



Cochrane
Library

Cochrane Database of Systematic Reviews

Rapamycin and rapalogs for tuberous sclerosis complex (Review)

Sasongko TH, Kademane K, Chai Soon Hou S, Jocelyn TXY, Zabidi-Hussin ZAMH

Sasongko TH, Kademane K, Chai Soon Hou S, Jocelyn TX, Zabidi-Hussin Z.
Rapamycin and rapalogs for tuberous sclerosis complex.
Cochrane Database of Systematic Reviews 2023, Issue 7. Art. No.: CD011272.
DOI: [10.1002/14651858.CD011272.pub3](https://doi.org/10.1002/14651858.CD011272.pub3).

www.cochranelibrary.com

Rapamycin and rapalogs for tuberous sclerosis complex (Review)

Copyright © 2023 The Cochrane Collaboration. Published by John Wiley & Sons, Ltd.

WILEY

TABLE OF CONTENTS

ABSTRACT	1
PLAIN LANGUAGE SUMMARY	2
SUMMARY OF FINDINGS	4
BACKGROUND	10
OBJECTIVES	13
METHODS	13
RESULTS	15
Figure 1.	16
Figure 2.	18
Figure 3.	19
DISCUSSION	25
AUTHORS' CONCLUSIONS	27
ACKNOWLEDGEMENTS	27
REFERENCES	28
CHARACTERISTICS OF STUDIES	42
DATA AND ANALYSES	61
Analysis 1.1. Comparison 1: Systemic administration of rapamycin or rapalogs versus placebo, Outcome 1: Response to tumour size (50% reduction from baseline)	62
Analysis 1.2. Comparison 1: Systemic administration of rapamycin or rapalogs versus placebo, Outcome 2: Response to skin lesions	63
Analysis 1.3. Comparison 1: Systemic administration of rapamycin or rapalogs versus placebo, Outcome 3: Increased creatinine levels	63
Analysis 1.4. Comparison 1: Systemic administration of rapamycin or rapalogs versus placebo, Outcome 4: Reduction in seizure frequency	64
Analysis 1.5. Comparison 1: Systemic administration of rapamycin or rapalogs versus placebo, Outcome 5: Change in attention and executive function - Stockings of Cambridge (CANTAB)	64
Analysis 1.6. Comparison 1: Systemic administration of rapamycin or rapalogs versus placebo, Outcome 6: Socialization and behaviour (Social Cognition - SRS)	64
Analysis 1.7. Comparison 1: Systemic administration of rapamycin or rapalogs versus placebo, Outcome 7: Adverse events	65
Analysis 2.1. Comparison 2: Topical administration of rapamycin or rapalogs versus placebo, Outcome 1: Improvement in skin lesions	67
Analysis 2.2. Comparison 2: Topical administration of rapamycin or rapalogs versus placebo, Outcome 2: Deterioration in skin lesions	67
Analysis 2.3. Comparison 2: Topical administration of rapamycin or rapalogs versus placebo, Outcome 3: Mean general improvement in facial angiofibroma at 12 weeks	68
Analysis 2.4. Comparison 2: Topical administration of rapamycin or rapalogs versus placebo, Outcome 4: Change in quality of life (combined DLQI and CDLQI)	68
Analysis 2.5. Comparison 2: Topical administration of rapamycin or rapalogs versus placebo, Outcome 5: Mean participant satisfaction at 12 weeks	69
Analysis 2.6. Comparison 2: Topical administration of rapamycin or rapalogs versus placebo, Outcome 6: Adverse events	69
ADDITIONAL TABLES	69
APPENDICES	71
WHAT'S NEW	72
HISTORY	73
CONTRIBUTIONS OF AUTHORS	73
DECLARATIONS OF INTEREST	73
SOURCES OF SUPPORT	73
DIFFERENCES BETWEEN PROTOCOL AND REVIEW	73
INDEX TERMS	74

[Intervention Review]

Rapamycin and rapalogs for tuberous sclerosis complex

Teguh Haryo Sasongko^{1,2}, Kumaraswamy Kademane³, Stanley Chai Soon Hou⁴, Tan Xin Yi Jocelyn⁴, ZAMH Zabidi-Hussin⁵

¹Department of Physiology, School of Medicine, International Medical University, Kuala Lumpur, Malaysia. ²Institute for Research, Development, and Innovation, International Medical University, Kuala Lumpur, Malaysia. ³Department of Pharmacology, Arunai Medical College and Hospital, Tiruvannamalai, Tamilnadu, India. ⁴Perdana University - Royal College of Surgeons in Ireland (RCSI) School of Medicine, Kuala Lumpur, Malaysia. ⁵Faculty of Medicine, University of Cyberjaya, Selangor, Malaysia

Contact: Teguh Haryo Sasongko, tghsasongko@gmail.com.

Editorial group: Cochrane Cystic Fibrosis and Genetic Disorders Group.

Publication status and date: New search for studies and content updated (conclusions changed), published in Issue 7, 2023.

Citation: Sasongko TH, Kademane K, Chai Soon Hou S, Jocelyn TX, Zabidi-Hussin Z. Rapamycin and rapalogs for tuberous sclerosis complex. *Cochrane Database of Systematic Reviews* 2023, Issue 7. Art. No.: CD011272. DOI: [10.1002/14651858.CD011272.pub3](https://doi.org/10.1002/14651858.CD011272.pub3).

Copyright © 2023 The Cochrane Collaboration. Published by John Wiley & Sons, Ltd.

ABSTRACT

Background

Potential benefits of rapamycin or rapalogs for treating people with tuberous sclerosis complex (TSC) have been shown. Currently everolimus (a rapalog) is only approved for TSC-associated renal angiomyolipoma and subependymal giant cell astrocytoma (SEGA), but not other manifestations of TSC. A systematic review needs to establish evidence for rapamycin or rapalogs for various manifestations in TSC. This is an updated review.

Objectives

To determine the effectiveness of rapamycin or rapalogs in people with TSC for decreasing tumour size and other manifestations and to assess the safety of rapamycin or rapalogs in relation to their adverse effects.

Search methods

We identified relevant studies from the Cochrane-Central-Register-of-Controlled-Trials (CENTRAL), Ovid MEDLINE and ongoing trials registries with no language restrictions. We searched conference proceedings and abstract books of conferences.

