A comprehensive review of mTOR-inhibiting pharmacotherapy for the treatment of non-infectious uveitis

Blair, J; Barry, Robert; Moore, David; Denniston, Alastair

DOI:

[10.2174/1381612823666170111125550](https://doi.org/10.2174/1381612823666170111125550)

Document Version

Peer reviewed version

Citation for published version (Harvard):

Blair, J, Barry, R, Moore, D & Denniston, A 2017, 'A comprehensive review of mTOR-inhibiting pharmacotherapy for the treatment of non-infectious uveitis', *Current pharmaceutical design*, vol. 23, no. 46.

<https://doi.org/10.2174/1381612823666170111125550>

[Link to publication on Research at Birmingham portal](#)

Publisher Rights Statement:

Checked for eligibility: 17/02/2017

Blair, J., et al. "A comprehensive review of mTOR-inhibiting pharmacotherapy for the treatment of non-infectious uveitis." *Current pharmaceutical design* (2017).

<http://www.eurekaselect.com/149162/article#>

DOI: 10.2174/1381612823666170111125550

General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.

Title: A comprehensive review of mTOR-inhibiting pharmacotherapy for the treatment of
non-infectious uveitis

Authors: Joshua Blair [1,2], Robert Barry [1,2,3], David J Moore [4], Alastair K Denniston
[1,2,3]

Affiliations:

¹Department of Ophthalmology, University Hospitals Birmingham NHS Foundation Trust,
Birmingham, United Kingdom

²Centre for Rare Diseases, Institute of Translational Medicine, Birmingham Health Partners,
United Kingdom

³Academic Unit of Ophthalmology, Institute of Inflammation & Ageing, University of
Birmingham, United Kingdom

⁴Institute of Applied Health Research, University of Birmingham, United Kingdom

Corresponding Author:

Alastair Denniston, PhD MRCP FRCOphth

Centre for Translational Inflammation Research, Institute of Inflammation & Ageing,
University of Birmingham, United Kingdom

Tel: +44 (0) 121 371 6905

Fax: +44 (0) 121 371 6918

Mob: +44 (0) 7876 557 443

Running title: mTOR inhibitors in uveitis

Key words: uveitis, mTOR inhibitors, mammalian target of rapamycin, immunosuppression

Abstract

Background: Non-infectious uveitis is a sight-threatening inflammatory disease that often necessitates prolonged use of high-dose corticosteroids, resulting in significant systemic side effects. There is a need for efficacious steroid-sparing immunomodulatory therapy for these patients, and the mTOR inhibitors (sirolimus and everolimus) may be contenders for this role.

Methods: A comprehensive review of preclinical and clinical research on mTOR inhibitors for non-infectious uveitis was performed. Articles were identified by a search of MEDLINE (PubMed/OVID) and EMBASE (OVID) the terms (*uveitis* OR *non-infectious uveitis*) AND (*mTOR inhibitor* OR *sirolimus* OR *everolimus*). Assessment of study aims, methods, efficacy outcomes and adverse events was performed.

Results: Seven pre-clinical and nine clinical studies were identified. One study in each group was on everolimus, the rest sirolimus. Preclinical studies have been performed in rabbit, rat, mouse and in-vitro models. Clinical studies range from comparative open-label trials to case reports, with reported clinical efficacy ranging from 40% to 100% depending on endpoint assessed. The overall rate of drug-related adverse events (such as ocular irritation, visual floaters, nausea and vomiting) was 0.640 events per patient-year with sirolimus, and 0.111 events per patient-year with everolimus.

Conclusion: Published evidence suggests that sirolimus and everolimus may be useful in the management of non-infectious uveitis. Both appear to be well tolerated, especially when

locally administered. Further high-quality RCTs adopting standardised end-points are required to definitively determine the efficacy of each agent.

Introduction

Uveitis (intraocular inflammation) is a serious, potentially blinding condition [1,2,3]. Uveitis is described by the anatomical pattern of involvement in the eye (anterior, intermediate, posterior or panuveitis), the clinical course of the inflammation (acute, recurrent or chronic), and by its aetiology (individual causes within the categories of infectious, non-infectious, masquerade) [4,5]. In many parts of the world (including the USA and Europe), non-infectious uveitis is a significant cause of blindness in the working age group. Most non-infectious uveitis appears to be autoimmune (or autoinflammatory) in nature [2], either occurring as isolated ocular inflammation (usually labelled 'idiopathic uveitis') or may be associated with systemic conditions in which immune dysregulation is evident (e.g. sarcoid uveitis, multiple-sclerosis associated uveitis, etc.) [4,5].

Currently, non-infectious uveitis is treated first-line with corticosteroids [6]. For disease restricted to the front of the eye (anterior uveitis) topical therapy may be sufficient, but for the more sight-threatening forms which affect more posterior structures (intermediate, posterior and panuveitis), local (injections or implants) or systemic therapy (usually as oral prednisolone) may be given. Corticosteroids are capable of quickly regaining control of the inflammatory state, but high doses are rarely used long-term due to the increased burden of side effects that occur [7]. Safe maintenance doses are generally considered to be less than 10mg of prednisolone per day [8].

As a result, patients who are not adequately controlled at safe maintenance levels of steroids, or cases of uveitis refractory to corticosteroid therapy, require an escalation of their treatment to include more powerful second-line therapeutics [8]. These may include

one or more of the drugs sometimes referred to as 'DMARD' (Disease Modifying Anti-Rheumatic Drug) agents, such as mycophenolate mofetil, azathioprine, methotrexate, ciclosporin and tacrolimus [7,8]. Current options include switching to or combining second-line agents, switching to or combining with a biological therapy (such as adalimumab), or using cytotoxic alkylating agents [5,8]. However, a significant proportion of cases at this stage still suffer from either poor inflammatory control or excessive side effects. As a result there is a growing need for alternative treatment regimens, whether systemic or local, which optimise inflammatory control without an overburden of side effects [5]. The challenges and morbidity of current treatment regimens are discussed in more detail in a number of reviews, highlighting the high patient cost of drug-related morbidity, particularly of excessive corticosteroid usage, and the urgent need for better therapies [5,9,10].

One area of recent interest are mTOR inhibitors, a group of immunosuppressant drugs with particular effects on the T-cell arm of the adaptive immune system [5,8]. The immune dysfunction in most non-infectious uveitis appears to be primarily T-cell mediated [2,11]. T-cell inhibition by calcineurin inhibitors such as ciclosporin or tacrolimus is already a well-established part of uveitis treatment strategy. The calcineurin inhibitors act to reduce IL-2 transcription and prevent the cascade of T-cell activation [12,13].

mTOR (mammalian Target Of Rapamycin) is a serine/threonine kinase that acts downstream of various cytokine receptors, including the IL-2 receptor, and influences many active cell processes [14]. mTOR action in cells is complex, integrating a range of cell signals to direct cell metabolism, which in the case of T-cells has been associated with cell differentiation [15]. T-cell activation has been associated with mTOR activity, while suppressed T-cell mTOR

activity in has been associated with quiescence, memory T-cell formation, and increased T-regulatory cells [15].

As a result, the mTOR system may be a desirable therapeutic target for the control of autoimmune conditions [15]. mTOR inhibitors, such as sirolimus (Rapamune; Pfizer Inc.) and everolimus (Zortress [USA]/Certican [EU and others]; Novartis International AG), bind to the FKBP12 protein, and the resulting complex acts to reduce the transcription of mTOR. This has a particular effect in T-cells on the mTORC1 complex, associated with signal transduction for T-cell activation [15]. This allows mTOR inhibitors to interfere with T-cell signal transduction, preventing cytokines such as IL-2 from having a proliferative and differentiating effect on T-cells [11,16].

Due to the pleiotropic actions of mTOR, mTOR inhibitors can have more wide-ranging pharmacological effects than calcineurin inhibitors. mTOR inhibitors have been shown to have antiviral properties, where studies have shown inhibitory effects in HIV infections [17,18,19]; anti-tumour properties, with studies detailing activity in prostate [20] and breast cancers [21,22]; and actions which reduce cell senescence [23].

