual gain (20/800 to 20/80) and vitreous haze grade. Vasculitis and retinitis had also resolved in three out of four eyes, and papillitis resolved in 1/1 eyes at 3 months. ERG showed no significance in photopic and scotopic b-wave amplitude and implicit time between baseline and final follow-up in those who received all nine doses.

**Pseudophakic CME.** A retrospective study of the Pan American Collaborative Study group on the effect of intravitreal infliximab (1 mg) in patients with refractory pseudophakic CME showed significant improvement in BCVA from  $1.14 \pm 0.59$  logMAR at baseline to  $0.51 \pm 0.35$  logMAR

( $p = 0.016$ ) and reduction in CFT from  $584 \pm 159$  microns to  $327 \pm 127$  ( $p = 0.011$ ) in seven patients at 6 months. All eyes had prior treatment with topical NSAIDs, topical intravitreal, or peribulbar corticosteroids or intravitreal anti-VEGF. The average number of injections was 2.7, and there was only a single episode of uveitis in one patient which responded to topical steroids.<sup>168</sup> Lack of control group limits the application of this study.

**Age-related macular degeneration.** The first study of intravitreal infliximab was done in patients with refractory wet AMD by Theodossiadis *et al.*<sup>145</sup> in which two injections of infliximab (1 and 2 mg) were given 2 months apart. All three patients showed significant improvement in BCVA and reduction in CFT. One patient who was on anticoagulants developed spontaneous vitreous hemorrhage which resolved at 2 months. However, three more studies on its use in AMD have noted significant intraocular inflammation which required management ranging from topical steroids to vitrectomy without any improvement in the outcome of the disease.<sup>169,170,188</sup> Freitas *et al.* studied the effect of a combination of intravitreal bevacizumab and infliximab in treatment-naïve CNV and found significant reduction in subretinal and intraretinal fluid and activity of CNV in five of six patients. Cataract progressed in four of five patients and one patient developed vasculitis and vitritis 1 month after the second injection.<sup>171</sup>

**Diabetic macular edema.** A single retrospective study on the use of intravitreal TNF-alpha inhibitors in refractory DME showed no significant change in BCVA or CMT with the use of intravitreal infliximab. However, 42% (8/19) of the patients who were injected with 2 mg/0.05 mL developed severe uveitis and three of them needed vitrectomy. None of the patients in the 1 mg group developed uveitis.<sup>172</sup>

**Side effects.** Ocular side effects of intravitreal infliximab can be immunogenic (uveitis) or retinotoxic (electrophysiological changes). A prospective study by Giganti *et al.* demonstrated increased complications with low-dose intravitreal infliximab (0.5 mg) when injected in two patients of CNV and two patients of DME. None of the patients showed improvement in BCVA, CFT, or activity of CNV, and all patients worsened on angiography. Three of the four patients developed optic disk leakage, one patient developed anterior chamber (AC) and vitreous cells with acute rise in IOP, and one patient developed vitreous opacities which persisted till 12

weeks. Electrophysiological tests showed decline in amplitude and increase latency in all patients. Microperimetry showed a steady decline in all patients ranging from 20% to 73% from baseline. Three patients also developed systemic antibodies to infliximab (HACA). Other studies in literature have shown complications, such as anterior uveitis, vitritis, vasculitis, CME, and panuveitis with use of intravitreal infliximab.<sup>173</sup>

#### *Adalimumab*

Adalimumab is another TNF-alpha inhibitor and a fully human recombinant monoclonal antibody IgG1 which has similar mechanism of action to infliximab.<sup>174</sup> It was first FDA-approved in the year 2002 for subcutaneous use, and the first reported use in literature was in the cases of juvenile rheumatoid arthritis.<sup>175</sup> Its first intravitreal use was in the cases of refractory macular edema following noninfectious uveitis.<sup>176</sup> The systemic half-life of adalimumab is 14 days and its molecular weight is 148 kDa.

*Animal studies.* Inflammation (AC reaction and vitreous cells), 30% reduction in amplitudes of a- and b-wave, and histological changes (inflammatory cell infiltration) were noted in rabbits with 1 mg dose.<sup>177</sup> However, a similar study done 3 years later showed no such changes in doses up to 10 mg except for significant decrease in photopic-wave ERG response in the 10 mg dose.<sup>178</sup> Two similar studies showed that doses up to 5 mg showed no adverse changes.<sup>179,180</sup> A study to look for cytotoxicity and genotoxicity of 0.5 mg adalimumab in rabbits found no detectable toxicity up to 60 days of injection.<sup>181</sup> Intravitreal adalimumab injected in rd10 mice (model of autosomal recessive retinitis pigmentosa) was found to prevent retinal degeneration in rats.<sup>182</sup> Effect of bevacizumab and adalimumab in laser-induced CNV in rats was studied and demonstrated that adalimumab reduced the number of laser-induced grade 4 lesions by more than 50%; however, it was not as effective as bevacizumab.<sup>183</sup>

