THE EFFECT OF DIFFERENT DOSING SCHEDULES OF INTRAVITREAL SIROLIMUS, A MAMMALIAN TARGET OF RAPAMYCIN (MTOR) INHIBITOR, IN THE TREATMENT OF NON-INFECTIOUS UVEITIS (AN AMERICAN OPHTHALMOLOGICAL SOCIETY THESIS)

By Quan Dong Nguyen, MD, MSc, Mohammad Ali Sadiq, MD (by invitation), Mohamed Kamel Soliman, MD (by invitation), Aniruddha Agarwal, MD (by invitation), Diana V. Do, MD (by invitation), Yasir J. Sepah, MBBS (by invitation)

ABSTRACT

Purpose: To determine if two different doses of intravitreal sirolimus, an mTOR inhibitor, can decrease inflammation and is safe in eyes with non-infectious posterior, intermediate, or panuveitis in the Sirolimus as a Therapeutic Approach UVEitis: Protocol-2 (SAVE-2) Study.

Methods: SAVE-2 is a prospective randomized, phase II, open-label interventional clinical trial conducted at 4 clinical centers in the United States. Eligible subjects were randomized into one of two treatments. Group 1 received 440µg of intravitreal sirolimus in study eyes on days 0, 30, 60, 90, 120, and 150; group 2 received 880µg of intravitreal sirolimus on days 0, 60, and 120. Fellow eyes were also eligible to receive sirolimus (of opposite dose to that of study eye). Primary endpoint of the study was at month 6 (M6).

Results: 24 subjects have been randomized in SAVE-2 and are included in the analysis. Vitreous haze decreased by ≥2 steps in 63.6% and 50% of patients in groups 1 and 2, respectively at M6 (p=0.695). Mean change in best-corrected visual acuity for subjects was +3.66 and -2.91 ETDRS letters in group 1 and 2, respectively. Among subjects with macular edema at baseline (n=13), the mean change in foveal thickness was -89.42µm in group 1 and +81.5µm in group 2 at M6.

Conclusions: Both low and high doses of intravitreal sirolimus were found to decrease vitreous haze in eyes with non-infectious uveitis. Low dose (440µg) sirolimus administered monthly may be more efficacious in reducing uveitic macular edema than high dose (880µg) administered every 2 months.

Trans Am Ophthalmol Soc 2016;114:T3[1-14].

INTRODUCTION

The primary goal in the management of non-infectious uveitis (NIU) is to suppress inflammation and achieve remission. NIU comprises a heterogeneous group of ocular inflammatory conditions that can differ in their clinical presentation, disease course and response to therapy.[1] Development and study of animal models of uveitis have contributed significantly to our understanding of the molecular mechanisms and various signaling pathways behind the loss of immune privilege of the eye.[2-4] Over the years, it has become increasingly clear that a broad range of activating and inhibitory targets are available to regulate the immune system that can be utilized for therapeutic benefit. Unfortunately, it is also the same exact multi-dimensional interplay of cellular pathways that makes it difficult to isolate and target a single pathway with reasonable success in controlling inflammation.

Consequently, since the 1950s, pan-blockade of cytokines via corticosteroids has been the first line of treatment for NIU. Both systemic and the local forms of corticosteroids are used to treat posterior uveitis; however, not all patients can tolerate their side effects.[5] The discovery of immunomodulatory therapy (IMT) has shifted the treatment paradigm of NIU more towards biologics, antimetabolites, and cytokine and interleukin inhibitors. Such therapies have not only been used to decrease the burden of corticosteroids but also to achieve sustained remission.[6] Drugs that primarily target T-cells, like cyclosporine and tacrolimus, have already been shown to have acceptable efficacy in cases of NIU where other therapies did not show significant improvement.[7-11]

Sirolimus, previously known as rapamycin, was isolated in the 1970's from *Streptomyces hygroscopicus* in soil samples from Easter Island. Sirolimus suppresses T-cell proliferation through the inhibition of interleukin (IL)-2, IL-4, and IL-15 employing calcium dependent or calcium independent pathways by binding to immunophilin FK binding protein 12 (FKBP-12) of the mammalian target of rapamycin (mTOR) receptor. Previously, the **S**irolimus as a Therapeutic Approach UVEitis (SAVE) Study was conducted as the first study worldwide to evaluate clinically the role of mTOR in ocular inflammation through assessing the safety and efficacy profile of locally administered sirolimus in patients with noninfectious intermediate, posterior, and panuveitis. The SAVE Study results were encouraging with more than 70% study subjects showing significant improvement of the inflammatory indices at the primary (month 6) and secondary (month 12) end points ($p<.05$).[12,13] The SAVE Study assessed the bioactivity and tolerability of 352-µg intravitreal (IVT) or 1320-µg subconjunctival (SC) sirolimus. In addition, subjects in the SAVE Study also demonstrated improvement in quality of life as measured through various parameters during the course of the 12-month study.[14]

Owing to better tolerability of the IVT administration and similar bioactivity of the IVT and SC injections, the SAVE-2 study was designed to determine whether two different doses of IVT sirolimus can decrease ocular inflammation and is safe in patients with NIU. Based on the safety and efficacy results from the preclinical and the phase I/II studies, the two IVT doses of sirolimus that were chosen to be evaluated in SAVE-2 are 440 microgram and 880 microgram. In the index manuscript, we present the results of the primary endpoint of the SAVE-2 Study to examine further the potential role of locally delivered sirolimus as an mTOR inhibitor for NIU.

Ocular Imaging Research and Reading Center (OIRRC), Omaha, Nebraska, USA

MATERIALS AND METHODS

STUDY DESIGN

The SAVE-2 Study was a multicenter, randomized, open-label clinical trial (clinicaltrials.gov identifier: NCT01280669), which evaluated the safety and efficacy of IVT sirolimus for the treatment of non-infectious intermediate, posterior or panuveitis. The Investigational New Drug (IND) for sirolimus to be studied in SAVE-2 was held by one of the authors (QDN) as the Study was investigator-led. Patients were enrolled at 4 selected sites within United States. The study adhered to the Declaration of Helsinki and was compliant with the Health Insurance Portability and Accountability Act of 1996. The study protocol was reviewed and approved by the Institutional Review Board (IRB)/Ethics Committee at each site. Written, informed consent was obtained from all patients enrolled in the study.

STUDY POPULATION

Patients with age ≥18 years, able to provide informed consent and attend all study visits, diagnosed with active, non-infectious intermediate, posterior or panuveitis, as determined by the investigator based on history, examination and laboratory evaluations, were enrolled in the study. Active uveitis was defined as having ≥ 1+ Vitreous Haze (VHZ: SUN Scale). Treatment-naïve (disease category 1) or patients receiving prednisone ≥10 mg/day (or equivalent dose of another corticosteroid) and/or or at least 1 systemic IMT other than corticosteroids (disease category 2) were included in the study. Patients deemed to have sufficient inflammation to require systemic treatment, as well as IVT treatment based on the investigator's decision, were enrolled in the study. Best-corrected visual acuity (BCVA) was required to be 20/400 or better [approximately 20 letters Early Treatment Diabetic Retinopathy Study (ETDRS) equivalent] in the study eye and 20/400 or better in the fellow eye (approximately 20 letters ETDRS equivalent).

Since all systemic IMT (other than corticosteroids) were to be discontinued 30 days prior to the administration of the first dose of the study drug, patients requiring systemic IMT for bilateral disease were excluded. Other key exclusion criteria included presence of concomitant ocular conditions such as diabetic retinopathy and macular degeneration, treatment with IVT injections (including but not limited to anti-vascular endothelial growth factors; anti-VEGF) 60 days prior to the baseline, intraocular surgery including vitreoretinal surgery within 90 days of baseline, intraocular pressure ≥25 mmHg in the study eye (glaucoma patients maintained on ≤2 topical medications with IOP <25 mmHg were allowed to participate), and presence of active or inactive ocular infections (including herpes and toxoplasmosis) and periocular infections in either eye. Patients with allergy to sirolimus, or those receiving strong enzyme inducers/inhibitors of CYP34A and P-gp (e.g. rifampin, ketoconazole), history of immunodeficiency, malignancy, metabolic dysfunction, pregnancy or lactation were excluded from the study as well.