Sirolimus and everolimus have been licensed for human applications for many years, with their main indications being transplant immunosuppression and for the treatment of mTOR-dependent tumours [14]. mTOR inhibition has also been shown to be of potential therapeutic use in diseases of autoimmune aetiology, with promise in pre-clinical studies for systemic lupus erythematosus [24], experimental allergic encephalomyelitis (as a model for multiple sclerosis) [25], rheumatoid arthritis [26] and type 1/insulin dependent diabetes mellitus [27].

Additionally, a recent trial studied sirolimus in the context of refractory autoimmune cytopenia, finding that the agent showed significant improvement for patients with

autoimmune lymphoproliferative syndrome [28]. The authors especially noted a decrease in the number of active T-cells, attributed to the relatively high degree of action that mTOR inhibitors have on T-cell activities [28]. The study concluded that sirolimus is a contender for steroid-sparing treatment in this autoimmune haematological context [28].

Other applications for which the role of mTOR inhibitors has been considered include the modulation of restenosis of coronary stents [29], and the prevention of post-traumatic epilepsy [30].

While calcineurin inhibitors are well-established in the treatment of uveitis, mTOR inhibitors have been little considered within ophthalmology until recently; use of mTOR inhibition outside of clinical trials has been largely viewed as a 'last line' option for refractory uveitis, when almost all other agents have failed or are contraindicated. This appears to be changing, as highlighted by the recent undertaking of an international multicentre randomised controlled trial in this area.

It is timely therefore to undertake a comprehensive review examining the evidence for the use of mTOR inhibitors in non-infectious uveitis. The review goes beyond the relatively narrow scope of the existing narrative summaries in this area [11,31] to include all preclinical and clinical research on mTOR inhibitors. This comprehensive approach also includes analysis of adverse events.

Materials and Methods

Searches were conducted of MEDLINE (1946 to April Week 4 2016; via PubMed and Ovid) and EMBASE (1974 to 2016 May 10; via Ovid), using the terms (*uveitis OR non-infectious*

uveitis) AND (mTOR inhibitor OR sirolimus OR everolimus). The searches had no limitations on publication date or language of publication.

From the search yield, any article which satisfied the following criteria was selected for this narrative review: the study intervention was an mTOR inhibitor, either sirolimus or everolimus; the disease context was non-infectious uveitis (of any anatomical category).

Pre-clinical (animal and in vitro studies) and clinical studies (comparative and non-comparative) were included; there were no age restrictions.

Review of the studies included detailing the type of research identified and findings with regard to the use mTOR inhibitors for non-infectious uveitis.

Adverse events, adverse reactions and study discontinuations were also assessed for and categorised. As per UK NHS National Institute for Health Research nomenclature, an

“Adverse Event” (AE) is one that is not necessarily deemed related to the study drug, an

“Adverse Reaction” (AR) is one that is deemed related to the study drug; and a “Serious

Adverse Event” (SAE) or “Serious Adverse Reaction” (SAR) is an AE or AR resulting in death or hospitalisation [32].

Results

Overview

Sixteen studies were identified, comprising nine clinical studies and seven preclinical studies (Table 1). Of the clinical studies, eight investigated sirolimus with a total of 46.87 patient-years of analysis; one study investigated everolimus with 18 patient-years of analysis.

Therefore the clinical studies included for review offer a combined total of 64.87 patient-years of analysis of mTOR inhibitors in non-infectious uveitis. Of the preclinical studies, six studies investigated sirolimus and one study investigated everolimus. The reported uveitis diagnoses of patients featured in the nine clinical studies are documented in Table 2.

Table 1. Details of included studies

Study	Drug	Dose	Model/Study Design
<i>Pre-clinical studies</i>			
Ideka et al. [33]	Sirolimus	0.03 to 0.2mg/kg/day	Animal study (rat; Experimental Autoimmune Uveitis [EAU])
Martin et al. [34]		0.01 mg/kg/day	Animal study (rat; EAU)
Roberge et al. [35]		1mg/kg/day	Animal study (rat; EAU)
Zhang et al. [36]		1.5µg to 7.5µg	Animal study (mouse; EAU)
Ohia et al. [37]		10mg/kg	Animal study (rabbit; Endotoxin-Induced Uveitis [EIU])
Yang et al. [38]		1-1000ng/ml	Human in-vitro study (peripheral blood mononuclear cells)
Hennig et al. [39]	Everolimus	5mg/kg	Animal study (mouse; EAU)
<i>Clinical studies</i>			
Vigil et al. [40]	Sirolimus	352µg intravitreal or 1320µg subconjunctival, as single doses on days 0, 60, 120; then as required from 6 to 12 months (maximum 3	Prospective, randomised, comparative open label trial
Ibrahim et al. [41]			Prospective, randomised, comparative open label trial

		additional doses)	
Nguyen et al. [42]		352µg intravitreal or 1320µg subconjunctival, as single doses on days 0, 60 and 120	Prospective, randomised, comparative open label trial
Sen et al. [43]		1320µg subconjunctival, single dose	Prospective non-comparative open label trial
Shanmuganathan et al. [44]		Oral 2 to 12mg per day	Prospective non-comparative open label trial
Phillips et al. [45]		Oral 1 to 4mg per day, to 4-12ng/ml blood trough levels	Case series
Nussenblatt et al. [46]		Oral 3 to 6mg per day	Case report
Nowosielska et al. [47]		Unknown	Case report
Heiligenhaus et al. [48]	Everolimus	Oral 1.5 to 2.5mg per day, to 3-8ng/ml serum trough levels	Prospective non-comparative open label trial

Table 2. Summary of clinical study patient diagnoses

Diagnosis	Patients receiving Sirolimus	Patients receiving Everolimus	Total
Behçet's Disease	3	0	3
Birdshot Chorioretinopathy	4	0	4
HLA-B27 related	1	0	1
Idiopathic Uveitis	28	12	40
Multifocal Choroiditis	2	0	2
Punctate Inner Choroiditis	3	0	3
Psoriasis related	1	0	1
Sarcoidosis	8	0	8
Secondary to Lyme Disease	1	0	1
Sympathetic Ophthalmia	1	0	1
Vogt-Koyanagi-Harada Disease	1	0	1
Total	53	12	65

Sirolimus – preclinical studies

Six preclinical studies were identified that evaluated sirolimus for use in uveitis. Three studies utilised a rat model [33,34,35]; one a mouse model [36]; one a rabbit model [37]; and one was an in-vitro study of collected human cells [38]. In all five animal studies sirolimus was delivered systemically by intravenous, intraperitoneal or intramuscular injection.

Four animal studies (Ideka et al. [33]; Martin et al. [34]; Roberge et al. [35]; Ohia et al. [37]) set sirolimus doses relative to weight, and delivered doses ranged from 0.01mg/kg/day to 10mg/kg in various trial arms. The fifth study by Zhang et al. [36] used absolute doses of sirolimus, administering 1.5µg and 7.5µg doses to mice.

Three animal studies (Ideka et al. [33]; Roberge et al. [35]; Ohia et al. [37]) used a control group of uveitis (EAU or EIU as per study design) with an equivalent sham injection in place of sirolimus delivery. Martin et al. [34] used a three-arm design where sirolimus injection with a sham ciclosporin injection was compared to ciclosporin with a sham sirolimus injection, and sirolimus and ciclosporin co-therapy, all groups on an EAU rat model. Zhang et al. [36] used a control group of EAU with no injection. Primary outcomes varied but were almost always included a histological evaluation of uveitis and analysis of T-cell number and function, accompanied by secondary outcomes of inflammatory cytokine levels, immunoglobulin assessments, and toxicity.