Date of the last searches: 15 July 2022.

Selection criteria

Randomised controlled trials (RCTs) or quasi-RCTs of rapamycin or rapalogs in people with TSC.

Data collection and analysis

Two review authors independently extracted data and assessed the risk of bias of each study; a third review author verified the extracted data and risk of bias decisions. We assessed the certainty of the evidence using GRADE.

Main results

The current update added seven RCTs, bringing the total number to 10 RCTs (with 1008 participants aged 3 months to 65 years; 484 males). All TSC diagnoses were by consensus criteria as a minimum. In parallel studies, 645 participants received active interventions and 340 placebo. Evidence is low-to-high certainty and study quality is mixed; mostly a low risk of bias across domains, but one study had a high risk of performance bias (lack of blinding) and three studies had a high risk of attrition bias. Manufacturers of the investigational products supported eight studies.

Systemic administration

Rapamycin and rapalogs for tuberous sclerosis complex (Review)

Copyright © 2023 The Cochrane Collaboration. Published by John Wiley & Sons, Ltd.

Six studies (703 participants) administered everolimus (rapalog) orally. More participants in the intervention arm reduced renal angiomyolipoma size by 50% (risk ratio (RR) 24.69, 95% confidence interval (CI) 3.51 to 173.41; $P = 0.001$; 2 studies, 162 participants, high-certainty evidence). In the intervention arm, more participants in the intervention arm reduced SEGA tumour size by 50% (RR 27.85, 95% CI 1.74 to 444.82; $P = 0.02$; 1 study; 117 participants; moderate-certainty evidence), and reported more skin responses (RR 5.78, 95% CI 2.30 to 14.52; $P = 0.0002$; 2 studies; 224 participants; high-certainty evidence). In one 18-week study (366 participants), the intervention led to 25% fewer seizures (RR 1.63, 95% CI 1.27 to 2.09; $P = 0.0001$) or 50% fewer seizures (RR 2.28, 95% CI 1.44 to 3.60; $P = 0.0004$); but there was no difference in numbers being seizure-free (RR 5.30, 95% CI 0.69 to 40.57; $P = 0.11$) (moderate-certainty evidence). One study (42 participants) showed no difference in neurocognitive, neuropsychiatry, behavioural, sensory and motor development (low-certainty evidence).

Total adverse events (AEs) did not differ between groups (RR 1.09, 95% CI 0.97 to 1.22; $P = 0.16$; 5 studies; 680 participants; high-certainty evidence). However, the intervention group experienced more AEs resulting in withdrawal, interruption of treatment, or reduced dose (RR 2.61, 95% CI 1.58 to 4.33; $P = 0.0002$; 4 studies; 633 participants; high-certainty evidence and also reported more severe AEs (RR 2.35, 95% CI 0.99 to 5.58; $P = 0.05$; 2 studies; 413 participants; high-certainty evidence).

Topical (skin) administration

Four studies (305 participants) administered rapamycin topically. More participants in the intervention arm showed a response to skin lesions (RR 2.72, 95% CI 1.76 to 4.18; $P < 0.00001$; 2 studies; 187 participants; high-certainty evidence) and more participants in the placebo arm reported a deterioration of skin lesions (RR 0.27, 95% CI 0.15 to 0.49; 1 study; 164 participants; high-certainty evidence). More participants in the intervention arm responded to facial angiofibroma at one to three months (RR 28.74, 95% CI 1.78 to 463.19; $P = 0.02$) and three to six months (RR 39.39, 95% CI 2.48 to 626.00; $P = 0.009$; low-certainty evidence). Similar results were noted for cephalic plaques at one to three months (RR 10.93, 95% CI 0.64 to 186.08; $P = 0.10$) and three to six months (RR 7.38, 95% CI 1.01 to 53.83; $P = 0.05$; low-certainty evidence). More participants on placebo showed a deterioration of skin lesions (RR 0.27, 95% CI 0.15 to 0.49; $P < 0.0001$; 1 study; 164 participants; moderate-certainty evidence). The intervention arm reported a higher general improvement score (MD -1.01, 95% CI -1.68 to -0.34; $P < 0.0001$), but no difference specifically in the adult subgroup (MD -0.75, 95% CI -1.58 to 0.08; $P = 0.08$; 1 study; 36 participants; moderate-certainty evidence). Participants in the intervention arm reported higher satisfaction than with placebo (MD -0.92, 95% CI -1.79 to -0.05; $P = 0.04$; 1 study; 36 participants; low-certainty evidence), although again with no difference among adults (MD -0.25, 95% CI -1.52 to 1.02; $P = 0.70$; 1 study; 18 participants; low-certainty evidence). Groups did not differ in change in quality of life at six months (MD 0.30, 95% CI -1.01 to 1.61; $P = 0.65$; 1 study; 62 participants; low-certainty evidence).

Treatment led to a higher risk of any AE compared to placebo (RR 1.72, 95% CI 1.10, 2.67; $P = 0.02$; 3 studies; 277 participants; moderate-certainty evidence); but no difference between groups in severe AEs (RR 0.78, 95% CI 0.19 to 3.15; $P = 0.73$; 1 study; 179 participants; moderate-certainty evidence).

Authors' conclusions

Oral everolimus reduces the size of SEGA and renal angiomyolipoma by 50%, reduces seizure frequency by 25% and 50% and implements beneficial effects on skin lesions with no difference in the total number of AEs compared to placebo; however, more participants in the treatment group required a dose reduction, interruption or withdrawal and marginally more experienced serious AEs compared to placebo.

Topical rapamycin increases the response to skin lesions and facial angiofibroma, an improvement score, satisfaction and the risk of any AE, but not severe adverse events.

With caution regarding the risk of severe AEs, this review supports oral everolimus for renal angiomyolipoma, SEGA, seizure, and skin lesions, and topical rapamycin for facial angiofibroma.

PLAIN LANGUAGE SUMMARY

Drugs that aim to relieve clinical symptoms of tuberous sclerosis complex

Review question

Can rapamycin or rapalogs reduce the severity of clinical symptoms in people with tuberous sclerosis complex?