*Noninfectious uveitis.* Hamam *et al.*<sup>184</sup> studied the efficacy of intravitreal adalimumab (1.5 mg/0.03 mL) in 13 eyes with active noninfectious uveitis. Each eye was given seven injections (Day 0, 2 weeks, and then every month). The last assessment was done 1 month after the last dose (26 weeks). Treatment failure was observed in one eye, seven eyes showed significant gain of vision (>2 lines on ETDRS), and five gained one line. Five of eight

eyes had complete resolution of CME, and three eyes with AC cells and nine eyes with vitreous haze had complete resolution of inflammation. FA score decreased in all patients from baseline and vascular sheathing was the first sign to respond followed by optic disk hyperfluorescence. No adverse electrophysiological changes were noted. Another study in patients with refractory CME did not show significant improvement in visual acuity or central macular thickness after three monthly injections and 6 months follow-up.<sup>176</sup>

*Behcet's disease.* Kheir *et al.*<sup>185</sup> studied the efficacy of intravitreal adalimumab in patients of Behcet's disease with breakthrough intraocular inflammation when on systemic adalimumab. Seven eyes of four patients were included in the study and a collective of 13 attacks were noted over a mean time of 4.1 months on systemic therapy. All 13 attacks resolved with intravitreal adalimumab, 3 attacks resolved with one injection, and 10 attacks resolved after more than one injection. VA returned to baseline in 4/6 attacks, AC cells resolved in 4/8 attacks, vitreous haze reduced in 11/12, and vascular leakage reduced in 5/7 attacks.

*Pseudophakic CME.* Farvardin *et al.*<sup>186</sup> studied the efficacy of 1 mg/0.04 mL of intravitreal adalimumab in pseudophakic CME and found that none of the patients had significant difference in visual acuity or central macular thickness at 1 and 4 weeks. One eye developed severe ocular inflammation and IOP rise which could not be controlled on topical steroids and intravitreal antibiotics; hence, vitrectomy was done.

*Macular edema due to other causes.* A case series of seven patients with macular edema of different etiologies (two pseudophakic macular edema, two DME, two AMD, and one central retinal vein occlusion (CRVO)) were treated with intravitreal bevacizumab and 2 mg intravitreal adalimumab (one to four injections each). The study showed improvement in BCVA of more than three early treatment of diabetic retinopathy study (ETDRS) lines in four of seven patients, and none developed any systemic or ocular complication.<sup>187</sup>

Studies on intravitreal adalimumab in neovascular AMD and diabetic macular edema by the Pan American Collaborative Retina Study Group showed no improvement in BCVA and central macular thickness. No AEs were also recorded in both studies.<sup>172,188</sup>

### *Golimumab*

Golimumab is a novel human monoclonal TNF-alpha antibody similar to adalimumab which has a systemic half-life of 14 days and molecular weight of 150 kDa. It is usually administered subcutaneously in cases of rheumatoid arthritis, psoriatic arthritis, and HLA-B27-related arthritis and has FDA approval for the same since 2009.<sup>189</sup> Intravitreal administration has not been reported, and only one animal study has been done in which three doses were studied in albino rabbits (5, 10, and 20 mg).<sup>190</sup> Light microscopy showed decreased cell count of outer nuclear layer (ONL) and inner nuclear layer (INL) at all three doses and showed no significant difference in ganglion cell layer (GCL). TUNEL staining showed increased percentage of TUNEL-positive cells in INL (all doses) and ONL (10 and 20 mg). The percentage of TUNEL-positive cells in GCL was not significantly different from that of controls. Caspase-3 staining (irreversible point of programmed cell death) was also significantly different in ONL and INL in all doses of golimumab, while in GCL, it was not. The most prominent ultrastructural change observed on electron microscopy of the golimumab-administered eyes was karyorrhexis in the nucleus and myelin figures in the inner segments of the photoreceptor cells.