All animal studies showed that sirolimus significantly suppressed experimental uveitis based on clinical and immunological markers of ocular inflammation compared to controls [33-37]. Ohia et al. noted that 10mg/kg intramuscular sirolimus was effective in reducing endotoxin induced uveitis (EIU) in rabbits [37]. In Lewis rats, Roberge found that intravenous sirolimus

could prevent induction of experimental autoimmune uveitis (EAU) at doses down to 0.1mg/kg/d [35]. In two other studies of EAU in Lewis rats, efficacy of sirolimus was shown when the drug was studied as a combination therapy [33,34]. Ikeda et al. found that prevention of clinical EAU was achieved in 20% eyes with tacrolimus (0.1mg/kg/d), 50% eyes with sirolimus (0.1mg/kg/d), and 100% eyes with both drugs [33]. Martin et al. conducted a similar study in which they showed that EAU was prevented in 100% eyes treated with sirolimus (0.01mg/kg/d) and ciclosporin (2mg/kg/d), whereas most developed the disease if treated with either agent alone [34]. They also demonstrated marked synergism between sirolimus and ciclosporin for inhibition of proliferation of retinal S-antigen primed lymphocytes *in vitro* [34]. The authors argue that this potential synergistic effect between mTOR inhibitors and calcineurin inhibitors may have implications for its clinical use [33,34].

One cautionary note comes from an EAU study in B10.RIII mice which compared low (1.5ug/d) vs high (7.5ug/d) sirolimus. Although high dose sirolimus significantly suppressed uveitis, low dose sirolimus appeared to worsen the uveitis compared to controls [36]. The authors suggest that low doses of sirolimus may alter T-cell dynamics to enhance the effector T-cell response, rather than inhibiting it, but the exact mechanism remains unclear [36]. This has not been repeated in other studies, and may be a feature specific to this model. Assuming that these six-week old mice were around 20g, these doses equate to around 0.075mg/kg/d and 0.375mg/kg/d, thus the lower dose which caused worsening of disease in the mouse is similar to the doses which cause significant benefit in the rat model [33,35].

In the sole human *in vitro* study, Yang et al. cultured peripheral blood mononuclear cells collected from uveitis patients with Vogt-Koyanagi-Harada Syndrome, in various concentrations of sirolimus ranging from 1 to 1000 ng/ml [38]. The study used control groups of the cells cultured in equivalent concentrations of dexamethasone. The authors found that sirolimus had a significantly greater effect at suppressing inflammatory cytokines than dexamethasone, and could completely suppress IL-17 production at doses as low as 10ng/ml [38].

Sirolimus – clinical studies

The clinical use of sirolimus for non-infectious uveitis is currently reported in three prospective open label trials (one Phase 2 randomised comparative open label trial, and two non-comparative Phase 2 trials) and three retrospective studies (one case series, and two case reports). The Phase 2 comparative open label trial (the SAVE study) comprises three separate reports. No blinded randomised controlled trials (RCTs) studying the use of sirolimus for non-infectious uveitis were identified by the searches.

In total these studies comprise 46.87 patient-years of analysis, using data collected from a total of 53 patients, comprising a wide range of uveitis diagnoses (documented in Table 2).

Of the three prospective studies, the primary outcome was generally based on clinical scores of inflammation and reflected the target group of uveitis patients (vitreous haze in one, anterior chamber cells in one, and a multi-component endpoint in one). Additional secondary outcomes included the reporting of adverse events, corticosteroid use, the need for additional therapy, and changes in fluorescein angiography. Locally administered sirolimus regimens were provided either by intravitreal injection or subconjunctival

injection. Local doses featuring in the studies ranged from 352µg as an intravitreal injection to 1320µg as a subconjunctival injection. Three studies used systemic sirolimus, all orally. Systemic doses ranged from 1mg to 12mg daily.

Due to the range of study designs, sirolimus regimens and trial endpoints, direct comparison of studies is problematic and meta-analysis is not possible. The data is therefore provided below as a narrative.

Intravitreal and subcutaneous injection of sirolimus

The use of intravitreal and subcutaneous administration of sirolimus was investigated in two prospective studies. The SAVE study [40,41,42] was a randomised open label trial comparing two monthly subcutaneous vs intravitreal sirolimus with a primary endpoint at 6 months, and then a further 6 months extension during which treatment could be continued at the investigator's discretion. The primary efficacy outcome was a reduction of vitreous haze by 2 points (in those with active uveitis at baseline), or the maintenance of no clinical vitreous haze (in those with inactive disease at baseline). The SAVE study comprised a total of 30 patient-years of analysis.

At six months, combining both sirolimus treatment groups together, the study reported that the primary efficacy outcome was met in 8/20 (40%) of those with active uveitis and 7/8 (88%) patients who were inactive at baseline [42]. At 12 months the primary efficacy outcome was met in 14/20 (70%) in the active uveitis patients, and 7/8 (88%) in the inactive patients [41]. There were no significant differences in efficacy between the intravitreal and subconjunctival groups [41,42].

Quality of life was assessed as a secondary outcome of the SAVE study, evaluated by the visual function questionnaire NEI VFQ-39 [40]. Both the intravitreal and subconjunctival groups reported improvements in pain over 12 months, but only the intravitreal group had a significant improvement in the overall composite score, which includes other life factors. The authors suggest that intravitreal administration of sirolimus may be superior for 'vision-related health' [40].

Evaluation of visual acuity and macular thickness showed a mixed picture. In terms of LogMAR VA, at six months 30% gained one or more lines, 50% remained stable, and 20% lost one or more lines; at 12 months these figures were 52%, 24%, and 24% respectively. Of those with macular oedema at baseline (n=11/20 of active uveitis), 6/11 showed improvement in central macular thickness (CMT) at month 3, but only 2/11 showed improvement at month 6; indeed the mean CMT for the macular oedema group showed no significant improvement at either the 6 month or the 12 month time-point [41,42]. At baseline 20 patients were taking corticosteroids, of whom 13/20 were taking $\geq 10\text{mg/d}$; at 6 months only 2 were still requiring $>10\text{mg/d}$; at 12 months only one patient was still requiring $>10\text{mg/d}$, and 5/20 (25%) were reported to have stopped their steroid use entirely [41].

There were 6/30 (20%) patient discontinuations from the SAVE study, of which one was due to drug failure, one due to death (not considered to be related to the study drug), one patient had progression of a pre-existing macular oedema, and three were loss of follow-up or patient transport issues [41]. Various adverse events (AEs) and reactions (ARs) were reported that are discussed below. Serious adverse events (SAEs) included perioperative

death, shoulder debridement, vitrectomy, infective myocarditis, myocardial infarction, and exacerbation of sarcoidosis. There were no serious adverse reactions (SARs).

The authors conclude that local sirolimus, both by intravitreal and subconjunctival injection, is safe and well-tolerated in patients with uveitis, and appears to be effective in reducing inflammation but that further work is needed to evaluate efficacy.

Sen et al. produced a non-comparative open label trial of subconjunctival sirolimus for anterior uveitis, studying a small population of 5 patients with 1.67 patient-years of analysis [43]. All 5 patients had non-infectious uveitis and were taking steroids. Sirolimus was administered as a one-off 1320µg subconjunctival injection. The primary outcome was a reduction of anterior chamber inflammation at 4 weeks, measured by a 2-step reduction in anterior cells. Efficacy was shown by meeting this outcome in 3/5 (60%) patients, with the other 2 patients showing a 1-step reduction in the 4 week period [43].

Secondary outcomes included visual acuity, fluorescein angiography, and retinal thickness. Visual acuity improved by at least one line in 4/5 (80%). No significant changes were reported regarding angiography or retinal thickness on OCT [43]. No patients discontinued the study. There were four ARs related to the subconjunctival injection process (injection site irritation (3) and chemosis (1)), but no SAEs or SARs [43]. Sen et al. conclude that sirolimus was well-tolerated and that its potential efficacy in uveitis should be explored in further studies [43].

Oral administration of sirolimus

The use of oral sirolimus is reported in one prospective study, one case series and two case-reports. A phase 2 non-comparative open label trial by Shanmuganathan et al. evaluated a

regimen of oral sirolimus on a population of eight patients with uveitis refractory to usual treatment, comprising 7.6 patient-years of analysis [44]. The regimen involved a starting dose of 4mg sirolimus per day, which could be altered in 2mg increments as dictated by blood sirolimus levels and clinical activity. Doses provided ranged from 2mg to 12mg per day [44].