Background

Tuberous sclerosis complex is a genetic disease caused by mutations in *TSC1* or *TSC2* genes that affects several organs such as the brain, kidneys, heart, lungs and skin. The incidence is one in approximately 6000. Previous studies have shown potential benefits of rapamycin or rapalogs for treating people with tuberous sclerosis complex. Although everolimus (a rapalog) is currently approved by the FDA (USA Food and Drug Administration) and EMA (European Medicines Agency) for tumours associated with tuberous sclerosis complex (renal angiomyolipoma and subependymal giant cell astrocytoma), the use of these drugs for treating other symptoms of the condition has not yet been established. This review aims to bring together clinical trials in this area to establish the clinical value of rapamycin and rapalogs for various symptoms of tuberous sclerosis complex.

Search date

Rapamycin and rapalogs for tuberous sclerosis complex (Review)

Copyright © 2023 The Cochrane Collaboration. Published by John Wiley & Sons, Ltd.

The evidence is current to: 15 July 2022.

Study characteristics

The review included 10 studies with 1008 people with tuberous sclerosis complex aged between three months and 65 years of age. However, one study involved five people with sporadic lymphangioleiomyomatosis (without tuberous sclerosis complex) who we could not remove from the analysis. Studies compared rapamycin or rapalogs with placebo (containing no active ingredient) and people were selected for one treatment or the other randomly. The duration of the studies was variable. Two studies were funded by Novartis Pharmaceuticals.

Key results

Oral everolimus (rapalog) increased the number of people who achieved a 50% reduction in the size of subependymal giant cell astrocytoma and renal angiomyolipoma, as well as 25% and 50% reduction in seizure frequency. Oral everolimus also showed benefit in terms of response to skin lesions. However, those who received the systemic treatment probably had a higher risk of experiencing any adverse events as compared to those who did not receive treatment. More people receiving the systemic treatment had severe adverse events and adverse events that cause them to withdraw from the trial, temporarily stop treatment or reduce their dose compared to people receiving placebo.

Topical rapamycin increased the proportion of people who reported response to any skin lesions and probably facial angiofibroma. Absence of topical rapamycin increased the proportion of people who reported deterioration to any skin lesions. The topical rapamycin was also shown to increase improvement score and satisfaction. However, those who received the topical treatment probably had a higher risk of experiencing any adverse events, but not severe adverse events, compared to those who did not receive treatment.

Certainty of the evidence

All of the included studies generally showed a low risk of bias in study design. Four of these studies showed high risk of bias in a few areas of study design, such as whether participants knew if they were given the treatment or placebo and incomplete data in the final analyses. In eight of the studies, we need important information from trial authors in several study design areas in order to make quality judgements on those areas, such as whether people knew which group they would be put into, whether participants knew if they were given the treatment or placebo, whether research personnel knew if participants receive the treatment or placebo, whether the person who assess the effect of intervention knew if participants receive the treatment or placebo, and incomplete data in final analyses. In eight studies, some authors who are employees, stock owners, consultants, or received grants from the manufacturers of the investigational products were involved in the study design, discussion, research, overseeing of data collection and data analysis and interpretation. These studies were also supported by manufacturers of the investigational products.

The evidence from studies using systemic administration showed mixed levels of certainty. We found high-certainty evidence for 50% reduction in the size of renal angiomyolipoma, response to skin lesions, and adverse events. Outcomes related to 50% reduction in the size of SEGA, frequency of seizure, and number of participants with increased creatinine level were judged as having moderate-certainty evidence. Outcomes related to participants' well-being were of low-certainty evidence.

The evidence from studies using topical administration also showed mixed levels of certainty. We found high-certainty evidence for improvements in any skin lesions. We found moderate-certainty evidence for adverse events. Outcomes on response to facial angiofibroma, cephalic plaque, and participants' well-being were of low-certainty evidence.

SUMMARY OF FINDINGS

Summary of findings 1. Summary of findings for systemic administration of rapalogs

Oral everolimus (rapalog) compared with placebo for people with TSC

Patient or population: people with TSC

Settings: outpatient

Intervention: oral everolimus (rapalog)

Comparison: placebo

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Placebo	Oral everolimus (rapalog)				
50% reduction of tumour size^a Renal angiomyolipoma (follow-up: mixed - 12 to 48 weeks)	See comment	450 per 1000 (see comment)	RR 24.7 (95% CI 3.5 to 173.4)	162 (2)	⊕⊕⊕⊕ high^c	P = 0.001 0/53 participants in the placebo group and 49/109 participants in the rapalog group experienced 50% reduction in tumour size. Assumed and corresponding risk are calculated as the event rates in each group.
	SEGA (follow-up: 12 weeks)	See comment 346 per 1000 (see comment)	RR 27.9 (95% CI 1.7 to 444.8)	117 (1)	⊕⊕⊕ moderate^{c,e}	P = 0.02 0/39 participants in the placebo group and 27/78 participants in the rapalog group experienced 50% reduction in tumour size. Assumed and corresponding risk are calculated as the event rates in each group.
Response to skin lesions^b Follow-up: 6 months	53 per 1000	307 per 1000 (122 to 769 per 1000)	RR 5.8 (95% CI 2.3 to 14.5)	224 (2)	⊕⊕⊕⊕ high^c	P = 0.0002