DESIGNATION OF THE STUDY EYE

In patients with bilateral uveitis, the eye with the more advanced disease activity was chosen as the *study* eye. If both eyes were equally affected, the study eye was chosen at the investigator's discretion prior to randomization. Fellow eye received standard of care with local therapies (e.g., IVT or periocular injection of triamcinolone acetonide) at the investigator's discretion. However, if the standard-of-care local therapies were contraindicated, proved ineffective, or refused by the patient, then the investigator could decide to administer sirolimus to the fellow eye. In such a scenario, fellow eyes were *not* counted towards of the study sample size and were assigned to the treatment arm *opposite* to the study eye.

In patients with unilateral disease, if the fellow eye developed active uveitis at any point during the study period, a similar approach as with bilateral disease was adopted i.e., if IVT sirolimus was chosen over standard-of- care local therapies due to contraindication, ineffectiveness or patient refusal, dose and regimen opposite to the study eye was administered.

RANDOMIZATION AND INTERVENTION

Randomization of the patients was stratified by 2 disease categories, i.e. treatment naïve (disease category 1) and active disease receiving prednisone ≥10 mg/day (or equivalent dose of another corticosteroid) and/or or at least 1 systemic IMT other than corticosteroids (disease category 2). Patients in each category were randomized into 1 of the 2 treatment arms in a ratio of 1:1.

During the treatment period (Day 0 to month 6), patients in Group 1 (440 µg, low-dose group) were assigned to receive IVT sirolimus 440 µg in the study eye on days 0, 30, 60, 90, 120 and 150. Patients in Group 2 (880 µg, high-dose group) received IVT sirolimus 880 µg in the study eye every 2 months on days 0, 60 and 120. During the follow-up period (month 6 to 12, not reported in this manuscript), treatment for the study or the fellow eye was optional and administered in case of relapse or partial response. Patients received the same dose and regimen of treatment in the follow-up period of the study as assigned at randomization.

20 µL of 2% (22 µg/µL sirolimus) and 4% (44 µg/µL sirolimus) injectable sirolimus solutions were obtained from sterile vials to deliver the 440 and 880 microgram doses, respectively. The drug manufacturer provided specially designed plastic syringes for the SAVE-2 Study; these syringes are also being used in the currently conducted phase 3 studies of sirolimus in uveitis. Each syringe has only one specific mark at 20 µL to allow the investigators/injectors to identify clearly and exactly where 20 µL is on the syringe.

VISIT SCHEDULE

At each study visit, evaluation of the patient included a detailed history, assessment of BCVA, IOP by applanation tonometry, slit-lamp biomicroscopy, indirect ophthalmoscopy, spectral domain optical coherence tomography (OCT), and adverse events. Fluorescein angiography was performed at days 0, 30 and 180.

RESPONSE TO THERAPY

Response to sirolimus was documented according to the following definitions: (1) *No Response:* Absence of any improvement when assessed using VHZ or VCC; or worsening of primary disease, (2) *Partial Response:* Improvement of at least 1 point in VHZ or VCC, (3) *Complete Response:* A grade of 0.5+ or less in VHZ and a grade of 0.5+ or less in VCC. Worsening of the primary disease was defined as at least a 2-point increase in VHZ or VCC compared to the baseline.

RESCUE CRITERIA

Rescue therapy was defined as any systemic treatment or local treatment to the study eye, other than IVT sirolimus, given in response to worsening of the primary disease. Rescue therapy was initiated, if any, one of the following criteria were met:

Worsening of primary disease in the *study* eye (for at least 1 month following the 3rd scheduled injection of sirolimus, i.e. at month 3 in Group 1, or for at least 2 months following the 2nd scheduled injection of sirolimus, i.e.at month 4 in Group 2).

No response in the *study* eye (for at least 1 month following 5th scheduled injection of sirolimus, i.e. at month 6 in Group 1), or for at least 2 months following 3rd scheduled injection of sirolimus, i.e. at month 6 in Group 2).

No response in the *fellow* eye (for at least 2 months following the last injection of sirolimus, despite receiving 2 doses).

Worsening of the primary disease in the *fellow* eye at least 2 months following the last sirolimus injection (if the *study* eye was randomized to Group 1) or at least 1 month following the last sirolimus injection (if the *study* eye was randomized to Group 2) despite at least 2 sirolimus injections.

Severe deterioration of vision in either eye as indicated by at least doubling of the visual angle as compared to baseline.

Patients were, however, required to follow-up for safety evaluation every 2 months. In addition, patients receiving rescue therapy were not eligible for treatment in the fellow eye or for optional treatments in the study eye during the follow-up period.

OUTCOME MEASURES

The primary endpoint of the study was at month 6. Post-randomization measures of BCVA (ETDRS letters read at 4m), central macular thickness (CMT), VHZ, vitreous cells, and amount of anterior cells were summarized by visit, treatment group, and disease category within each treatment group. For those patients receiving prednisone or other corticosteroid therapy at baseline, the daily dose of prednisone (or other corticosteroid) was summarized by visit, treatment group, and disease category within treatment group. For patients who are receiving IMT prior to entering the study (and discontinuing the IMT), information about the IMT was collected as part of the patient medical history for this study. In addition, during the study, if the patients require rescue therapy with systemic IMT, for the study or fellow eyes, information on treatment dosing, frequency, and monitoring, as dictated by the treating investigators, was collected as part of the study.

Efficacy parameters at the study endpoint included proportion of patients receiving a complete or partial response in the study eye, reduction or prevention of recurrences in the study eye (frequency of attacks during the first 6-month period), and the change in BCVA, CMT, VHZ, VCC and anterior chamber cells compared to baseline.

Safety parameters that were recorded included adverse events (AE), serious adverse events (SAE), post-injection vision and IOP assessment, liver function tests, physical examination and vital signs. Ocular and non-ocular AEs were analyzed in total as well as subsets based on relationship to study drug and severity assignment.

STATISTICAL ANALYSIS

The sample size per dose group was chosen based on clinical judgment. Since the study was primarily designed to determine the safety and dosing regimen of IVT sirolimus, formal sample size calculations were not performed. Baseline and demographic details (mean and standard deviation) were summarized by treatment groups and disease categories. Non-parametric tests were used to analyze the data. Quantitative data was analyzed for significance within groups using Wilcoxon Signed Rank test and between groups using Mann Whitney U test. A p value of $<.05$ was considered to be statistically significant. Statistical analysis was performed using GraphPad Prism 6® (GraphPad Inc., La Jolla, CA, USA).

RESULTS

PATIENT POPULATION

Twenty-four subjects were enrolled in the study. Mean age of the subjects was 49.6 ± 15.4 years. The study population consisted of 7 males (29%), 23 patients were Caucasian (96%). All the patients enrolled in the SAVE-2 study had active uveitis, of which 13 subjects (54%) received *no* prior treatment (treatment-naïve: disease category 1). 11 subjects (46%) had active uveitis and were receiving prednisone ≥10 mg/day (or equivalent dose of another corticosteroid) and/or at least 1 systemic IMT other than corticosteroids (disease category 2). At baseline, 14 patients (58%) were diagnosed with posterior uveitis, 6 (25%) with intermediate uveitis and 4 (17%) with panuveitis. The most common diagnosis was birdshot chorioretinopathy. The baseline demographic characteristics of the study population and among the study groups are summarized in Table 1.

Of the 24 patients enrolled in the study, 3 patients did not complete the primary endpoint visit at month 6. One patient was lost to follow-up after month 3 (Group 1, Category 2) and a second patient (Group 2, Category 2) chose a different therapeutic option and therefore, exited the study at month 4. The third patient required rescue therapy at month 4 (Group 1, Category 1) due to worsening of inflammation. Since all the subjects completed their month 3 visit, their efficacy data was carried forward to month 6. In the final analysis, 11 patients were included in Group 1 (of which 7 patients belonged to category 1) and 13 patients in Group 2 (of which 6 patients belonged to category 1).