The primary outcome was improvement in one or more of symptoms, visual acuity, specific signs of inflammation and reduction of corticosteroids. Efficacy was reported in 5/8 (63%) patients all of whom were able to reduce their steroid doses to below 10mg per day; in two of these patients sirolimus appeared to induce a state of uveitis remission with successful tapering of all treatment (including sirolimus). The treatment was deemed to have failed in the remaining 3 patients due to symptomatic progression of the uveitis and an AR, and one patient discontinued the study due to an AR [44]. There were no SAEs or SARs. The rate of ARs was relatively high in this study and generally occurred when trough levels were significantly above that recommended; there is no established recommendation for uveitis but for renal transplantation the recommended level is <20ng/ml [44]. The authors conclude that oral sirolimus appears to be effective in treatment-resistant uveitis but that further study is needed [44].

Phillips et al. report a case-series of 8 patients with uveitis who were treated with oral sirolimus using doses of 1 to 4mg per day to achieve blood trough levels of 4-12ng/ml (significantly lower than the levels used in transplantation) [45]. Treatment was for an average of 44.5 weeks. The authors reviewed records for levels and flare-ups of eye inflammation and steroid use as primary outcomes, alongside symptoms, visual acuity, and available imaging [45]. Efficacy was reported in 4/8 (50%) patients as improving all primary

outcomes; however 3 of these required a sirolimus and methotrexate co-therapy to achieve this [45]. Of the other 4 patients, 1 discontinued as a loss to follow up, and 3/8 (38%) were deemed to have failed on sirolimus treatment as they discontinued due to ARs [45]. All 3 ARs (a DVT, excessive nausea and vomiting, and a case of thrombocytopenia) were deemed to be side effects of the sirolimus treatment; however none were SARs [45]. Phillips et al. conclude that although it appeared to be effective in some cases of uveitis, its use might be limited by the high rate of adverse events even at low doses [45].

Two case reports outlining the use of sirolimus in uveitis were identified [46,47].

Nussenblatt reported the use of oral sirolimus in a patient with Punctate Inner Choroiditis (PIC) that had previously been treated with intraocular steroids [46]. Sirolimus was used at an initial loading dose of 6mg, followed by a regime of 2mg every 2 days for 3 weeks, and then 4mg every 2 days for 4 months [46]. The authors report that a notable reduction in retinal thickness was detected, and improvement of appearance on fluorescein angiography also occurred, indicating improvement of the inflammatory state [46]. These changes continued for at least 5 months after the treatment stopped, and throughout the follow up period the patient's visual acuity remained stable [46].

The second case report documents a case of acute idiopathic uveitis which was refractory to corticosteroids [47]. This case was then subsequently treated with a combination therapy of sirolimus and cyclophosphamide, which is reported to have led to remission and retention of visual acuity [47]; however the full text of this case report was unobtainable and is not included in further analysis.

Everolimus – preclinical study

One relevant preclinical study of the use of everolimus in uveitis was identified. In the EAU mouse model Hennig et al. evaluated oral everolimus given at a dose of 5mg/kg, starting either two days before or 14 days after induction of EAU [39]. Treatment with everolimus significantly reduced the frequency and severity of development of EAU when compared to controls, which was associated with an increase in regulatory T-cells and reduction in the levels of inflammatory cytokines [39].

Everolimus – clinical study

There are no RCTs reported for the use of everolimus for non-infectious uveitis in humans. There is one phase 2 non-comparative open label clinical trial which included 12 patients with idiopathic uveitis (18 patient-years of analysis). The trial used a twice daily everolimus regimen titrated upwards from 1.5mg to 2.5mg max daily dose to achieve a serum trough level of 3-8ng/ml, with doses guided by blood measurements [48]. The primary outcome was a clinical evaluation for the inactivity of uveitis (based on any of AC cells, vitreous snowballs and chorioretinal lesions). Additional secondary outcomes assessed were the complications, the clinical course of disease, the use of alternative treatments (including steroids) and reporting of adverse events. The primary efficacy endpoints was met in 12/12 (100%) patients at 3 months, and 6/11 (55%) at 12 months [48].

At baseline all patients were taking ciclosporin and high doses of steroids. The study found that everolimus allowed reduction of both of these drugs [48]. All patients reduced steroid doses by 12 months, with complete withdrawal in 4/12 (33%) and doses less than 10mg/day in 8/12 (67%). Ciclosporin could be withdrawn in 3/12 (25%) and was reduced to less than

half dose in 8/12 (67%) [48]. The study also assessed visual acuity, vitreous cells, vitreous haze, and fluorescein angiography as secondary outcomes. However none of these displayed statistically significant changes [48].

One patient (8.33%) discontinued prior to the end of the trial, attributed to a patient decision not related to the everolimus therapy. A number of AEs and ARs were also reported which are included below. There were no SAEs or SARs.

The authors conclude that everolimus appears effective for refractory uveitis, and recommend that a full RCT be done to further evaluate its use for uveitis [48].

Study Discontinuations and Adverse Events

An important marker of the safety of these drugs in humans is, the rate of discontinuation (and the reasons for this including adverse drug reactions), and the overall rate and severity of adverse events.

Overall from the clinical studies, 12 study discontinuations occurred: 11 from sirolimus studies, and 1 from the everolimus study. The sirolimus clinical studies totalled 53 patients and 46.87 patient-years of analysis; therefore the discontinuation rate of sirolimus patients is 20.8% of patients, or 0.235 discontinuations per patient-year. The discontinuation rate for oral sirolimus was 27.8% of patients, or 0.329 discontinuations per patient-year; for local (subconjunctival or intravitreal) sirolimus the rate was 17.1% of patients, or 0.189 discontinuations per patient-year. The everolimus clinical study was made up of 12 patients and 18 patient-years of analysis; therefore the discontinuation rate of everolimus is 8.3% of patients, or 0.056 discontinuations per patient-year.

It should be noted that many of the discontinuations are not due to treatment failure or an adverse reaction to the drug, but related to patient choice or practical aspects that affected their ability to continue (illustrated in Table 3). Only 4/12 discontinuations were deemed to be caused by the study drug, comprising a case of Deep Vein Thrombosis (DVT), a case of excessive nausea and vomiting, a case of thrombocytopenia, and a case of scleroderma-like skin changes. Taking these discontinuations in isolation, the sirolimus discontinuation rate becomes 7.5% of patients and 0.085 per patient-year; there were no drug-related discontinuations for everolimus.

Table 3. Clinical study discontinuations by reported cause

Cause of discontinuation	Discontinuations on sirolimus	Discontinuations on everolimus	Total
<i>Ophthalmic reasons</i>			
Lack of clinical improvement	1	0	1
Exacerbation of macular oedema	1	0	1
<i>Medical reasons</i>			
Deep Vein Thrombosis	1	0	1
Nausea +/- vomiting	1	0	1
Scleroderma	1	0	1
Thrombocytopenia	1	0	1
Unrelated death	1	0	1
<i>Other reasons</i>			
Loss to follow up	3	0	3
Patient decision	0	1	1
Patient transport problem	1	0	1
Total	11	1	12

In addition to discontinuations, various adverse events and reactions (AEs and ARs) were reported. Most reports (and all of the serious reports) were deemed to be unrelated to the

study drug. In total there were 65 reported AEs; 53 occurring with sirolimus therapy and 12 with everolimus, representing an adverse event rate of 1.131 per patient-year with sirolimus, and 0.666 per patient-year with everolimus.

However, of the 65 AEs, only 32 were thought to have a high likelihood of having been caused by the studied drug or delivery method and considered as ARs (Table 4). 30 ARs occurred in the sirolimus studies, and 2 in the everolimus study. This represents an adverse reaction rate of 0.640 per patient-year for sirolimus (0.505 per patient-year for local sirolimus, 0.921 per patient-year for oral sirolimus) and 0.111 per patient-year for everolimus.