Frequency of seizure Follow-up: 18 weeks	Number of participants with 100% reduction in seizure frequency	8 per 1000	42 per 1000 (6 to 325 per 1000)	RR 5.30 (95% CI 0.69 - 40.57)	366 (1)	⊕⊕⊕ moderate^{c,e}	These outcomes were analysed from only one out of 3 studies reporting seizure frequency (French 2016 (EXIST 3)). We could not analyse data from Overwater 2016 that used cross-over design until we obtain data from the first period prior to crossing the arms. Neither could we analyse data from Franz 2013 (EXIST 1) that only reported the median change of seizure frequency and where a large proportion of participants did not experience seizures at baseline.
	Number of participants with 50% reduction in seizure frequency	151 per 1000	344 per 1000 (217 to 544)	RR 2.28 (95% CI 1.44 to 3.60)	366 (1)	⊕⊕⊕ moderate^e	
	Number of participants with 25% reduction in seizure frequency	378 per 1000	616 per 1000 (480 to 790)	RR 1.63 (95% CI 1.27 to 2.09)	366 (1)	⊕⊕⊕ moderate^e	
Number of participants with an increased creatinine level Follow-up: 6 months		77 per 1000	949 per 1000 (2 to 118)	RR 0.16 (95% CI 0.02 - 1.53)	118 (1)	⊕⊕⊕ moderate^e	
FEV₁/FVC ratio		This outcome was not reported.					
Outcomes relevant to participants' well-being	Change in attention and executive function: mean change in CANTAB score (follow-up: 6 months)	Mean score change in the placebo group was -0.58 (SD 1.11)	Mean score change in the rapalog group was 1.26 points lower (2.03 lower to 0.49 lower)	NA	42 (1)	⊕⊕ low^d	P = 0.001. Participants receiving placebo showed less deterioration in attention and executive function. A higher score is better. The outcome was reported as difference in mean score change.
	Socialization and behaviour (SRS): mean score (follow-up: 6 months)	Mean score in the placebo group was 81.15 (SD 18.86)	Mean score in the rapalog group was 4.98 points lower (17.34 lower to 7.38 higher)	NA	42 (1)	⊕⊕ low^d	P = 0.43. A higher score is better. The outcome was reported as difference in mean score.
Adverse events Follow-up: mixed (12 to 48 weeks)	Any adverse events	793 per 1000	892 per 1000	RR 1.09 (0.97-1.22)	680 (5)	⊕⊕⊕⊕ high	P = 0.16 180/227 from the placebo group and 404/453 from the treatment group experienced any adverse event.

Adverse events leading to dose reduction, interruption or withdrawal	76 per 1000	207 per 1000	RR 2.61 (95% CI 1.58 to 4.33)	633 (4)	⊕⊕⊕⊕ high	P = 0.0002 16/212 from placebo group and 87/421 experienced adverse events leading to dose reduction, interruption or withdrawal.
Severe adverse events	105 per 1000	233 per 1000	RR 2.35 (95% CI 0.99 to 5.58)	413 (2)	⊕⊕⊕⊕ high	P = 0.05 14/134 from the placebo group and 65/279 from the treatment group experienced severe adverse events.

*The basis for the **assumed risk** was the event rate in the placebo group unless otherwise stated in the comments. The **corresponding risk** (and its 95% CI) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: confidence interval; **FEV₁:** forced expiratory volume in 1 second; **FVC:** forced vital capacity; **RR:** risk ratio; **SEGA:** subependymal giant cell astrocytoma; **TSC:** tuberous sclerosis complex.

GRADE Working Group grades of evidence

High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.

Moderate certainty: we are moderately confident in the effect estimate; the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.

Low certainty: our confidence in the effect estimate is limited; the true effect may be substantially different from the estimate of the effect.

Very low certainty: we have very little confidence in the effect estimate; the true effect is likely to be substantially different from the estimate of effect.

This summary of finding table was generated based on analyses from two of the included studies (Bissler 2013 (EXIST 2), Franz 2013 (EXIST 1)) which used oral (systemic) administration of everolimus (rapalog). Five of the participants from Bissler study were diagnosed with sporadic lymphangiomyomatosis (without TSC) which we could not separate from the current analysis (we will attempt to do this for a future update of the review) (Bissler 2013 (EXIST 2)).

- Response to tumour size is defined as reduction in at least 50% reduction from baseline in sum of volumes of target tumour in participant.
- Definition of 'response' to skin lesions was not mentioned in the two studies analysed (Bissler 2013 (EXIST 2); Franz 2013 (EXIST 1)).
- No downgrading of evidence: certainty levels were not downgraded although there are large CIs. The number of participants is relatively large for a rare disease, thus judgement about the certainty of evidence (particularly judgements about precision) may be based on the absolute effect. Unless there is other reason for downgrading, certainty rating was considered 'high' because the outcome was appropriately assessed.
- Downgraded twice to low due to (1) imprecision caused by small number of participants and (2) indirectness because the result came from only 1 study which made it difficult to ascertain generalisability.
- Downgraded once to moderate due to indirectness because the result came from only 1 study which made it difficult to ascertain generalisability.

Summary of findings 2. Summary of findings for topical administration of rapamycin

Topical rapamycin compared with placebo for people with TSC

Patient or population: people with TSC

Settings: outpatient

Intervention: topical rapamycin

Comparison: placebo

Outcomes		Illustrative comparative risks* (95% CI)		Relative ef- fect (95% CI)	No of par- ticipants (studies)	Certainty of the evi- dence (GRADE)	Comments
		Assumed risk	Corresponding risk				
		Placebo	Topical Ra- pamycin				
50% reduction in tumour size		This outcome was not reported					
Response to skin lesion	Improve- ment of any skin lesion (follow-up: 6 months)	250 per 1000	730 per 1000	RR 2.72 (95% CI 1.76 to 4.18)	187 (2)	⊕⊕⊕⊕ high	P < 0.00001 16/59 from the placebo group and 94/128 from the treatment group ex- perienced response to any skin le- sion.Similarly, the number of partici- pants with worsening skin lesion was also reported in 1 study (164 partici- pants) as RR 0.27 (95% CI 0.15 to 0.49).
	Facial an- giofibroma (follow-up: 3 to 6 months)	See comment	600 per 1000	RR 39.39 (95% CI 2.48 to 626.00)	62 (1)	⊕⊕ low ^c	0/32 from the placebo group and 18/30 from the treatment group ex- perienced response to facial angiofi- broma at 3 to 6 months; similarly, at 1 to 3 months follow-up, RR 28.74 (95% CI 1.78 to 463.19).
	Cephalic plaque (fol- low-up: 3 to 6 months)	60 per 1000	460 per 1000	RR 7.38 (95% CI 1.01 to 53.83)	29 (1)	⊕⊕ low ^c	1/16 in the placebo group and 6/13 in the treatment group experienced response to cephalic plaque at 3 to 6 months; it was also reported at 1 to 3 months, RR 10.93 (95% CI 0.64 to 186.08).
Frequency of seizure	This outcome was not reported.						
Creatinine level	This outcome was not reported.						

FEV₁/FVC ratio This outcome was not reported.