TABLE 1: DEMOGRAPHIC CHARACTERISTICS AND BASELINE DETAILS OF THE STUDY PARTICIPANTS

	TOTAL (*n* = 24)	**GROUP 1(*n* = 11) 440-µg SIROLIMUS**	**GROUP 2 (*n* = 13) 880-µg SIROLIMUS**
Gender n (%)			
Male	7 (29)	5 (45)	2 (15)
Female	17 (71)	6 (55)	11 (85)
Age [year (±SD)]	49.6 (±15.4)	46.3 (±18.5)	52.3 (±14.4)
Race n (%)			
White	23 (96)	11 (100)	12 (92)
Hispanic	1 (4)	0 (0)	1 (8)
Disease category n (%)			
Category 1: active without treatment	13 (54)	7 (64)	6 (46)
Category 2: active with treatment	11 (46)	4 (36)	7 (54)
Anatomical location n (%)			
Intermediate	6 (25)	2 (18)	4 (30.5)
Posterior	14 (58)	7 (64)	7 (54)
Panuveitis	4 (17)	2 (18)	2 (15.5)
Underlying disease (*n*)			
Birdshot choroidopathy	7	2	5
Multifocal choroiditis	3	1	2
VKH	1	0	1
Sarcoidosis	1	0	1
Idiopathic	12	8	4
Central Macular thickness (CMT)			
Macular edema n (%)	13 (54)	7 (63)	6 (46)
Category 1	7 (54)	4 (57)	3 (50)
Category 2	6 (46)	3 (43)	3 (50)
CMT (mean ± SD)	360 (±118)	414 (±131)	314 (±86)
Category 1	394 (±135)	432 (±152)	351 (±111)
Category 2	319 (±82)	382 (±98)	283 (±48)
CMT in patients without ME (mean ± SD)	287 (±58)	330 (±67)	262 (±38)
CMT in patients with ME (mean ± SD)	422 (±122)	461 (±139)	375 (±89)
Corticosteroid use [n (%)]	10 (42)	4 (36)	6 (46)
Corticosteroid dose (mg/day)			
Category 1 (mean ± SD)	NA	NA	NA
Category 2 (mean ± SD)	17 (±10.3)	21.3 (±13.1)	14.2 (±8)
Prior IMT use [n (%)]	11 (46)	4 (36)	7 (64)
BCVA in ETDRS score (at 4 meters)			
Category 1 (mean ± SD)	35.8 (±17.6)	28 (±17.6)	45 (±13.4)
Category 2 (mean ± SD)	38.5 (±13.4)	28 (±12.1)	44.7 (±10.1)

Category 1 indicates treatment-naïve patients. Category 2 indicates patients receiving prednisone ≥10 mg/day (or equivalent dose of another corticosteroid) and/or or at least 1 systemic immunomodulatory therapy (IMT) other than corticosteroids.

EFFICACY ANALYSIS

Vitreous Haze Scores

At month 3, 13 subjects (54.1%) showed a reduction of ≥2 steps in VHZ (five in Group 1 and eight in Group 2). Eleven subjects (45.8%) either showed no change or a reduction of less than 2 steps in VHZ (6 in Group 1 and 5 in Group 2). Figure 1 shows the change in the VHZ scores in both the groups at month 3. At month 6, 14 subjects (58.3%) showed a reduction of ≥2 steps in VHZ (seven in Group 1 and seven in Group 2). Ten subjects (41.7%) either showed no change or a reduction of less than 2 steps in VHZ (4 in Group 1 and 6 in Group 2) (Figure 2).

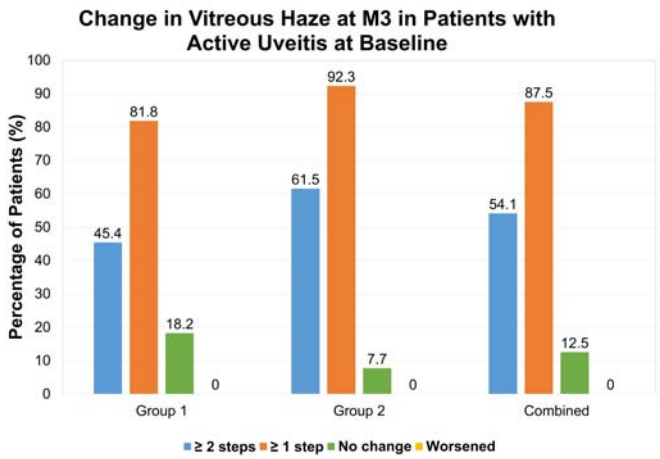

FIGURE 1

Graphical representation of the changes in vitreous haze among all subjects in the SAVE-2 study (group-wise and combined) at month 3 of the study.

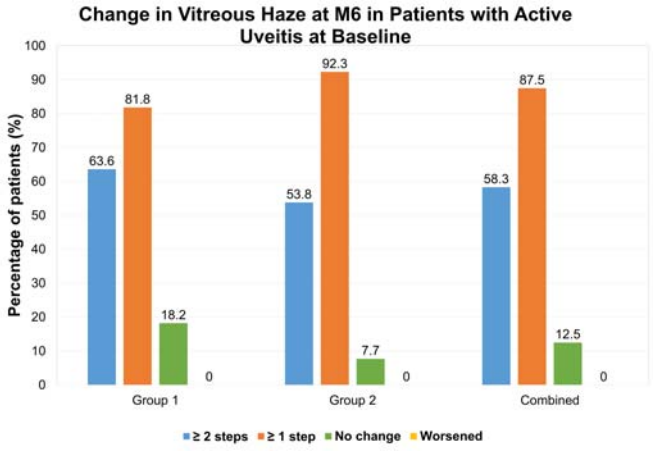

FIGURE 2

Changes in vitreous haze among all subjects in the SAVE-2 study (group-wise and combined) at month 6 (primary end-point) of the study.

In comparing both groups, the difference in VHZ reduction between the two groups was *not* statistically significant at either months 3 or 6. However, the reduction in VHZ from baseline to months 3 and baseline to month 6 was statistically significant in *both* groups ($p<.05$, Wilcoxon signed rank test).

Comparing the two disease categories at month 3, seven subjects in category 1 (53.8%) showed a reduction of ≥2 steps in VHZ (3 in group 1 and 4 in group 2) compared to six subjects (54.5%) in category 2 (2 in group 1 and 4 in group 2). Meanwhile, six subjects in category 1 (46.1%) showed either no change or a reduction of less than 2 steps in VHZ (4 in group 1 and 2 in group 2) compared to five (45.4%) subjects in category 2 (2 in group 1 and 3 in group 2).

Similarly, on comparison at month 6, nine subjects in category 1 (69.2%) showed a reduction of ≥2 steps in VHZ (5 in group 1 and 4 in group 2) compared to five subjects (45.4%) in category 2 (2 in group 1 and 3 in group 2). Meanwhile, four subjects in category 1 (30.7%) showed either no change or a reduction of less than 2 steps in VHZ (2 in group 1 and 2 in group 2) compared to six (54.5%) subjects in category 2 (two in group 1 and four in group 2).

Patients in both category 1 and 2 showed significant reduction in VHZ at both months 3 and 6; however, the differences between the two categories in VHZ reduction were not significant at either time points.

Vitreous Cell Counts

The mean VCC at baseline was 1.7 ± 0.83 (1.91 ± 0.83 and 1.69 ± 0.85, in groups 1 and 2, respectively). At month 3, 20 subjects (83.3%) showed a reduction in VCC (nine in group 1 and eleven in group 2). VCC either increased or showed no change in four subjects (16.6%) at month 3 (two in group 1 and two in group 2).

At month 6, 22 (91.7%) subjects showed a reduction in VCC (ten in group 1 and twelve in group 2). VCC either increased or showed no change in two subjects (8.3%) at month 6 (one in group 1 and one in group 2). Figure 3 demonstrates the change in the VCC in both the groups at months 3 and 6.

Visual Acuity

Mean BCVA at baseline in the study population was 37 ± 15.5 letters (28 ± 15.2 and 45 ± 11.2 letters in Groups 1 and 2, respectively). Mean BCVA was 35.8 ± 17.6 letters and 38.5 ± 13.4 letters in categories 1 and 2, respectively. The change in the BCVA in groups 1 and 2 is shown in Figures 4 and 5, respectively.