Most ARs were encountered using oral routes of administration, and tended to be systemic rather than ocular. In comparison, ARs of the locally administered drugs were dominated by expected consequences of the injection method, rather than the studied drug [41].

Gastrointestinal effects accounted for 4/16 (25%), and dermatological effects 5/16 (31%), of ARs reported in patients receiving mTOR inhibitors orally. Gastrointestinal effects, dermatological effects, DVT and thrombocytopenia are known side effects of mTOR inhibitors when taken orally [44,45].

Other ARs encountered that are more unusual include 2 cases of headaches, and 1 case each of deranged LFTs, myalgia, recurrent infection, and scleroderma. These comprise 6/16 (38%) of the ARs reported in patients taking mTOR inhibitors orally.

Table 4. Adverse drug reactions and procedure-related adverse events reported in the included clinical studies

Adverse Reaction	IVT sirolimus	SCJ sirolimus	Oral sirolimus	Oral everolimus	Total
Abdominal pain	0	0	1	1	2
Acne	0	0	1	0	1
Chemosis	0	1	0	0	1
DVT	0	0	1	0	1
Eczema	0	0	1	0	1
Erythema nodosum	0	0	1	0	1
Floater	4	0	0	0	4
Headache	0	0	1	1	2
LFT derangement	0	0	1	0	1
Myalgia	0	0	1	0	1
Nausea +/- vomiting	0	0	2	0	2
Ocular pain	1	0	0	0	1
Recurrent respiratory infection	0	0	1	0	1
Scleroderma-like changes	0	0	1	0	1
SCJ injection site irritation	0	9	0	0	9
Seborrheic dermatitis	0	0	1	0	1
Subconjunctival haemorrhage	1	0	0	0	1
Thrombocytopenia	0	0	1	0	1
Total	6	10	14	2	32

Note: some adverse reactions may have occurred in the same patient.

IVT is Intravitreal; SCJ is Subconjunctival; DVT is Deep Vein Thrombosis; LFT is Liver Function Test.

Discussion

This comprehensive review summarises the available evidence for the use of mTOR inhibitors in the management of non-infectious uveitis. At present, potential use of sirolimus and everolimus in uveitis treatment pathways is limited by the paucity of RCTs in this field.

The studies evaluated here provide some evidence that sirolimus and everolimus may be effective in suppressing active uveitis, and reducing the dose of corticosteroid or other immunomodulatory therapy required to achieve disease control. Reported efficacy across these endpoints ranges from 40% to 100%. Only one preclinical study documented a worsening of uveitis with sirolimus, however this only occurred only at very low doses, and as discussed above may be a feature of the animal model [36].

Sirolimus and everolimus appear to be well tolerated; the incidence of side effects was significantly higher in patients taking an mTOR inhibitor orally rather than by intravitreal or subconjunctival injection (0.921 per patient-year and 0.505 per patient-year, respectively) attributed to the wider systemic absorption of the drugs by this method of administration. Local administration is thus likely to become the preferred method of use in the future management of uveitis. On the whole, mTOR inhibition appears to be well tolerated, with no drug-related serious adverse reactions occurring in the included studies.

It is interesting to compare the adverse event rate to the major alternatives for corticosteroid-sparing therapy in uveitis. The recent VISUAL I randomised controlled trial for the use of adalimumab in uveitis reported an AE rate of 1052.4 per 100 patient-years (10.52 per patient-year) [49]. This is significantly higher than our current review's suggested AE rate for mTOR inhibitors, which is 1.131 per patient-year for sirolimus and 0.666 per patient-

year for everolimus. However it should be recognised that this is not a direct comparison within the same study, and that there are differences in the populations sampled and the methodologies used.

A recent trial of methotrexate and mycophenolate mofetil in non-infectious uveitis reported AEs in 33/41 patients receiving methotrexate, and 32/39 patients receiving mycophenolate mofetil, with a follow up of 6 months [50]. The trial comprises an analysis of 20.5 patient-years for methotrexate and 19.5 patient-years for mycophenolate mofetil, as a result representing a methotrexate AE rate of 1.608 per patient-year and mycophenolate mofetil AE rate of 1.641 per patient-year. Both of these rates are higher, although in the same order of magnitude, than those found for the mTOR inhibitors in this review, although again the same cautions apply. Overall however, these studies do suggest that mTOR inhibitors seem to have acceptable rates of adverse events in the studied populations.

Corticosteroids currently form the mainstay of treatment in uveitis, used both as 'rescue therapy' for acute disease flares, and as long-term therapy in chronic disease.

Corticosteroids have many desirable features, including speed of onset and efficacy in both local and systemic forms: in the MUST trial disease inactivity was reported in 88% eyes randomised to the fluocinolone acetonide implant and in 71% eyes randomised to systemic corticosteroid usage [51]. They do however have an unfavourable side-effect profile, with adverse systemic effects being more common and of greater severity with increasing dose and duration of use. There has been a longstanding desire to develop alternative immunomodulatory therapies with improved side-effect profiles whilst achieving similar efficacy to corticosteroids; these agents are often referred to as "steroid-sparing agents", as they often enable reduction in concurrent steroid dosage. Unfortunately, currently available

steroid-sparing agents are often associated with different, but equally severe side effects, and in many cases lack both the speed of onset and efficacy of corticosteroids.

In general, locally administered agents achieve rapid onset and as demonstrated in this review, tend to be associated with fewer systemic side-effects; locally administered mTOR inhibitors therefore offer an attractive option as potential steroid-sparing immunomodulatory therapy in uveitis.

The potential for mTOR inhibitors in this role is being recognised, as reflected by the level of ongoing study into the area. The SAKURA study (NCT01358266) is a phase 3 multicentre, randomised and double-blinded study of sirolimus for active non-infectious uveitis [52,53]. The SAKURA study is expected to report initial efficacy data in late 2016, with an associated safety study due to report in 2017 [52,54]. This will be the largest study of mTOR inhibition in uveitis to date (in terms of patient population and patient-years of analysis) and is likely to provide robust evidence to guide further use of sirolimus in uveitis treatment protocols.

Screening of international trial registries also identified two other studies which were registered but for which the results have not been published. The SAVE-2 trial (NCT01280669) is registered as a randomised phase 2 trial comparing 440µg to 880µg intravitreal sirolimus [55]; this was registered in 2011 and there are currently no updates on ClinicalTrials.gov so it is not clear if this is ongoing. Another trial was registered on EudraCT in 2006 outlining an investigation into oral everolimus, but this is recorded as having been cancelled [56]. The potential relevance of mTOR inhibitors to health policy in regard to uveitis is shown by the fact that in 2015 the National Institute for Health and Care

Excellence (NICE), a public body that guides clinical practice in the UK, commissioned a 'Multiple Technology Appraisal' to evaluate a number of agents for the management of non-infectious uveitis which would include intravitreal sirolimus; however in 2016 intravitreal sirolimus was withdrawn from this particular MTA, which is now focussed on the use of subcutaneous adalimumab and an intravitreal dexamethasone implant (Ozurdex) [57]. The outcome of this appraisal is scheduled to be published in July 2017.

As mentioned above, one preclinical study identified a worsening of uveitis with low dose sirolimus. It is therefore interesting to note that a potentially pro-inflammatory effect of sirolimus has also been described by Valle et al. in a type 1 diabetes mellitus NOD (non-obese diabetic) mouse model [58]. The study aimed to investigate sirolimus as an immunomodulatory adjunct for diabetic mice treated with anti-CD3 antibodies. However they encountered significant worsening of glycaemic control and disruption of T cell tolerance [58]. The authors suggest that this may be due to the inhibition of IL-2 transduction by sirolimus, which they state is necessary for the effects of the anti-CD3 therapy in the mouse model [58].