Outcomes relevant to participants' well-being	Mean change in QoL (combined DLQI and CDLQI)	Mean (SD) change in QoL score in the placebo group was -0.5 (2.3).	Mean change in QoL score in the intervention group was 0.30 points higher (1.01 lower to 1.61 higher).	62 (1)	⊕⊕ low^c	
	Follow-up: 6 months					
	Mean score of participant satisfaction	Child group	Mean (SD) score in the placebo group was 3.67 (1.37).	Mean score in the intervention group was 1.5 points lower (2.69 lower to 0.31 lower).	18 (1)	⊕⊕ low^c
	Follow-up: 12 weeks	Adult group	Mean (SD) score in the placebo group was 3.17 (1.33).	Mean score in the intervention group was 0.25 points lower (1.52 lower to 1.02 higher).	18 (1)	⊕⊕ low^c
Adverse events	Any adverse events	426 per 1000	733 per 1000 (469 to 1000)	RR 1.72 (95% CI 1.10 to 2.67)	277 (3)	⊕⊕⊕ moderate^b
	Severe adverse events	53 per 1000	41 per 1000 (10 to 167)	RR 0.78 (95% CI 0.19 to 3.15)	179 (1)	⊕⊕⊕ moderate^d

*The basis for the **assumed risk** is provided in footnotes. The **corresponding risk** (and its 95% CI) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: confidence interval; **CDLQI:** Child dermatology life quality index; **DLQI:** Dermatology life quality index; **FEV₁:** forced expiratory volume in 1 second; **FVC:** forced vital capacity; **ITT:** intention-to-treat; **QoL:** quality of life; **RR:** risk ratio; **TSC:** tuberous sclerosis complex.

GRADE Working Group grades of evidence

High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.

Moderate certainty: we are moderately confident in the effect estimate; the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.

Low certainty: our confidence in the effect estimate is limited; the true effect may be substantially different from the estimate of the effect.

Very low certainty: we have very little confidence in the effect estimate; the true effect is likely to be substantially different from the estimate of effect.

- a. The study reported only the percentages and we inferred the absolute numbers of responders in each treatment arm from the percentages. While there are 5 dropouts, there is no information from which treatment arm these came from. The ITT principle is in effect for the analysis, assuming all participants completed the protocol.
- b. We downgraded once to moderate due to imprecision caused by substantial heterogeneity.
- c. We downgraded twice to low due to (1) imprecision caused by low number of participants and (2) indirectness because the result came from only 1 study which made it difficult to ascertain generalisability.
- d. We downgraded once to moderate due to indirectness because the result came from only 1 study which made it difficult to ascertain generalisability

BACKGROUND

Description of the condition

Tuberous sclerosis or tuberous sclerosis complex (TSC) (OMIM#191100; OMIM#613254) is a genetic disease that affects several organs such as the brain, kidneys, heart, lungs and skin (NINDS 2013). The primary manifestation is as a consequence of growth of non-malignant tumours in the various systems described. The incidence has been reported to be one in approximately 6000 (Osborne 1991). However, its true incidence is not known because of a number of undiagnosed cases consisting mostly of mildly affected or asymptomatic individuals (Osborne 1991).

Two disease-causing genes have been identified by positional cloning, *TSC1* (van Slegtenhorst 1997) and *TSC2* (ECTSC 1993). The *TSC1* gene is located on chromosome 9q34, and encodes the protein, hamartin (130 kDa, 1164 amino acids) (van Slegtenhorst 1997). The *TSC2* gene is located on chromosome 16p13.3, and encodes another protein, tuberlin (180 kDa, 1807 amino acids) (ECTSC 1993). However, 10% to 25% of people with TSC showed no *TSC1/TSC2* mutation as identified by conventional genetic testing (Northrup 2013). A report of 53 people with TSC with no mutation identified, reported that mosaicism was observed in the majority (58%) and then followed by intronic mutations, which were seen in 40% of the study population. Two mutations were even identified in skin tumour biopsies only, and were not seen at appreciable frequency in blood or saliva DNA (Tyburczy 2015). These genetic abnormalities are generally inherited in an autosomal dominant manner. Nevertheless, in more than 60% of cases sporadic mutations are known to occur (van Slegtenhorst 1999).

The defective production of hamartin is caused by *TSC1* mutations (NINDS 2013). Mutations on the *TSC2* gene lead to the defective production of another protein, tuberlin and are usually related to more severe manifestations (Dabora 2001). Both *TSC1* and *TSC2* are tumour suppressor genes, the defect of which will lead to an uncontrolled proliferation of benign tumours called tubers or hamartomas in various sites (Gao 2001). People with TSC present at different ages with a variety of clinical manifestations. Common presenting symptoms include skin lesions, seizures, learning disabilities or manifestations of tumours affecting organs such as the brain, heart, eyes, or the kidneys (Napolioni 2008). The disease can be diagnosed clinically by assessing individuals against major or minor criteria, depending on the signs and symptoms present (Northrup 2021).

Investigation of somatic mutations in a variety of TSC hamartomas supports classification of the *TSC1* and *TSC2* as tumour suppressor genes (Cheadle 2000). Interaction between hamartin and tuberlin has a stoichiometry of 1:1 and is stable; a tight binding interaction between tuberlin and hamartin forming a tumour suppressor heterodimer has been elucidated (Kwiatkowski 2003). This complex inhibits the mammalian target of rapamycin (mTOR) which is a key regulator in the signalling pathway of cell proliferation and organ size (Kwiatkowski 2003). It has been reported that hamartin-tuberlin complex regulates mTOR via hydrolysis of Rheb-GTP into its inactive GDP bound state, Rheb-GDP (Rosner 2004; Tee 2003).

Diagnostic criteria for TSC were recently updated (Northrup 2021) from its 2013 (Northrup 2013) and 1998 (Roach 1998) consensus.

The disease is diagnosed either genetically or clinically (Northrup 2021). Genetic diagnostic criteria include the identification of either a *TSC1* or *TSC2* pathogenic mutation in DNA from normal tissue. A pathogenic mutation is defined as a mutation that clearly inactivates the function of the *TSC1* or *TSC2* proteins (e.g. out-of-frame indel or nonsense mutation), prevents protein synthesis (e.g. large genomic deletion), or is a missense mutation whose effect on protein function has been established by functional assessment (Hoogeveen-Westerveld 2012; Hoogeveen-Westerveld 2013; LOVD *TSC1*; LOVD *TSC2*). Clinical diagnostic criteria include the major and minor clinical features that enable clinicians to clinically diagnose TSC (Northrup 2021). The 2013 consensus (Northrup 2013) featured 'cortical dysplasia' as a replacement to 'cortical tubers' in 1998 diagnostic criteria (Roach 1998). The most recent consensus (Northrup 2021) specified "multiple cortical tubers and/or radial migration lines" to replace the more general term "cortical dysplasias".