At month 3, 9 patients (37.5%) gained 1 or more lines of BCVA (5 in Group 1 and 4 in Group 2). Out of these 9 patients, 4 patients gained 2 or more lines of BCVA (2 in group 1 and 2 in group 2) and 3 patients gained 3 or more lines of BCVA (1 in group 1 and 2 in group 2). Three patients lost 1 or more lines of BCVA (1 in group 1 and 2 in group 2). Twelve subjects (50%) showed no changes in BCVA at month 3 (5 in group 1 and 7 in group 2).

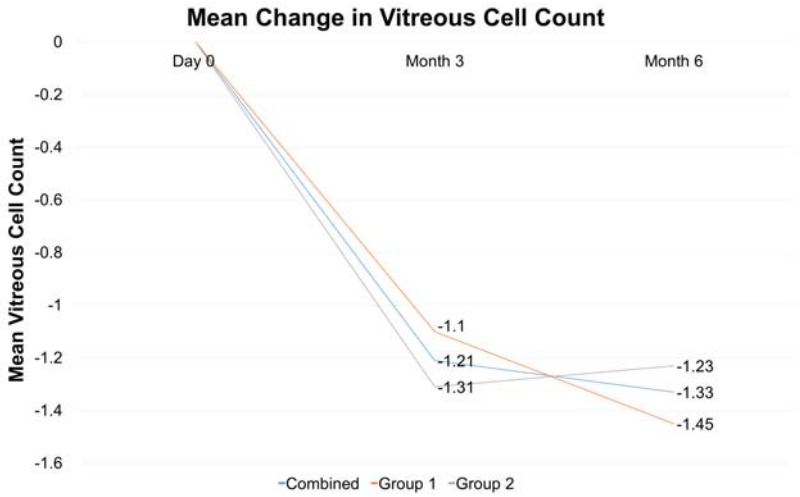

FIGURE 3

Mean decrease in vitreous cell count among all subjects in the SAVE-2 study (group-wise and combined) from baseline to month 6.

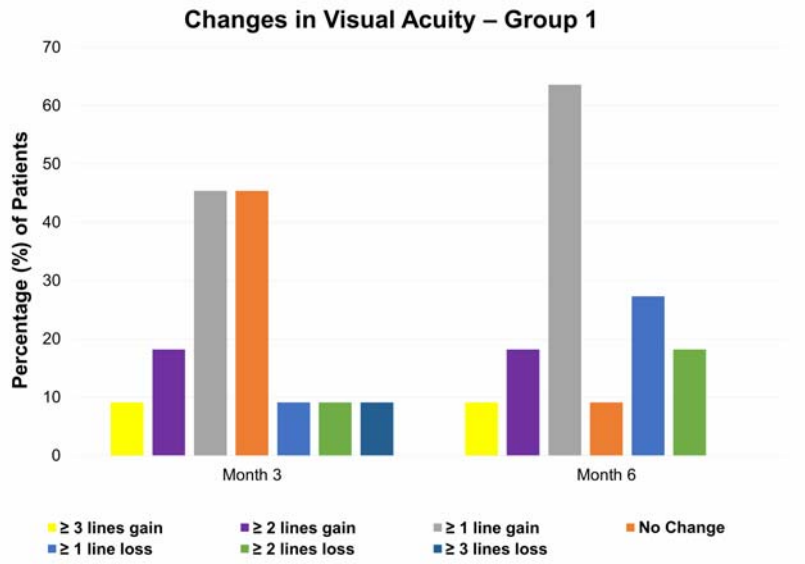

FIGURE 4

Changes in best-corrected visual acuity (ETDRS letters) among the SAVE-2 study subjects in group 1 (440-µg Sirolimus) at months 3 and 6.

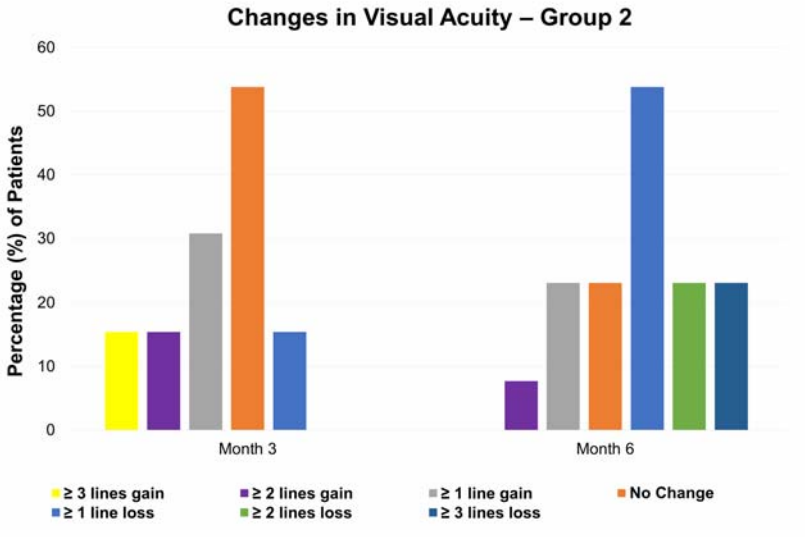

FIGURE 5

Changes in best-corrected visual acuity (ETDRS letters) among the SAVE-2 study subjects in group 2 (880-µg Sirolimus) at months 3 and 6.

At month 6, 10 subjects (41.6%) gained 1 or more lines of BCVA (7 in Group 1 and 3 in Group 2). Out of these 10 subjects, 3 subjects gained 2 or more lines of BCVA (two in Group 1 and one in Group 2) and 1 subject gained 3 or more lines of BCVA (Group 1). Ten subjects lost 1 or more lines of BCVA (three in Group 1 and seven in Group 2). Of these 10 subjects, 5 lost 2 or more lines of BCVA (two in Group 1 and three in Group 2) and three lost 3 or more lines of BCVA (all in Group 2). Among the three patients that

lost ≥ 3 lines of ETDRS BCVA, two had macular edema at baseline that demonstrated initial improvement; however, they then developed recurrent macular edema. Figure 6 shows the change in the BCVA among the patients in both groups.

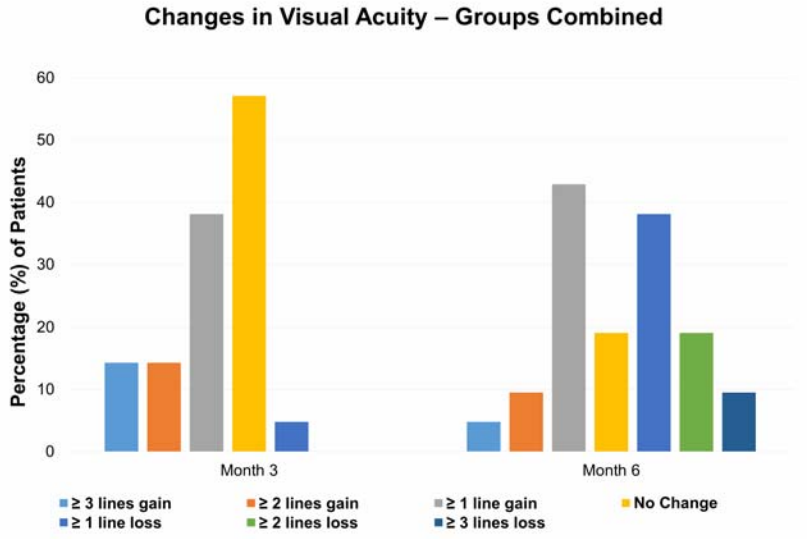

FIGURE 6

Comparison of the mean changes in best-corrected visual acuity (ETDRS letters) for all SAVE-2 study subjects (in both the groups combined) at months 3 and 6.

None of the study groups showed a statistically significant change in BCVA from baseline at both months 3 and 6 ($p>.05$; Wilcoxon rank sum test). There was also no statistically significant difference between the two treatment categories in mean change in BCVA from baseline to months 3 and 6.

Response to Treatment

At month 3, five subjects (1 from Group 1 and 4 from Group 2) achieved complete response to treatment (a grade of 0.5+ or less in VHZ and a grade of 0.5+ or less in VCC). Meanwhile, 19 subjects (10 from Group 1 and 9 from Group 2) achieved a partial response to treatment (improvement of at least 1 point in VHZ or VCC). There were no patients that showed no response to treatment (absence of any improvement when assessed using VHZ or VCC).