However, the results of this study have been contradicted by a more recent study by Perl et al. [59]. Perl et al. found that sirolimus and anti-CD3 co-therapy resulted in improved long-term control of diabetic NOD mice, although documented a similar initial worsening of glycaemic state. The authors discussed the Valle et al. study, and suggested that the previous results may be caused by a directly toxic action on pancreatic beta cells posed by sirolimus, rather than an immune mediated effect [59]. However Perl et al. concluded that the ability of sirolimus to provide immune tolerance to beta cells in the setting of diabetes is

unclear [59]. Further research may therefore be required to establish if a pro-inflammatory sirolimus effect occurs outside of a mouse model.

It is also intriguing to note that the synergistic effect of mTOR inhibition provided alongside calcineurin inhibitors described in pre-clinical study by Ideka et al. [33] and Martin et al. [34] is not solely limited to experimental uveitis. Ideka et al. added tacrolimus, while Martin et al. added ciclosporin, and both of these calcineurin inhibitors have been documented elsewhere to provide benefit for autoimmune disease in combination with mTOR inhibition [60,61]. Warner et al. have shown synergistic action between sirolimus and ciclosporin for the treatment of systemic lupus erythematosus in a mouse model [60], while Shapiro et al. have shown synergism between sirolimus and tacrolimus for NOD mouse models of type 1 diabetes mellitus [61]. This indicates a potential for co-therapy that could extend to human autoimmune disease, which may have an effect on reducing adverse events.

While considering preclinical studies of experimental uveitis treatments, it is worthwhile mentioning other mechanisms of current interest in this field, in addition to immunomodulation via mTOR inhibition. Of particular recent interest is the immunomodulatory molecule VGX-1027, which seems to exhibit properties that antagonise the activation of the Toll-Like Receptor 4 (TLR4) [62]. It is thought that this occurs by reducing antigen presentation [62], while studies have also shown effects on intracellular signalling pathways [63]. Preclinical trials of VGX-1027 have shown efficacy for models of autoimmune disease, particularly inflammatory bowel disease, type 1 diabetes mellitus and rheumatoid arthritis [62]. From an ophthalmological viewpoint, Mangano et al. have studied

VGX-1027 use in a Lewis rat model of uveitis, and found evidence for a reduction of ocular inflammation [64].

Another mechanism of interest regards the role of carbon monoxide in inflammatory disease; exogenous CO may assist in the control of autoimmune conditions [65]. Fagone et al. have studied the use of the CO-releasing molecule CORM-A1 in a rat model of uveitis, and found a significant improvement of ocular inflammation [65].

Both of these mechanisms are in the early stages of being potential contenders for non-infectious uveitis treatment modalities.

This review is particularly limited by the low evidence level of the literature currently available. Most studies included were non-comparative trials, and of the few comparative studies included, none were masked increasing the risk of performance and detection biases. Additionally, the field of study of mTOR inhibition for uveitis is relatively small, reflected in the low number of studies that could be included, and the low combined patient-years of analysis that this review represents (64.87 patient-years of combined clinical analysis). This is due to a combination of short follow-up periods and small study populations, both of which are restrictions in the available literature in this field currently. As a result, our ability to derive robust conclusions regarding wider use of mTOR inhibitors in uveitis is limited.

The wide range of mTOR inhibitor doses featured in this review is also a potential limitation. Doses were particularly varied for studies of oral sirolimus, which ranged from 1mg sirolimus per day in a case series by Phillips et al. [45], to 12mg sirolimus per day in a trial by Shanmuganathan et al. [44]. Further research into a sirolimus dose-response relationship in

the setting of non-infectious uveitis may be needed to investigate for the relevance of this variable.

Full texts for two studies identified by the searches were unable to be obtained for this review, despite attempts to contact the authors and publishing bodies directly. Since these comprised only one preclinical study [37] and a single case report [47], these would not have significantly contributed to the evidence, and there is no indication in the abstracts that they would have contradicted the conclusions of other included studies.

The varied aetiologies of patients with non-infectious uveitis is an underlying difficulty when undertaking research in this field. Uveitis is a heterogeneous group of disorders rather than a single aetiological entity, an aspect which is seen within the studies presented in this review (illustrated in Table 2).

As interest in this field grows, a more consistent range of uveitis diagnoses may emerge, and consideration of efficacy of interventions, such as the mTOR inhibitors, in clearly defined subgroups may inform the development of subgroup-targeted therapies and improved outcomes for patients.

Conclusion

In conclusion, this comprehensive review provides some support for the use of mTOR-inhibitors in the management of non-infectious uveitis, being well tolerated, particularly when delivered locally. Whilst the review found limited evidence for the efficacy of sirolimus and everolimus, there is a clear need for further study in this area, particularly in the form of well-designed RCTs. To this end, we await the results of the SAKURA study which will

provide much-needed evidence of the potential role for mTOR inhibitors in contemporary treatment protocols for the management of non-infectious uveitis.

References

- [1] Suttorp-Schulten MS, Rothova A. The possible impact of uveitis in blindness: a literature survey. *Br J Ophthalmol*. 1996 Sep; 80(9): 844-8.
- [2] Curnow SJ, Scheel-Toellner D, Jenkinson W, Raza K, Durrani OM, Faint JM, et al. Inhibition of T cell apoptosis in the aqueous humor of patients with uveitis by IL-6/soluble IL-6 receptor trans-signalling. *J Immunol*. 2004 Oct 15; 173(8): 5290-7.
- [3] Lightman S, Towler H. *Uveitis*. 1st ed. United Kingdom: BMJ Publishing Group; 1998. ISBN: 9780727912022.
- [4] Jabs DA, Nussenblatt RB, Rosenbaum JT, Standardization of Uveitis Nomenclature (SUN) Working Group. Standardization of uveitis nomenclature for reporting clinical data. Results of the First International Workshop. *Am J Ophthalmol*. 2005 Sep; 140(3): 509-16.
- [5] Barry RJ, Nguyen QD, Lee RW, Murray PI, Denniston AK. Pharmacotherapy for uveitis: current management and emerging therapy. *Clin Ophthalmol*. 2014 Sep 22; 8: 1891-911.
- [6] Mikhail M, Sallam A. Novel Intraocular Therapy in Non-infectious Uveitis of the Posterior Segment of the Eye. *Med Hypothesis Discov Innov Ophthalmol*. 2013 Winter; 2(4): 113-20.
- [7] Salzmann J, Lightman S. The potential of newer immunomodulating drugs in the treatment of uveitis: a review. *BioDrugs*. 2000 Jun; 13(6): 397-408.
- [8] Jabs DA, Rosenbaum JT, Foster CS, Holland GN, Jaffe GJ, Louie JS et al. Guidelines for the use of immunosuppressive drugs in patients with ocular inflammatory disorders: recommendations of an expert panel. *Am J Ophthalmol*. 2000 Oct; 130(4): 492-513.
- [9] Lee K, Bajwa A, Freitas-Neto CA, Metzinger JL, Wentworth BA, Foster CS. A comprehensive review and update on the non-biologic treatment of adult noninfectious uveitis: part I. *Expert Opin Pharmacother*. 2014 Oct; 15(15): 2141-54.