### *Natalizumab*

Natalizumab is the first selective adhesion molecule inhibitor which has been developed. It attaches to A4B1 integrin and inhibits leukocyte interaction with endothelial receptors, such as VCAM-1 and mucosal addressin cell adhesion molecule-1 and subsequently arresting their translocation and cellular inflammation.<sup>191</sup> It has been approved by FDA for the treatment of multiple sclerosis (MS)<sup>192</sup> and Crohn's disease.<sup>193</sup> In a study by Chawla *et al.*<sup>194</sup> on the safety of intravitreal natalizumab in rabbit eyes, three different doses (0.625, 1.25, and 2.5 mg) were injected intravitreally. The low and the intermediate dose did not show any ERG changes from baseline; however, the high dose (2.5 mg) showed reduction of the amplitude of both a- and b-wave. Electron microscopy of the retina of the high-dose group showed alterations in the outer plexiform (deformed synaptic ribbons) and inner nuclear layer (loss of essential organelles, vacuolation in bipolar neurons with damaged and swollen mitochondria).

### *Etanercept*

Etanercept is a soluble decoy receptor and binds to TNF1 and TNF2 receptor, thus preventing

the binding of TNF-alpha and blocking its proinflammatory effects. It consists of two p75 TNF receptors attached to the Fc portion of human IgG.<sup>195</sup> Etanercept was FDA-approved in 1998 for rheumatoid arthritis, JIA, psoriatic arthritis, and ankylosing spondylitis. Recently, it has been approved for plaque psoriasis in pediatric patients. The systemic half-life of this drug is 4–6 days and molecular weight is 150 kDa.

Animal studies showed no signs of toxicity on clinical evaluation, ERG, and histological examination at doses ranging from 100 µg to 2.5 mg.<sup>196</sup> Peak value of etanercept in the vitreous was noted till 4 weeks after injection, which started declining thereafter.<sup>197</sup> The effect of intravitreal etanercept (0.125 µg) was studied after inducing penetrating ocular injury in rabbit eyes.<sup>198</sup> Fundus examination, USG B-scan, and histopathological examination of etanercept-treated eyes showed reduced formation of PVR changes, such as vitreous haze, epiretinal membrane, retinal folds, and retinal detachment at 30 days compared to controls. RT-PCR showed significant decrease in connective tissue growth factor (CTGF), while TGF- $\alpha$  expression was the same. The study concluded that intravitreal etanercept can be potentially used to prevent PVR formation in retinal detachments. The first and only human trial of intravitreal etanercept was done in patients with diabetic macular edema in which seven eyes were treated with two doses of 2.5 mg/0.1 mL intravitreal etanercept 2 weeks apart.<sup>199</sup> There was no significant change in visual acuity and retinal hemorrhages at the end of 3 months.

### *Rituximab*

Rituximab is a chimeric mouse-human monoclonal IgG1 antibody against CD20 antigen which is found on the surface of normal and malignant B lymphocytes. Given that most cases of PVRL or PNC SL are of the CD20-positive DLBCL type, rituximab has shown good results as both systemic and an intravitreal agent.<sup>24</sup> It was first FDA-approved in 1997 as a single intravenous infusion agent in cases of B-cell non-Hodgkin's lymphoma. The aqueous and vitreous half-life of rituximab (1 mg) is 5.3 and 4.7 days, respectively, and was seen in the vitreous even at 72 days. The aqueous clearance rate of the drug when injected intravitreally is 1.2 mL/min which shows that even anterior spread of PIOL can be treated with intravitreal administration.<sup>200</sup> As per a report by Singh and Peereboom,<sup>201</sup> the drug has a low vitreous half-life and needs to be administered more

frequently (two weekly). A single injection of 1 mg/0.1 mL rituximab has been found to penetrate all layers of the retina.<sup>202</sup>

*Animal studies.* Animal studies have proven the efficacy of intravitreal rituximab in treating eyes with human CD20-transfected murine lymphomatous B cells.<sup>203</sup> A dose of 1 mg/0.1 mL has shown no evidence of clinical or histological signs of toxicity in rabbits.<sup>204</sup> Another study evaluated ERG, VEP, histology, and GFAP expression in 12 rabbits with 1 mg rituximab and found no significant changes. However, GFAP was mildly expressed in 6 of the 12 rabbits showing some amount of retinal stress.<sup>205</sup>