At month 6, the primary endpoint of the study, 8 subjects (5 from Group 1 and 3 from Group 2) achieved complete response to treatment. Meanwhile, 16 (6 from Group 1 and 10 from Group 2) patients achieved a partial response to treatment (Table 2). There were no patients that showed no response to treatment.

TABLE 2: RESPONSE TO INTRAVITREAL SIROLIMUS TREATMENT AMONG EYES WITH NON-INFECTIOUS POSTERIOR, INTERMEDIATE, OR PANUVEITIS AT MONTHS 3 AND 6

	MONTH 3	MONTH 6
Complete response [n (%)]	5 (20.8)	8 (33.3)
Partial response [n (%)]	19 (79.2)	16 (66.6)

* The table indicates data for both Group 1 (440-µg Sirolimus) and Group 2 (880-µg Sirolimus) combined.
**Complete response: A grade of 0.5+ or less in vitreous haze and a grade of 0.5+ or less in vitreous cell count. Partial response: Improvement of at least 1 point in vitreous haze or vitreous cell count.

Central Macular Thickness and Macular Edema

At baseline, 13 (54%) patients had macular edema (7 in group 1 and 6 in group 2). The average CMT of patients with macular edema was 422 µm (±122) at baseline. The mean CMT in patients without macular edema (n = 11) did not show changes from baseline in any patient, either at month 3 or at month 6, with an average thickness of 287 µm (±58), 270 µm (±65), and 280 µm (±87) at baseline, month 3, and month 6, respectively. In patients with macular edema at baseline, CMT in group 1 decreased from an average of 461 µm (±139) at baseline to 403 µm (±148) at month 3 and 419 µm (±160) at month 6, a mean change of -58 and -42.0 µm at months 3 and 6, respectively. Group 2 showed reduction of CMT from 375 µm (±89) at baseline to 313 µm (±66) at month 3 and increased to 457 µm (±204) at month 6, a mean change of -62 and +82 µm at months 3 and 6, respectively.

At month 3, nine patients (five in Group 2) showed improvement of their macular edema with three patients showing substantial reduction of their CMT. However, at month 6, only two patients continued to improve to complete resolution of edema (both belonged to Group 2), five patients (3 in Group 2) developed recurrence of macular edema and two patients (Group 1) maintained their initial

improvement.

Two patients (one in each group) had shown no change in their CMT at month 3 and month 6. The remaining two patients (Group 1) had worsened edema at month 3 which continued to worsen in one patient while completely resolved in the other at month 6. The changes of CMT from baseline were not statistically significant at either month 3 or 6. Figure 7 shows the change in the CMT in representative subjects from both groups at months 3 and 6.

Corticosteroids and Immunomodulatory Therapy Use

At baseline, ten of 24 subjects were receiving corticosteroids (42%, four in Group 1 and six in Group 2). All ten subjects were receiving ≥ 10mg/day of prednisone. Prior to screening, eleven subjects (45.8%) were receiving IMT (five in Group 1 and six in Group 2). Six patients (25%) were receiving both corticosteroids and IMT therapy prior to screening.

Only two patients at Month 3 were receiving systemic steroids (at a dose of 7.5 and 5 mg/day). Both the patients belonged to Group 2 and Category 2. Of these, only one patient was continued on steroids at 5 mg/day at Month 6. Figure 8 shows the corticosteroid-sparing effect of sirolimus.

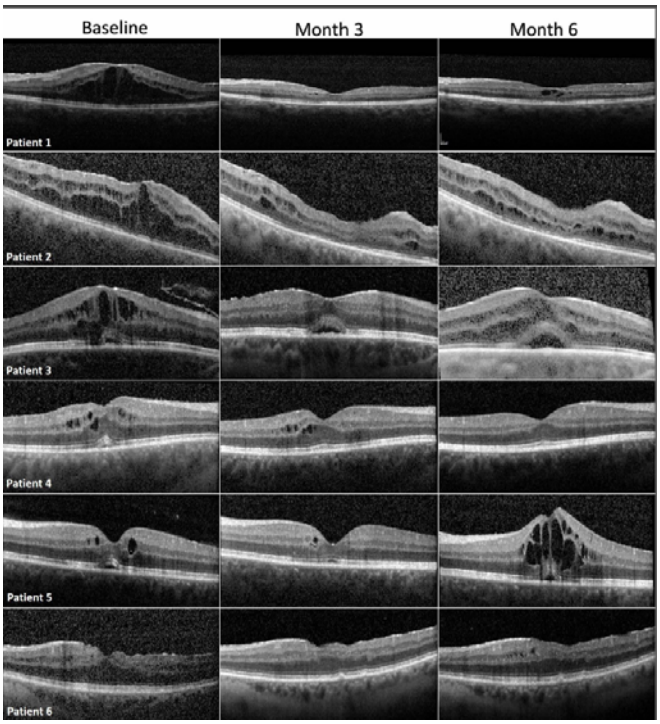

FIGURE 7

Spectral domain optical coherence tomography scans of the macula of six representative study subjects with macular edema at baseline in Group 1 (patients #1-3) (low dose sirolimus 440 µg) and Group 2 (patients #4-6) (high dose sirolimus 880 µg) at baseline, month 3 and month 6 visits. Patients #1 – 4 showed a decrease in macular edema until month 6. Patient #5 showed decrease in macular edema at month 3. However, there was increase in macular edema at month 6. Patient #6 showed an increase in macular edema from baseline until the visit at month 6.

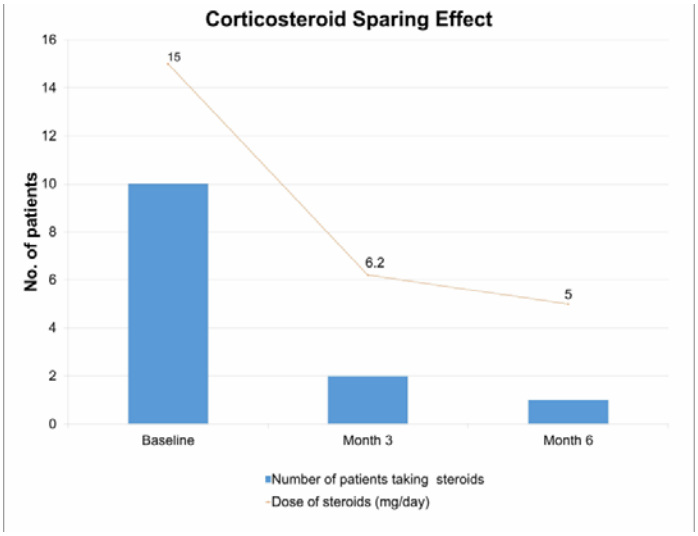

FIGURE 8

Graphical representation of the corticosteroid-sparing beneficial effects of sirolimus. The line represents the changes in the mean dose of corticosteroids among all study subjects in category 2. The columns represent the number of patients receiving oral steroids.

Safety Analysis

Prior to the primary endpoint at month 6, the total number of IVT injections received by all the patients taken together were 101 in the study eyes and 47 in the fellow eyes. Patients in group 1 received 62 injections in the study eye and 12 in the fellow eye.

Systemic Adverse Events

The most common systemic AE was sinus infection. Other systemic AEs noted were rash, arthritis and cardiovascular events such as premature ventricular contractions and atrial fibrillation. None of these events were noted to be related to the study drug (sirolimus). The systemic AEs were termed as mild to moderate and resolved without sequelae. The details of systemic AEs are listed in Table 3.