- [10] Lee K, Bajwa A, Freitas-Neto CA, Metzinger JL, Wentworth BA, Foster CS. A comprehensive review and update on the biologic treatment of adult noninfectious uveitis: part II. *Expert Opin Biol Ther*. 2014 Nov; 14(11): 1651-66.
- [11] Agarwal A, Rajagopalan N, Hassan M, Sadiq MA, Soliman MK, Afridi R, et al. Sirolimus for Retinal and Uveitic Diseases. *Dev Ophthalmol*. 2016; 55: 276-81.
- [12] Barrett K, Brooks H, Boitano S, Barman S. Ganong's Review of Medical Physiology. 23rd Ed. USA: McGraw-Hill Companies Inc Lange Medical; 2010. ISBN: 978-0-07-160568-7. P.76
- [13] Cantrell DA, Smith KA. The interleukin-2 T-cell system: a new cell growth model. *Science*. 1984 Jun 22; 224(4655): 1312-6.
- [14] Pópulo H, Lopes JM, Soares P. The mTOR signalling pathway in human cancer. *Int J Mol Sci*. 2012; 13(2): 1886-918.
- [15] Pierdominici M, Vacirca D, Delunardo F, Ortona E. mTOR signaling and metabolic regulation of T cells: new potential therapeutic targets in autoimmune diseases. *Curr Pharm Des*. 2011 Dec; 17(35): 3888-97.
- [16] Hu S, Chen M, Wang Y, Wang Z, Pei Y, Fan R, et al. mTOR Inhibition Attenuates Dextran Sulfate Sodium-Induced Colitis by Suppressing T Cell Proliferation and Balancing TH1/TH17/Treg Profile. *PLoS One*. 2016 Apr 29; 11(4): e0154564.
- [17] Nicoletti F, Fagone P, Meroni P, McCubrey J, Bendtzen K. mTOR as a multifunctional therapeutic target in HIV infection. *Drug Discov Today*. 2011 Aug; 16(15-16): 715-21.
- [18] Donia M, McCubrey JA, Bendtzen K, Nicoletti F. Potential use of rapamycin in HIV infection. *Br J Clin Pharmacol*. 2010 Dec; 70(6): 784-93.
- [19] Nicoletti F, Lapenta C, Donati S, Spada M, Ranazzi A, Cacopardo B, Mangano K, Belardelli F, Perno C, Aquaro S. Inhibition of human immunodeficiency virus (HIV-1)

infection in human peripheral blood leucocytes-SCID reconstituted mice by rapamycin. Clin Exp Immunol. 2009 Jan; 155(1): 28-34.

[20] Fagone P, Donia M, Mangano K, Quattrocchi C, Mammana S, Coco M, Libra M, McCubrey JA, Nicoletti F. Comparative study of rapamycin and temsirolimus demonstrates superimposable anti-tumour potency on prostate cancer cells. Basic Clin Pharmacol Toxicol. 2013 Jan; 112(1): 63-9.

[21] Sokolosky ML, Stadelman KM, Chappell WH, Abrams SL, Martelli AM, Stivala F, Libra M, Nicoletti F, Drobot LB, Franklin RA, Steelman LS, McCubrey JA. Involvement of Akt-1 and mTOR in sensitivity of breast cancer to targeted therapy. Oncotarget. 2011 Jul; 2(7): 538-50.

[22] Steelman LS, Martelli AM, Cocco L, Libra M, Nicoletti F, Abrams SL, McCubrey JA. The therapeutic potential of mTOR inhibitors in breast cancer. Br J Clin Pharmacol. 2016 Nov; 82(5): 1189-1212.

[23] Gu Z, Tan W, Ji J, Feng G, Meng Y, Da Z, Guo G, Xia Y, Zhu X, Shi G, Cheng C. Rapamycin reverses the senescent phenotype and improves immunoregulation of mesenchymal stem cells from MRL/lpr mice and systemic lupus erythematosus patients through inhibition of the mTOR signaling pathway. Aging (Albany NY). 2016 May; 8(5): 1102-14.

[24] Warner LM, Adams LM, Sehgal SN. Rapamycin prolongs survival and arrests pathophysiologic changes in murine systemic lupus erythematosus. Arthritis Rheum. 1994 Feb; 37(2): 289-97.

[25] Donia M, Mangano K, Amoroso A, Mazzarino MC, Imbesi R, Castrogiovanni P, Coco M, Meroni P, Nicoletti F. Treatment with rapamycin ameliorates clinical and histological signs of protracted relapsing experimental allergic encephalomyelitis in Dark Agouti rats and induces expansion of peripheral CD4+CD25+Foxp3+ regulatory T cells. J Autoimmun. 2009 Sep; 33(2): 135-40.

- [26] Carlson RP, Baeder WL, Caccese RG, Warner LM, Sehgal SN. Effects of orally administered rapamycin in animal models of arthritis and other autoimmune diseases. *Ann N Y Acad Sci.* 1993 Jun 23; 685: 86-113.
- [27] Baeder WL, Sredy J, Sehgal SN, Chang JY, Adams LM. Rapamycin prevents the onset of insulin-dependent diabetes mellitus (IDDM) in NOD mice. *Clin Exp Immunol.* 1992 Aug; 89(2): 174-8.
- [28] Bride KL, Vincent T, Smith-Whitley K, Lambert MP, Bleasing JJ, Seif AE, Manno CS, Casper J, Grupp SA, Teachey DT. Sirolimus is effective in relapsed/refractory autoimmune cytopenias: results of a prospective multi-institutional trial. *Blood.* 2016 Jan 7; 127(1): 17-28.
- [29] Dasari TW, Patel B, Saucedo JF. Systematic review of effectiveness of oral sirolimus after bare-metal stenting of coronary arteries for prevention of in-stent restenosis. *Am J Cardiol.* 2013 Nov 1; 112(9): 1322-7
- [30] Guo D, Zeng L, Brody DL, Wong M. Rapamycin attenuates the development of posttraumatic epilepsy in a mouse model of traumatic brain injury. *PLoS One.* 2013 May 14; 8(5): e64078
- [31] Pleyer U, Thureau SR. sirolimus for the treatment of noninfectious uveitis. *Expert Opin Pharmacother.* 2016; 17(1): 127-35.
- [32] NHS National Institute for Health Research. Clinical Trials Toolkit - Safety Reporting Assessment Flowchart. [internet] 2014 [cited 11 June 2016]. Available from: http://www.ct-toolkit.ac.uk/_data/assets/pdf_file/0011/35021/safety-reporting-assessment-flowchart.pdf
- [33] Ikeda E, Hikita N, Eto K, Mochizuki M. Tacrolimus-rapamycin combination therapy for experimental autoimmune uveoretinitis. *Jpn J Ophthalmol.* 1997 Nov-Dec; 41(6): 396-402.

- [34] Martin DF, DeBarge LR, Nussenblatt RB, Chan CC, Roberge FG. Synergistic effect of rapamycin and cyclosporin A in the treatment of experimental autoimmune uveoretinitis. *J Immunol*. 1995 Jan 15; 154(2): 922-7.
- [35] Roberge FG, Xu D, Chan CC, de Smet MD, Nussenblatt RB, Chen H. Treatment of autoimmune uveoretinitis in the rat with rapamycin, an inhibitor of lymphocyte growth factor signal transduction. *Curr Eye Res*. 1993 Feb; 12(2): 197-203.
- [36] Zhang Z, Wu X, Duan J, Hinrichs D, Wegmann K, Zhang GL et al. Low dose rapamycin exacerbates autoimmune experimental uveitis. *PLoS One*. 2012; 7(5): e36589.
- [37] Ohia EO, Mancino M, Kulkarni PS. Effects of steroids and immunosuppressive drugs on endotoxin-uveitis in rabbits. *J Ocul Pharmacol*. 1992 Winter; 8(4): 295-307.
- [38] Yang K, Wen J, Liu X, Kijlstra A, Chen L, Chi W et al. Inhibitory effect of rapamycin and dexamethasone on production of IL-17 and IFN-gamma in Vogt-Koyanagi-Harada patients. *Br J Ophthalmol*. 2009 Feb; 93(2): 249-53.
- [39] Hennig M, Bauer D, Wasmuth S, Busch M, Walscheid K, Thanos S, Heiligenhaus A. Everolimus improves experimental autoimmune uveoretinitis. *Exp Eye Res*. 2012 Dec; 105: 43-52.
- [40] Vigil EM, Sepah YJ, Watters AL, Sadiq MA, Ansari M, Bittencourt MG, 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. *J Ophthalmic Inflamm Infect*. 2015 Apr; 5: 13.
- [41] Ibrahim MA, Sepah YJ, Watters A, Bittencourt M, Vigil EM, Do DV, Nguyen QD. One-Year Outcomes of the SAVE Study: Sirolimus as a Therapeutic Approach for UVEitis. *Transl Vis Sci Technol*. 2015 Mar 10; 4(2): 4.