The new consensus for TSC clinical diagnostic criteria defines only possible and definitive diagnosis (Northrup 2013; Northrup 2021) without a probable diagnosis as defined in the old consensus (Roach 1998). A definitive diagnosis can be made when there are either two major features or one major feature with two or more minor features. A possible diagnosis can be made when there are either one major feature or two or more minor features (Northrup 2013; Northrup 2021). Major features in the most recent consensus include hypomelanotic macules (three or more, at least 5 mm diameter), angiofibromas (three or more) or fibrous cephalic plaque, ungual fibromas (two or more), shagreen patch, multiple retinal hamartomas, multiple cortical tubers and/or radial migration line, subependymal nodules, subependymal giant cell astrocytoma, cardiac rhabdomyoma, lymphangioleiomyomatosis (LAM) and angiomyolipomas (two or more). It is of note, however, findings on the combination of LAM and angiomyolipomas still require another feature for a definitive diagnosis (Northrup 2021). Minor features include 'confetti' skin lesions, dental enamel pits (more than three), intraoral fibromas (two or more), retinal achromic patch, multiple renal cysts, nonrenal hamartomas, and sclerotic bone lesions (Northrup 2021).

Tumours of major clinical attentions include those involving the heart, brain, kidney and lung. Generally, tumour manifestations of TSC occur later in life, except for cardiac rhabdomyomas. Studies estimated that up to 70% to 90% of children with rhabdomyomas have TSC, and at least 50% of children with TSC have rhabdomyomas. Nearly 100% of fetuses with multiple rhabdomyomas have TSC, underscoring the practical importance of identifying additional tumours at the time of fetal assessment for diagnosis and prognosis (Hinton 2014).

There are three types of brain lesions in TSC - cortical dysplasia (cortical tubers and cerebral white matter radial migration lines), subependymal nodules (SEN; formed in the walls of the ventricles) and subependymal giant cell astrocytoma (SEGA; develops from SEN and grow such that they may block the flow of fluid within the brain, causing a build-up of fluid and pressure and leading to headaches and blurred vision) (NINDS 2013).

Along this line, seizures are prevalent symptoms among individuals with TSC, affecting up to 85% (Chu-Shore 2010; Curatolo 2015a). In fact, around 66% of people with TSC experience seizures during their first year of life, usually as infantile spasms or focal seizures (Chu-Shore 2010; Curatolo 2015a). Notably, early-onset

epilepsy that goes untreated is linked to a greater likelihood of neurodevelopmental impairments, such as intellectual disability and autism spectrum disorder (Curatolo 2015a). The seizures linked to TSC can be focal, multifocal, or generalised, and are typically challenging to manage (Curatolo 2015b).

Renal problems in TSC, including angiomyolipomas (which occur in 80% of people with TSC) and multiple renal cysts, comprise the second leading cause of premature death after severe intellectual disability (Shepherd 1991). In people with TSC, LAM is associated with interstitial expansion of the lung with benign-appearing smooth muscle cells that infiltrate all lung structures (Johnson 2010; McCormack 2010).

Long-term morbidity that requires life-long institutional care is also of concern in TSC. Best estimates from epidemiological populations suggest that about 45% of individuals with TSC have intellectual disability (Joinson 2003). Rates of self-injury and aggression in children with TSC were 27% and 50%, respectively. These are high but not significantly different from rates in children with Down syndrome or other syndrome groups (Eden 2014). In addition, 30 out of 45 women who were diagnosed with TSC as adults, actually met the clinical criteria for TSC in childhood. Although these women had minimal morbidity during childhood, they were at risk of life-threatening pulmonary and renal manifestations (Seibert 2011).

Once the diagnosis of TSC is established and initial diagnostic evaluations completed, continued surveillance is necessary to monitor the progression of known problems or lesions and the emergence of new ones. Although some manifestations that appear during childhood do not cause significant problems in adulthood and vice versa, some others may be present throughout the entire life-span of the individual, such as epilepsy and TSC-associated neuropsychiatric disorders (TAND) (Krueger 2013).

Recent recommendations for provision of co-ordinated care of people with TSC specified that to allow TSC clinics to fulfil a pluripotent role, they need to offer or have access to a range of core services, including genetic testing and genetic counselling, neurology and neuroimaging, nephrology, urology, general and interventional radiology services, clinical psychology, psychiatry, and developmental paediatrics, collaboration with patient and family organisations, and collaboration with an individual's community physician (general practitioner) (Annear 2019).

Description of the intervention

Rapamycin (sirolimus) and rapalogs (analogs of rapamycin) are first generation inhibitors of mTOR. This is a protein kinase that controls cell growth, proliferation, and survival. Often mTOR signalling is up-regulated in cancer and there is a great interest in developing drugs that target this enzyme. Until recently, rapamycin sensitivity was the major criterion used to identify mTOR-controlled effects (Ballou 2008).

During the 1980s, rapamycin (sirolimus) was discovered to show an anti-cancer activity (Faivre 2006). However, due to its unfavourable pharmacokinetic properties, the development of rapamycin for the treatment of cancer was not successful at that time (Yuan 2009). Later on, analogs of rapamycin (rapalogs) with more favourable pharmacokinetic properties and reduced immunosuppressive effects were discovered (Faivre 2006). These

include temsirolimus (CCI-779), everolimus (RAD001), Biolimus A9 and zotarolimus (ABT-578) (Falotico 2005; Brachmann 2009). Both biolimus and zotarolimus are drug-eluting coronary stents for preventing coronary artery restenosis.