TABLE 3: SYSTEMIC ADVERSE EVENTS AMONG PATIENTS ENROLLED IN THE SAVE-2 STUDY UP TO THE PRIMARY ENDPOINT (MONTH 6)

PATIENT ID	ADVERSE EVENT	STUDY VISIT	STUDY GROUP	DISEASE CATEGORY
01-004	Sinus Infection	Month 3	2	2
	Sinus Infection	Month 5		
01-006	Migraine	Month 1	1	2
01-009	Shingles	Month 1	2	1
	Premature Ventricular Contractions	Month 1		
01-011	Sinus Infection	Month 2	1	1
01-012	Atrial Fibrillation	Month 2	2	2
01-013*	Pedal Edema	Month 1	2	2
	Upper body rash	Month 4		
01-016*	Sinus Infection	Month 1	2	1
03-002*	Arthritis	Month 3	1	1
04-002	Gastric Ulcer	Month 4	2	2

* These patients received bilateral injections of sirolimus at same study visit.
** Group 1 includes patients who received the 440-µg dose of Sirolimus and Group 2 includes patients who have received 880-µg Sirolimus
† Category 1 indicates treatment-naïve patients. Category 2 indicates patients receiving prednisone ≥10 mg/day (or equivalent dose of another corticosteroid) and/or at least 1 systemic immunomodulatory therapy (IMT) other than corticosteroids.

Ocular Adverse Events

The most common local ocular AE was ocular pain or redness followed by vitreous floaters. Four eyes developed anterior uveitis during the study; among them, three eyes (two in Group 2 and one in Group 1) had anterior uveitis as part of their panuveitis at time of study enrollment. The anterior inflammation was noted in 3 eyes after a single injection of high-dose sirolimus (Group 2) whereas in 1 eye after 2 injections of low-dose sirolimus (Group 1). In Group 1, 6 study eyes out of 11 were phakic at baseline (54.5%) and in Group 2, 9 eyes out of 13 were phakic at baseline (69.2%). Progression of cataract (one grade) was noted in 4 study eyes, of which 3 belonged to Group 2 (high-dose). Two patients developed progression of cataract (one grade) in the treated fellow eyes.

The mean baseline IOP in the study eye was 14.1 ± 3.04 and 15.15 ± 3.23 in Group 1 and Group 2, respectively. At month 6, the mean IOP in the study eye measured 15.2 ± 3.47 and 16.30 ± 4.23, respectively. An increase in IOP was noted in 2 eyes. The IOP remained below 35 mm Hg for all the eyes in the study. None of the patients required any intervention for the rise in IOP up to month 6 endpoint. The details of the local ocular AEs are listed in Table 4.

Safety of Bilateral Injections

Among the patients enrolled in the study, 19 (79%) patients had bilateral active uveitis, of which 8 belonged to Group 1. Of the 19 patients with bilateral diseases, 9 patients were treatment-naïve (i.e. disease category 1). Six patients had bilateral macular edema at the time of enrollment. Fourteen patients (out of 19 with bilateral diseases) received bilateral injections of sirolimus. All bilateral injections were given on the same day; each eye was prepared and injected as completely separate and independent processes as per

protocol of the study. At the primary end point of the study (month 6), a total of 27 bilateral injections (12 in group 1 and 15 in group 2) had been administered.

Bilateral IVT injections of sirolimus were well tolerated by all patients and the AEs encountered were rare. Systemic AEs were reported only in 3 patients receiving bilateral injections (Table 2). Local AEs were found in only 2 of the fellow eyes, and were similar to those reported in the study eye (Table 3).

TABLE 4. LOCAL OCULAR ADVERSE EVENTS AMONG PATIENTS ENROLLED IN THE STUDY EYES AND FELLOW EYES UP TO THE PRIMARY ENDPOINT (MONTH 6) IN THE SAVE-2 STUDY

PATIENT ID	ADVERSE EVENT (STUDY EYE)	ADVERSE EVENT (FELLOW EYE)	STUDY VISIT	STUDY GROUP*	DISEASE CATEGORY†
01-006	Ocular Pain		Month 2	1	2
	Redness		Month 2		
01-009	Anterior Uveitis		Month 1	2	1
	Increased IOP		Month 1		
01-012	Anterior Uveitis		Month 1	2	2
01-014	Cataract Progression		Month 4	2	2
01-016	Floaters		Month 1	2	1
01-018	Increased IOP		Month 6	2	1
	Cataract Progression		Month 6		
03-001		Floater	Day 15	2	1
	Photophobia	Photophobia	Day 15		
		Decreased vision	Day 15		
	Ocular Pain	Ocular Pain	Month 1		
	Cataract Progression		Month 1		
		Cataract Progression	Month 2		
	Anterior Uveitis	Ocular Pain	Month 2		
		Redness	Month 2		
		Blurry vision	Month 2		
		Blurry vision	Month 4		
		Ocular Pain	Month 4		
03-002	Cataract Progression		Month 1	1	1
	Ocular pain		Month 1		
		Cataract Progression	Month 2		
		Decreased vision	Month 3		
		Positive Watzke-Allen Sign	Month 3		
	Elevated IOP		Month 5		
	Floaters		Month 6		
04-001	Ocular Pain		Month 1	1	1
04-005	Anterior Uveitis		Day 14	1	2
	Ocular Hypertension		Month 2		

* Group 1 includes patients who received the 440-µg dose of Sirolimus and Group 2 includes patients who have received 880-µg Sirolimus

† Category 1 indicates treatment-naïve patients. Category 2 indicates patients receiving prednisone ≥10 mg/day (or equivalent dose of another corticosteroid) and/or at least 1 systemic immunomodulatory therapy (IMT) other than corticosteroids.

DISCUSSION

The breakthrough in the treatment of NIU was made when Gordon et al[15] demonstrated the dramatic effects of pituitary adrenocorticotrophic hormone (corticosteroids) therapy in 6 patients with anterior uveitis. While the benefits of corticosteroids in managing uveitis cannot be disputed, the wide range of risks and complications associated with long term corticosteroids therapy cannot be ignored. Significant effort to reduce the need for long term corticosteroids therapy along with better understanding of the human immune system have led to the development of systemic IMT such as cyclophosphamide, methotrexate, and azathioprine, among others.[5] Among the IMT agents, IVT methotrexate has been shown to have some success in controlling ocular inflammation.[6] However, level-one data on the use of IVT methotrexate in the treatment of NIU is scarce at best. Systemic IMTs also have a number of AEs, including life-threatening complications that require aggressive management.

Keeping in mind the risks associated with systemic IMT and with the use of any form of corticosteroids, we have designed the **S**irolimus as a Therapeutic Approach UVEitis (SAVE) Study to investigate clinically the role of mammalian target of rapamycin (mTOR) receptor in NIU by assessing the safety and efficacy profile of sirolimus, an mTOR receptor inhibitor, as a first-line therapeutic agent or as a steroid sparing agent in patients with noninfectious intermediate, posterior, and pan-uveitis.[12,13] Results of the primary and secondary endpoints from the SAVE Study indicated that sirolimus delivered bimonthly either IVT or SC has bioactivity as an IMT and corticosteroid-sparing agent and aids in reducing VHZ and cells and improving BCVA. The SAVE Study also demonstrated that the IVT injection may be better tolerated by patients compared to the SC injection, as ocular discomfort was frequently associated with the SC injection. Thus, from the results of the SAVE Study, it can be concluded that IVT route of administration of sirolimus is the most appropriate approach for treatment of patients with uveitis. The next logical step following the SAVE Study was to determine the most effective dose and treatment regimen of IVT sirolimus. In the SAVE Study, we showed that 352 μg of sirolimus in 16 μL for IVT injection was effective in reducing intraocular inflammation; however, the volume of 16 μL needed to be delivered in a very specialized syringe. Therefore, the volume was adjusted to 20 μL for better feasibility, and thus delivered 440 μg in SAVE-2. In addition, we also elected to evaluate the maximum formulated dose of 880 μg in the SAVE-2 Study to detect any possible additional benefits for the patients.