[42] Nguyen QD, Ibrahim MA, Watters A, Bittencourt M, Yohannan J, Sepah YJ 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. *J Ophthalmic Inflamm Infect*. 2013 Feb 11; 3(1): 32.

[43] Sen HN, Larson TA, Meleth AD, Smith WM, Nussenblatt RB. Subconjunctival sirolimus for the treatment of chronic active anterior uveitis: results of a pilot trial. *Am J Ophthalmol*. 2012 Jun; 153(6): 1038-42.

[44] Shanmuganathan VA, Casely EM, Raj D, Powell RJ, Joseph A, Amoaku WM, Dua HS. The efficacy of sirolimus in the treatment of patients with refractory uveitis. *Br J Ophthalmol*. 2005 Jun; 89(6): 666-9.

[45] Phillips BN, Wroblewski KJ. A retrospective review of oral low-dose sirolimus (rapamycin) for the treatment of active uveitis. *J Ophthalmic Inflamm Infect*. 2010 Dec 7; 1(1): 29-34.

[46] Nussenblatt RB, Coleman H, Jirawuthiworavong G, Davuluri G, Potapova N, Dahr SS et al. The treatment of multifocal choroiditis associated choroidal neovascularization with sirolimus (rapamycin). *Acta Ophthalmol Scand*. 2007 Mar; 85(2): 230-1.

[47] Nowosielska A, Czarnecki W, Brydak-Godowska J, Dróbecka-Brydak E, Nowacka E, Lao M, Durlik M. Acute, idiopathic, bilateral uveitis with periphlebitis--case report. *Klin Oczna*. 2005; 107(4-6): 372-5.

[48] Heiligenhaus A, Zurek-Imhoff B, Roesel M, Hennig M, Rammrath D, Heinz C. Everolimus for the treatment of uveitis refractory to cyclosporine A: a pilot study. *Graefes Arch Clin Exp Ophthalmol*. 2013 Jan; 251(1): 143-52.

[49] Jaffe GJ, Dick AD, Brézín AP, Nguyen QD, Thorne JE, Kestelyn P, Barisani-Asenbauer T, Franco P, Heiligenhaus A, Scales D, Chu DS, Camez A, Kwatra NV, Song AP, Kron M, Tari S,

Suhler EB. Adalimumab in Patients with Active Noninfectious Uveitis. N Engl J Med. 2016 Sep 8; 375(10): 932-43.

[50] Rathinam SR, Babu M, Thundikandy R, Kanakath A, Nardone N, Esterberg E, Lee SM, Enanoria WT, Porco TC, Browne EN, Weinrib R, Acharya NR. A randomized clinical trial comparing methotrexate and mycophenolate mofetil for noninfectious uveitis. Ophthalmology. 2014 Oct; 121(10): 1863-70.

[51] Multicenter Uveitis Steroid Treatment (MUST) Trial Research Group, Kempen JH, Altaweel MM, Holbrook JT, Jabs DA, Louis TA, Sugar EA, Thorne JE. Randomized comparison of systemic anti-inflammatory therapy versus fluocinolone acetonide implant for intermediate, posterior, and panuveitis: the multicenter uveitis steroid treatment trial. Ophthalmology. 2011 Oct; 118(10): 1916-26. Erratum: Ophthalmology. 2012 Feb; 119(2): 212.

[52] ClinicalTrials.gov, US National Institutes of Health. Study Assessing Double-masked Uveitis Treatment (SAKURA). [internet] 2015 [cited 11 June 2016] Available from: <https://clinicaltrials.gov/ct2/show/NCT01358266>

[53] EU Clinical Trials Register. A Phase III, Multinational, Multicenter, Randomized, Double-Masked, Study Assessing the Safety and efficacy of Intravitreal Injections of DE-109 (three doses) for the Treatment of active, Non-Infectious Uveitis of the Posterior Segment of the eye. [internet] 2011 [cited 11 June 2016] Available from: <https://www.clinicaltrialsregister.eu/ctr-search/trial/2011-001595-19/GB>

[54] ClinicalTrials.gov, US National Institutes of Health. A Phase IIIb, Multinational, Multicenter, Open-Label Extension Study Assessing the Long-Term Safety of PRN Intravitreal Injections of DE-109 in Subjects With Non-Infectious Uveitis of the Posterior Segment of the

Eye Who Have Participated in the SAKURA Development Program (32-009). [internet] 2014 [cited 11 June 2016] Available from: <https://clinicaltrials.gov/ct2/show/NCT02251938>

[55] ClinicalTrials.gov, US National Institutes of Health. Intravitreal Sirolimus as Therapeutic Approach to Uveitis (SAVE-2). [internet] 2011 [cited 11 June 2016] Available from: <https://clinicaltrials.gov/ct2/show/NCT01280669>

[56] EU Clinical Trials Register. Single center study on safety and efficacy of everolimus in patients with endogenous intermediate and posterior uveitis. [internet] 2007 [cited 11 June 2016] Available from: <https://www.clinicaltrialsregister.eu/ctr-search/trial/2006-004876-10/DE#P>

[57] National Institute for Health and Care Excellence (NICE). Uveitis (posterior segment, non-infectious) - adalimumab and dexamethasone [ID763]. [internet] 2016 [cited 11 June 2016] Available from: <https://www.nice.org.uk/guidance/indevelopment/gid-ta10007>

[58] Valle A, Jofra T, Stabilini A, Atkinson M, Roncarolo MG, Battaglia M. Rapamycin prevents and breaks the anti-CD3-induced tolerance in NOD mice. Diabetes. 2009 Apr; 58(4): 875-81.

[59] Perl S, Perlman J, Weitzel RP, Phang O, Hsieh MM, Tisdale J. Addition of rapamycin to anti-CD3 antibody improves long-term glycaemia control in diabetic NOD mice. PLoS One. 2013 Jun 24; 8(6): e67189.

[60] Warner LM, Cummons T, Nolan L, Sehgal SN. Sub-therapeutic doses of sirolimus and cyclosporin A in combination reduce SLE pathologies in the MRL mouse. Inflamm Res. 1995 Aug; 44 Suppl 2: S205-6.

[61] Shapiro AM, Suarez-Pinzon WL, Power R, Rabinovitch A. Combination therapy with low dose sirolimus and tacrolimus is synergistic in preventing spontaneous and recurrent autoimmune diabetes in non-obese diabetic mice. Diabetologia. 2002 Feb; 45(2): 224-30.

[62] Fagone P, Muthumani K, Mangano K, Magro G, Meroni PL, Kim JJ, Sardesai NY, Weiner DB, Nicoletti F. VGX-1027 modulates genes involved in lipopolysaccharide-induced Toll-like receptor 4 activation and in a murine model of systemic lupus erythematosus. *Immunology*. 2014 Aug; 142(4): 594-602.

[63] Stojanovic I, Cuzzocrea S, Mangano K, Mazzon E, Miljkovic D, Wang M, Donia M, Al Abed Y, Kim J, Nicoletti F, Stosic-Grujicic S, Claesson M. In vitro, ex vivo and in vivo immunopharmacological activities of the isoxazoline compound VGX-1027: modulation of cytokine synthesis and prevention of both organ-specific and systemic autoimmune diseases in murine models. *Clin Immunol*. 2007 Jun; 123(3): 311-23.

[64] Mangano K, Sardesai NY, Quattrocchi C, Mazzon E, Cuzzocrea S, Bendtzen K, Meroni PL, Kim JJ, Nicoletti F. Effects of the immunomodulator, VGX-1027, in endotoxin-induced uveitis in Lewis rats. *Br J Pharmacol*. 2008 Nov; 155(5): 722-30.

[65] Fagone P, Mangano K, Mammana S, Cavalli E, Di Marco R, Barcellona ML, Salvatorelli L, Magro G, Nicoletti F. Carbon monoxide-releasing molecule-A1 (CORM-A1) improves clinical signs of experimental autoimmune uveoretinitis (EAU) in rats. *Clin Immunol*. 2015 Apr; 157(2): 198-204.