Rapamycin ($C_{51}H_{79}NO_{13}$) is a macrolide compound that was isolated in 1975 from *Streptomyces hygroscopicus* found in a soil sample on Easter Island. It prevents activation of T cells and B cells by inhibiting their response to interleukin-2 (IL-2). It is an FDA-approved drug for immunosuppression after organ transplantation. Rapamycin also possesses both antifungal and antineoplastic properties. Rapamycin is administered orally once daily, with or without food as a tablet or a solution with a maximum daily dose of 40 mg (RxList 2015a). The most common adverse reactions (at least 30%) observed with rapamycin in clinical studies are peripheral oedema, hypertriglyceridaemia, hypertension, hypercholesterolaemia, creatinine increase, constipation, abdominal pain, diarrhoea, headache, fever, urinary tract infection, anaemia, nausea, arthralgia, pain and thrombocytopaenia (RxList 2015a).

Topical rapamycin has been used for facial angiofibromas in people with TSC (Haemel 2010; Mutizwa 2011). There are various preparations for topical rapamycin that have been reported with concentrations ranging from 0.1% to 1% (Madke 2013). An irritation and burning sensation is the most common side effect seen after topical administration of rapamycin. Individuals should be prescribed topical hydrocortisone 0.1% cream or desonide 0.05% lotion along with liberal emollients to counteract any irritation and ensure compliance. It is practical to use commercially available oral solution of rapamycin (1 mg/mL) as a topical formulation since compounding pharmacies are not always readily accessible and the stability and efficacy of compounded preparation cannot be ensured (Madke 2013).

Temsirolimus ($C_{56}H_{87}NO_{16}$) has a molecular weight of 1030.30 and is non-hygroscopic. It is insoluble in water and soluble in alcohol. It has no ionisable functional groups, and its solubility is independent of pH. In vitro studies using renal cell carcinoma cell lines, temsirolimus inhibited the activity of mTOR and resulted in reduced levels of the hypoxia-inducible factors HIF-1 and HIF-2 alpha, and the vascular endothelial growth factor. It has been indicated for the treatment of advanced renal cell carcinoma with a recommended dose of 25 mg infusion over a 30- to 60-minute period once a week. The most common adverse reactions (at least 30%) are rash, asthenia, mucositis, nausea, oedema, and anorexia. The most common laboratory abnormalities (at least 30%) are anaemia, hyperglycaemia, hyperlipaemia, hypertriglyceridaemia, lymphopenia, elevated alkaline phosphatase, elevated serum creatinine, hypophosphataemia, thrombocytopenia, elevated aspartate aminotransferase (AST), and leukopenia. Temsirolimus is contraindicated in individuals with bilirubin more than 1.5 times of upper limit of normal range. (RxList 2015b).

Everolimus ($C_{53}H_{83}NO_{14}$) has a molecular weight of 958.2. It has been indicated for the treatment of post-menopausal women with advanced hormone receptor-positive, HER2-negative breast cancer, adults with progressive neuroendocrine tumours of pancreatic origin (PNET), adults with advanced renal cell carcinoma (RCC), adults with renal angiomyolipoma, SEGA and TSC. However, further follow-up of people with TSC is still required to determine long-term outcomes. The recommended

dose of everolimus tablets is 10 mg, to be taken once daily at the same time every day. The most common adverse reactions (incidence at least 30%) were stomatitis, infections, rash, fatigue, diarrhoea, and decreased appetite. The most common laboratory abnormalities (incidence at least 50%) were hypercholesterolaemia, hyperglycaemia, increased AST, anaemia, leukopenia, thrombocytopenia, lymphopenia, increased alanine transaminase (ALT), and hypertriglyceridaemia (RxList 2016).

While non-randomised trials showed that rapamycin or rapalogs reduce the size of TSC-associated tumours in humans, such as angiomyolipoma, LAM and SEGA, tumour regression does not occur in all cases and tumour regrowth is generally observed with the cessation of treatment (Bissler 2008; Cardamone 2014; Franz 2006). Furthermore, evidence on the effect of rapamycin (Canpolat 2014; Cardamone 2014) or rapalogs (Krueger 2013; Muncy 2009) in improving epilepsy is not without opposition (Overwater 2014; Wiemer-Kruel 2014). Rapamycin or rapalogs have also been tested on other manifestations of TSC with mostly promising results, albeit with a variable degree of evidence. Reports described improvement in facial angiofibroma (Foster 2012; Truchuelo 2012; Wataya-Kaneda 2012; Wheless 2013), cardiac rhabdomyoma (Tiberio 2011), renal cell carcinoma (Pressey 2010) but not the optic nerve tumour (Sparagana 2010) among people with TSC. An open study of 10 people with TSC-associated facial angiofibroma reported sustained improvement in erythema and in the size and extension of the lesions. Rapamycin plasma levels remained below detection limits (0.3 ng/mL) in all cases. The formula was well-tolerated with no local or systemic adverse effects (Salido 2012). Although the response results in early human trials are encouraging, conflicting evidence is present, and it is possible that a longer term use of rapamycin may be more effective. Identification of other active drugs is also of interest to improve the response rate or durability of response, or both (Lee 2009).

Rapamycin or rapalogs are extensively metabolized by O-demethylation or hydroxylation (or both) in the intestinal wall and liver and undergo counter-transport from enterocytes of the small intestine into the gut lumen. Seven major metabolites, including hydroxy, demethyl, and hydroxydemethyl, are identifiable in whole blood. Some of these metabolites are also detectable in plasma, faecal, and urine samples (RxList 2015a; RxList 2015b; RxList 2016).

In the human and rat liver, rapamycin or rapalogs is metabolized primarily by cytochrome P-450 3A4 (CYP3A4) (Sattler 1992). Rapamycin or rapalogs are substrates for both CYP3A4 and P-glycoprotein 1 (P-gp). Inducers of CYP3A4 and P-gp may decrease rapamycin or rapalogs concentrations whereas inhibitors of CYP3A4 and P-gp may increase their concentrations. Drugs and agents that could increase the concentration of rapamycin or rapalogs in the blood include cyclosporine, bromocriptine, cimetidine, cisapride, clotrimazole, danazol, diltiazem, fluconazole, protease inhibitors (e.g., for HIV and hepatitis C that include drugs such as ritonavir, indinavir, boceprevir, and telaprevir), metoclopramide, nicardipine, troleandomycin, verapamil, grapefruit (RxList 2015a), Seville oranges, limes and pomelos (Tanzi 2013). Drugs and agents that could decrease rapamycin or rapalogs concentrations include carbamazepine, phenobarbital, phenytoin, rifapentine, St. John's Wort (*Hypericum perforatum*).