*PVRL (non-Hodgkin's type).* There are studies that have established the benefit of intravitreal rituximab in PVRL both as a single agent<sup>206,207</sup> or as a combination therapy with intravitreal MTX.<sup>208,209</sup> However, alternating doses of MTX and rituximab have been more attractive as it decreases the side effects of MTX.<sup>15</sup> In a study by Itty *et al.*,<sup>210</sup> a patient who developed recurrence after multiple intravitreal MTX responded on shifting to rituximab, and remission was seen after 10 injections. Following which MTX and rituximab were injected alternatively every week for 3 months and the aqueous biopsy taken after 3 months showed no relapse. However, 6 months later, the patient showed relapse and intravitreal MTX was started again. In a 5-year retrospective study by Giuffrè *et al.*<sup>211</sup> of 59 eyes of 31 patients of vitreoretinal lymphoma, 81% were found to develop CNS lymphoma. However, 9 eyes with isolated PVRL underwent intravitreal therapy alone and 32 eyes with concurrent PNCSL underwent a combination of both intravitreal and systemic chemotherapy. MTX and rituximab were injected alternatively every week for 4 weeks followed by two weekly for 3 months. None of the patients developed any complication except for four eyes with IOP rise which was controlled with topical medications. There is a single-case report of a case of choroidal lymphoma showing no response to intravitreal rituximab but achieving complete regression with external beam radiation therapy (EBRT).<sup>212</sup> In a study by Hashida *et al.*,<sup>213</sup> eyes that developed corneal epitheliopathy with intravitreal MTX were shifted to weekly injections of intravitreal rituximab for 1 month and these were repeated again in case of a recurrence. Clinical improvement was seen in all patients; however, recurrence was seen in 55% of the patients within 3 months and these needed to be treated again.

They also showed that the pike-like keratic precipitates and retinal lesions resolved with rituximab which did not resolve with MTX. IOP rise (60%) and anterior uveitis (35%) were the only adverse effects noted in these patients which were controlled with topical medications. In a study by Larkin *et al.*,<sup>214</sup> (48 eyes of which 33 eyes were treatment-naïve) 18 eyes were treated with rituximab alone and the rest were either combined with intravitreal MTX or systemic chemotherapy. Meanwhile, 31 eyes demonstrated complete remission in the study. The complications associated with rituximab were cataract in nine eyes (18.8%); elevated IOP in two eyes (4.2%); granulomatous anterior uveitis in one eye (2.1%); vitreous hemorrhage in one eye (2.1%); and rhegmatogenous retinal detachment in one eye (2.1%). A study by Cicinelli *et al.*<sup>215</sup> showed reduction of OCT features, such as hyperreflective dots, fuzzy borders, and intraretinal infiltration during remission with rituximab.

*Lymphoma relapse and rituximab.* A patient reported to have bilateral ocular relapse (left eye worse than right) of lymphoma after 10 years of parotid gland DLBCL underwent EBRT in the left eye and 12 monthly intravitreal rituximab injections in the right. The right eye showed complete remission after 12 months and BCVA improved to 20/20.<sup>216</sup> In another report, a 58-year old lady with relapse of lymphoma underwent weekly intravitreal injections for 4 weeks and subsequently did not show evidence of relapse even after 12 months of the last dose.<sup>217</sup>

Three monthly doses of intravitreal rituximab following systemic chemotherapy and intravitreal bevacizumab were given to one patient with paraneoplastic maculopathy (serous macular detachment) following an immunoproliferative disorder. However, no significant improvement in visual acuity or reduction of subretinal fluid was noted.<sup>218</sup>

A single case of occlusive vasculopathy has been reported post intravitreal rituximab therapy for intraocular lymphoma.<sup>219</sup>

#### *Ublituximab*

Ublituximab (a glycoengineered anti-human CD20 antibody) was injected intracerebrally and intravitreally in murine models of lymphoma-transfected cells at doses of 1, 5, and 20 µg, and a similar dose of 20 µg of rituximab was kept as



control. Doses of 5 and 20 µg showed pronounced effect compared to 1 and 20 µg doses showed better tumor rejection and survival rates compared to those receiving 20 µg of rituximab.<sup>220</sup>

### Conclusion

Intravitreal use of many immunosuppressants and biologics seem to be promising with good safety profile. MTX has been a frontline drug in intraocular lymphoma along with rituximab. The use of intravitreal immunosuppressants and biologics in noninfectious uveitis is gaining popularity in the last decade. As most of these agents do not increase IOP, they may find use in cases where intravitreal steroids cannot be given. Drugs, such as cyclosporine, tacrolimus, golimumab, natalizumab, and etanercept, have shown good safety profile in animal studies. Human studies and clinical trials are needed to look for their safety, tolerability, and efficacy. Many of these agents need to be injected at frequent intervals to achieve long-term benefit. Future development of innovative biodegradable drug delivery systems for these agents would increase their usage. Further studies need to be designed as controlled prospective studies using objective parameters, blinded observers, and measurements performed by an independent operator who is unaware of the treatment received.

### Author contribution(s)

**Nasiq Hasan:** Conceptualization; Formal analysis; Methodology; Writing – original draft; Writing – review & editing.

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