Systemic administration of sirolimus has been evaluated by other investigators in treating various forms of NIU. Phillips et al,[16] evaluated the role of oral low-dose (1-4mg/day) sirolimus for the treatment of active NIU in a retrospective study. Primary outcome measures were determined by the ability of the drug to decrease the intraocular inflammation, corticosteroids requirement, and the frequency of flares. A total of 8 patients were treated with oral low-dose sirolimus for severe chronic uveitis. Improvement of all primary outcome measures was observed in 4 patients. However, sirolimus monotherapy was successful in only 1 patient, the remaining 3 received a combination of sirolimus and methotrexate. Thus, it was concluded that sirolimus may have a limited role in severe uveitis as an adjunct corticosteroid-sparing agent. In contrast, Shanmuganathan et al.[17] have reported that sirolimus (4 mg daily increased in increments of 2 mg) was effective in 5 out of 8 patients with refractory severe NIU. Shanmuganathan et al. had defined successful treatment as improvement of ≥2 lines of Snellen acuity, symptomatic improvement, regression of vasculitis, reduction in the amount of steroid use, and/or reduction in the number of inflammatory cells. Treatment in 3 patients was considered failure due to intolerable side effects (primarily gastrointestinal or cutaneous). It is important to note that the number of patients in both studies was smaller compared to the SAVE and SAVE-2 studies and that sirolimus was administered orally in these previous case series. It is possible that systemically administered sirolimus does not have appropriate penetration of the blood-retinal barrier at lower doses, compared to the higher doses where sirolimus is able to suppress the inflammation in the posterior segment. Such challenge with the retinal barrier confronted by systemic administration might be the reason for a wide array of responses to sirolimus in different studies. Higher systemic dose, however, does predispose individuals to intolerable side effects as reported by Shanmuganathan et al.[17] An indirect indication of poor blood-retinal barrier penetration of sirolimus is the absence or near absence of systemic sirolimus following local (IVT and SC) administration in both preclinical and clinical studies. Additionally, both the current SAVE-2 Study and the SAVE Study have demonstrated that systemic side effects of sirolimus can be avoided with the use of IVT sirolimus.

In the SAVE-2 Study, the 880-μg dose (administered every 8 weeks) did not seem to have any added benefits in controlling the inflammation or extending the duration compared to the 440 μg dose (administered every 4 weeks). There might be higher incidence of anterior uveitis with the higher dose (3 events in Group 2 compared to 1 event in Group 1), although the number of events was small for any appropriate conclusion. It has been observed that IVT injection of triamcinolone suspension causes ocular inflammation, including sterile endophthalmitis, which are partly mediated by a local innate immune reaction to drug particles.[18-20] Such finding that the lower 440-μg dose of sirolimus may have the best benefit-to-risk profile rather than the higher dose of 880 μg perhaps can be explained by the hormesis phenomenon/effect. It is unlikely that this dose response (where the 880-μg dose does not provide any additional benefit over the 440-μg dose) can be readily explained pharmacologically, because exposure to sirolimus in the target tissues, retina/choroid, is anticipated to be similar between 440-μg and 880-μg doses based on the non-clinical pharmacokinetic data that has been demonstrated. Similar to IVT triamcinolone, after IVT injection, sirolimus drug particles aggregate and become a depot. Sirolimus is dissolved from the depot and diffused into the target tissues. Thus, a more plausible explanation for these observed results may be partly related to a non-specific innate immune response that is prominent in the 880-μg dose due to the higher concentration (and hence larger amount of particle numbers) with this dose.

The observed inflammatory responses (i.e. anterior uveitis) may be dose-dependent and such a dose-dependency may be obtained in a similar dose range (from 440 to 880 μg). The only difference in physicochemical properties between precipitated sirolimus particles of 440 and 880 μg should be the amount of the drug (more precisely, particle numbers), because the vitreous environment of randomized eyes should be generally similar between two dose groups, under which sirolimus precipitates following IVT injection.

Therefore, as seen with triamcinolone, it is highly likely that number of sirolimus particles in the vitreous body qualitatively determines the degree of a local innate immune reaction, leading to ocular inflammation and subsequently reduced efficacy of IVT sirolimus at the higher dose. The amount of drug particles separated from the depot after IVT injection with the 880-µg dose stimulate the surrounding ocular tissues around the depot, such as iris-ciliary body and retina, leading to activation of local innate immune system and subsequently a local reaction resulting in exacerbated inflammation. Such differences may determine the severity of local reaction in the vitreous body. Furthermore, the drug particles would lose such characteristics governing a local reaction over time as the drug is eliminated from the vitreous body, suggesting that this reaction is transient in nature despite maintained therapeutic levels in the target tissues. Thus, this local nonspecific immune reaction to drug particles would mask or reduce its anti-inflammatory efficacy (of the higher 880-µg dose). It is imperative to note that the proposed and presented hypothesis of precipitated sirolimus leading to local inflammatory reaction has not been proven by published studies. If such hypothesis is correct, the net clinical efficacy outcome observed with the 880-µg dose is most likely manifested by its genuine anti-inflammatory effect minus a pro-inflammatory effect due to a local reaction, resulting in potentially lower efficacy at 880 µg than 440 µg.

In comparison with other local IMTs available for treatment of NIU, such as methotrexate, sirolimus seems to be well-tolerated, with minimal local and systemic AEs, even with the administration of bilateral injections in a large number of study subjects. The major side effects with sirolimus reported in the current study are ocular discomfort and redness that subsides with symptomatic therapy. Unlike other agents such as methotrexate that may lead to corneal epithelial damage, corneal complications were not observed with sirolimus. None of the patients in the SAVE-2 Study developed infectious endophthalmitis, similar to the SAVE Study. Other AEs including floaters may be related to the presence of the drug particles in the vitreous. Isolated increases in the IOP were noted on two occasions after administration of the drug. The IOP did not rise beyond 35mm of Hg in either case and did not require any intervention. Such rise in IOP is not uncommon in eyes receiving IVT injections. However, as the volume to deliver 440 µg or 880 µg of sirolimus is very small (20 µl) compared to other IVT pharmacologic agents (often 50 µl), ocular hypertension has not been a concern with IVT sirolimus therapy.

Over half of the study subjects in SAVE-2 had macular edema at study entry, which is consistent with the general uveitic population, where 1/3 to ½ of the patients with uveitis would have uveitic macular edema. IVT sirolimus, at either 440 or 880 µg, can lead to reduction (of different degrees) in retinal edema, as seen by the decrease in retinal thickness on OCT after injection. The challenge, we have learned, is that the duration and patterns of bioactivity of sirolimus against uveitic macular edema varies tremendously, as illustrated in Figure 7 of six representative subjects. When the measures were taken at month 3, both the low and high doses showed similar results in improving macular edema. Patients in both groups just received IVT sirolimus at month 2 and thus demonstrated beneficial, appropriate responses. When the subjects were examined at month 6, subjects in Group 1 just received IVT sirolimus at month 5 and thus the edema continued to improve. Subjects in Group 2 have been without sirolimus for two months, and thus saw recurrence of macular edema, accompanied by worsening of visual acuity. There were no vitreous cells that returned at month 6, implying that the clinical inflammation was controlled with sirolimus, but the macular edema was not for a long interval. The response of uveitic macular edema to sirolimus therapy in the SAVE-2 Study may be interpreted keeping in mind the pathophysiology of retinal edema in ocular inflammation. Patients with uveitis may develop macular edema in the absence of ocular inflammation due to presence of other contributory factors such as vitreomacular interface abnormalities, microstructural retinal blood-retinal-barrier damage, and irreversible retinal pigment epithelial injury.[21] Such patients may require additional forms of therapy (including anti-angiogenic therapy) for uveitic macular edema apart from anti-inflammatory agents such as sirolimus.

Thus, the 30 patients from the SAVE Study and 24 patients from the SAVE-2 Study have demonstrated that repeated locally administered sirolimus (a specific antagonist of mTOR) in eyes with NIU lead to reduction in VHZ, VCC, macular edema, and subsequent (whenever possible based on vision at baseline) improvement in visual acuity along with a decrease need for systemic corticosteroids. Such findings strongly suggest that mTOR plays an important role in the pathogenesis of NIU, at least in selected types of uveitis as evaluated in the studies.

Similar to any other phase I or II clinical trial, the SAVE-2 study is not without limitations. Certainly, a control arm with standard of care may illustrate better the effects of IVT sirolimus. However, as standard of care in uveitis can be quite protean, hence, it was not thought to be appropriate to have a controlled arm in the phase II study. In addition, since the SAVE-2 Study was primarily designed to determine the safety and appropriate dosing regimen of IVT sirolimus, a control group was not included. Such a control group may have permitted head-to-head comparison of sirolimus to other standard-of-care treatments. Another limitation of the index study is the imbalance in the baseline CMT values between the two treatment groups (approximately 100 µm difference at baseline). Such difference may affect the assessment of response of macular edema to the two doses of sirolimus. While the number of patients with macular edema in the two groups was similar (7 out of 11 patients in group 1 and 6 out of 13 patients in group 2), the effect of higher dose of sirolimus on uveitic macular edema must be interpreted with caution. Other limitations of the SAVE-2 Study include a relatively short follow-up and a modest sample size of study subjects. Thus, one should only consider certain trends of results as needing to be confirmed, and not necessarily as definite conclusions.

The SAVE-2 Study has generated a large amount of novel information, but it also raises several questions. We have learned that repeated IVT injections of sirolimus can be well tolerated and can be effective in reducing intraocular inflammation as measured by various markers. Either the low dose or high dose of sirolimus may be effective. Bilateral IVT injections of sirolimus are also well tolerated with no apparent evidences of additional ocular or systemic risks. Side effects from systemic administration of sirolimus have been reported. None was observed in subjects receiving bilateral treatment with IVT sirolimus in SAVE-2, suggesting that very little, if any, of sirolimus circulates systemically after IVT administration to mount a systemic effect. However, are the different patterns of response to IVT sirolimus in different study subjects because of different levels of mTOR receptors, for example, in these

patients? What is the optimal timing for treatments to eliminate uveitic macular edema and decrease the risk of recurrence? Is monthly injection of sirolimus the best overall regimen, or is it better to be as needed after the initial intense treatment period? How strong is the anti-angiogenic property of an mTOR inhibitor like sirolimus? There was reduction in retinal/macular edema noted, but the amount of reduction is not as robust as we have seen at times with locally delivered corticosteroids or anti-VEGF therapy. Are there any clinically identifiable predictive features that guide the use of sirolimus in clinical practice? The most important question raised by SAVE-2 is whether IVT of sirolimus can provide long-term benefit in patients with NIU. The reduction in VHZ, cells, edema, and the improvement in visual acuity at certain time points is suggestive. However, randomized controlled trials that will span longer period of time is needed to determine the ultimate role of sirolimus for patients with NIU. SAVE and SAVE-2 have supported mTOR as an important stimulus for intraocular inflammation and have planted the seed for an mTOR inhibitor to be of potential therapeutic benefits. Now, other clinical trials should confirm the role of IVT sirolimus for NIU, and such trials are being conducted.

ACKNOWLEDGEMENTS

A. Funding/Support: The SAVE-2 Study is supported in part by an unrestricted grant from Research to Prevent Blindness (RPB) and by an unrestricted educational grant from Santen, Inc. The study drug (sirolimus) was provided by the drug manufacturer.

B. Financial Disclosures: Dr. Nguyen chairs the SAKURA Study Steering Committee and serves on the Santen Scientific Advisory Board. The remaining disclosures are listed in the ICJME forms.

C. Contribution of Authors: The authors alone are responsible for the content and preparation of this manuscript. There are no ghost authors or other writing assistance.

D. Other Acknowledgements: The authors wish to thank the investigators, coordinators, study staff, and patients from the Wilmer Eye Institute (Johns Hopkins University), the Massachusetts Eye Research and Surgery Institution (MERSI), the Truhlsen Eye Institute (University of Nebraska Medical Center) and the Texas Retina Associates for assisting with the conduct of the SAVE-2 Study.

REFERENCES

1. Caspi RR. Understanding autoimmune uveitis through animal models. The Friedenwald Lecture. *Investigative ophthalmology & visual science.* 2011;52(3):1872-1879.
2. Whitcup SM, Chan CC, Li Q, Nussenblatt RB. Expression of cell adhesion molecules in posterior uveitis. *Archives of ophthalmology.* 1992;110(5):662-666.
3. Wakefield D, Easter J, Penny R. Immunological abnormalities in patients with untreated retinal vasculitis. *The British journal of ophthalmology.* 1986;70(4):260-265.
4. Loh AR, Acharya NR. Incidence rates and risk factors for ocular complications and vision loss in HLA-B27-associated uveitis. *American journal of ophthalmology.* 2010;150(4):534-542 e532.
5. Nguyen QD, Hatef E, Kayen B, et al. A cross-sectional study of the current treatment patterns in noninfectious uveitis among specialists in the United States. *Ophthalmology.* 2011;118(1):184-190.
6. Taylor SR, Habot-Wilner Z, Pacheco P, Lightman SL. Intraocular methotrexate in the treatment of uveitis and uveitic cystoid macular edema. *Ophthalmology.* 2009;116(4):797-801.
7. Jabs DA, Rosenbaum JT, Foster CS, et al. Guidelines for the use of immunosuppressive drugs in patients with ocular inflammatory disorders: recommendations of an expert panel. *American journal of ophthalmology.* 2000;130(4):492-513.
8. Ikeda E, Hikita N, Eto K, Mochizuki M. Tacrolimus-rapamycin combination therapy for experimental autoimmune uveoretinitis. *Japanese journal of ophthalmology.* 1997;41(6):396-402.
9. Martin DF, DeBarge LR, Nussenblatt RB, Chan CC, Roberge FG. Synergistic effect of rapamycin and cyclosporin A in the treatment of experimental autoimmune uveoretinitis. *Journal of immunology (Baltimore, Md. : 1950).* 1995;154(2):922-927.
10. Kilmartin DJ, Forrester JV, Dick AD. Tacrolimus (FK506) in failed cyclosporin A therapy in endogenous posterior uveitis. *Ocular immunology and inflammation.* 1998;6(2):101-109.
11. Sloper CM, Powell RJ, Dua HS. Tacrolimus (FK506) in the treatment of posterior uveitis refractory to cyclosporine. *Ophthalmology.* 1999;106(4):723-728.
12. Nguyen QD, Ibrahim MA, Watters A, et al. Ocular tolerability and efficacy of intravitreal and subconjunctival injections of sirolimus in patients with non-infectious uveitis: primary 6-month results of the SAVE Study. *Journal of ophthalmic inflammation and infection.* 2013;3(1):32.
13. Ibrahim MA, Sepah YJ, Watters A, et al. One-Year Outcomes of the SAVE Study: Sirolimus as a Therapeutic Approach for UVEitis. *Translational vision science & technology.* 2015;4(2):4.
14. Vigil EM, Sepah YJ, Watters AL, et al. Assessment of changes in quality of life among patients in the SAVE Study - Sirolimus as therapeutic Approach to uVEitis: a randomized study to assess the safety and bioactivity of intravitreal and subconjunctival injections of sirolimus in patients with non-infectious uveitis. *Journal of ophthalmic inflammation and infection.* 2015;5:13.
15. Gordon DM. The treatment of chronic uveitis; preliminary comments on chronic degenerative diseases. *Archives of ophthalmology.* 1959;62:400-413.
16. Phillips BN, Wroblewski KJ. A retrospective review of oral low-dose sirolimus (rapamycin) for the treatment of active uveitis. *Journal of ophthalmic inflammation and infection.* 2011;1(1):29-34.

17. Shanmuganathan VA, Casely EM, Raj D, et al. The efficacy of sirolimus in the treatment of patients with refractory uveitis. *The British journal of ophthalmology.* 2005;89(6):666-669.
18. Ahn SJ, Kim TW, Ahn J, Huh JW, Yu HG, Chung H. Associated factors and treatment outcome of presumed noninfectious endophthalmitis occurring after intravitreal triamcinolone acetonide injection. *Graefe's archive for clinical and experimental ophthalmology = Albrecht von Graefes Archiv fur klinische und experimentelle Ophthalmologie.* 2013;251(3):715-723.
19. Otsuka H, Kawano H, Sonoda S, Nakamura M, Sakamoto T. Particle-induced endophthalmitis: possible mechanisms of sterile endophthalmitis after intravitreal triamcinolone. *Investigative ophthalmology & visual science.* 2013;54(3):1758-1766.
20. Dodwell DG, Krimmel DA, de Fiebre CM. Sterile endophthalmitis rates and particle size analyses of different formulations of triamcinolone acetonide. *Clinical ophthalmology (Auckland, N.Z.).* 2015;9:1033-1040.
21. Guex-Crosier Y. The pathogenesis and clinical presentation of macular edema in inflammatory diseases. *Documenta ophthalmologica. Advances in ophthalmology.* 1999;97(3-4):297-309.