In addition, verapamil concentration could increase when given with rapamycin or rapalogs. Immunosuppressants may affect

response to vaccination. Therefore, during treatment with rapamycin or rapalogs, vaccination may be less effective. The use of live vaccines should be avoided, including, but not limited to, measles, mumps, rubella, oral polio, bacillus Calmette-Guérin (BCG), yellow fever, varicella, and TY21a typhoid (RxList 2015a).

Death, unfortunately, is a major caveat in everolimus administration. A meta-analysis involving 2,997 people with cancer receiving everolimus reported that there was a small but significant increase in the odds of a treatment-related fatal events (Wesolowski 2014).

How the intervention might work

Disruption of mTOR signalling is at the centrepiece of tuberous sclerosis pathophysiology. Such disruption results in TSC-related manifestations, namely tumours (hamartomas), epilepsy, and neuropsychiatric disorders.

As uncontrolled proliferation of benign tumours in various organs is part of the pathophysiology, due to the loss of tumour suppressor genes, inhibitors of such processes is a logical step in tumour suppression. Cell growth and proliferation function is largely regulated in its final pathway, by a set of processes involving a common regulatory mTOR protein. This protein regulates vital cell growth processes, receives external signals from growth factors, hormones, and proteins. It then gives the 'on' or 'off' signals for the cell to grow and divide. In normal circumstances, mTOR combines with several other cellular components to form two distinct complexes, termed mTORC1 and mTORC2 (Bhaskar 2007).

Inhibiting mTOR kinase was thought to be a useful approach to systemic therapy for TSC or LAM (or both) because rapamycin has been shown to normalise dysregulated mTOR signalling in cells that lack normal hamartin or tuberin (Gao 2001; Goncharova 2002; Inoki 2002; Kwiatkowski 2002; Manning 2002; Potter 2002).

Excessive activation of mTOR leads to abnormally large dysplastic neurons, disrupts the formation of dendrites and axons, increases excitatory synaptic currents, reduces myelination, and disrupts the cortical laminar structure (Feliciano 2013; McMahon 2012; Meikle 2008; Talos 2008; Weston 2012; Zeng 2008). Mutations in upstream pathway genes of mTOR, such as STRAD α , DEPDC5, and PI3K, can cause dysregulated mTOR activity, leading to cortical malformation, which is subsequently linked to the development of epilepsy and seizures (Wong 2016). These mechanisms underlie benefits of mTOR inhibitor for seizure manifestation in TSC.

A detailed look into the mode of action of rapamycin discovered that it binds to the cytosolic protein *FK-binding protein 12* (FKBP12). The sirolimus-FKBP12 complex inhibits the mTOR from forming the mTORC1, thus inhibiting cell proliferation. A previous study utilising cohorts of *Tsc2*^{+/-} mice and mouse model of *Tsc2*-null tumours showed that treatment with an mTOR kinase inhibitor (CCI-779, a rapamycin analogue) reduced the severity of TSC-related disease without significant toxicity (Lee 2005).

The mechanisms by which rapamycin or rapalogs inhibits mTOR are not fully understood, but rapamycin associates with FKBP12 to bind to the FRB (FKBP12-rapamycin-binding) domain of mTOR. Binding of the rapamycin-FKBP12 complex to mTOR can destabilise the mTORC1 complex and interfere with the activation of mTOR by phosphatidic acid. Several new compounds are available to inhibit

mTOR, either by interfering with complex formation (FKBP12-dependent or FKBP12-independent) or by directly inhibiting the catalytic domain of mTOR ([Ehninger 2011](#)).

Why it is important to do this review

There is currently no established therapy for TSC, despite the life-threatening morbidities of the disorder. Previous studies have shown potential clinical applications of rapamycin for TSC. Although everolimus (a rapalog) is currently FDA- and EMA-approved for TSC-associated renal angiomyolipoma, SEGA, and partial-onset seizures, applications for other manifestations of TSC are yet to be established. This review aims to bring together clinical trials in this area to establish the clinical value of rapamycin or rapalogs for various manifestations in TSC. This is an update of a previously published Cochrane Review ([Sasongko 2016](#)).

OBJECTIVES

To determine the effectiveness of rapamycin or rapalogs administration in people with tuberous sclerosis complex (TSC) for decreasing the size of TSC-related tumours and other manifestations and to assess the safety of rapamycin or rapalogs relating to their adverse effects.

METHODS

Criteria for considering studies for this review

Types of studies

We included randomised or quasi-randomised studies; studies using quasi-randomised methods, such as alternation, were eligible for inclusion if there was sufficient evidence that the treatment and control groups were similar at baseline.

Types of participants

People with known TSC as proven by the clinical features designated in the revised consensus on TSC diagnostic criteria (genetic or clinical (or both) manifestations) ([Northrup 2013](#)). We planned to include individuals diagnosed using older diagnostic criteria ([Hyman 2000](#); [Roach 1998](#); [Roach 1999](#)) if we could not ascertain compliance with the latest diagnostic criteria through communications with trial authors.

Types of interventions

Rapamycin or rapalogs designed to reduce any TSC-associated symptoms in people with TSC compared to placebo or any standard treatments, applied systemically or topically.

Types of outcome measures

We planned to assess the following outcome measures.

Primary outcomes

1. Tumour size (any unit of analysis found)

Secondary outcomes

1. Skin lesion response
2. Aneurysm size for angiomyolipomas (any unit of analysis found)
3. Frequency of seizure (times)*

4. Forced expiratory volume at one second (FEV₁) / forced vital capacity (FVC) ratio
5. Creatinine level (mg/dL)
6. Other outcomes relevant to participants' well-being*
7. Any reported adverse effect or toxicity

*Please refer to '[Differences between protocol and review](#)' for information about this *post hoc* addition to outcomes.

Search methods for identification of studies

We searched for all relevant published and unpublished trials without restrictions on language, year or publication status. We did not restrict the searches by language as long as an English translation was available for non-English reports.

Electronic searches

We searched the following databases and trial registries:

1. Cochrane Central Register of Controlled Trials (CENTRAL; 2022, Issue 7) in the Cochrane Library (searched 15 July 2022);
2. MEDLINE Ovid (1946 to 15 July 2022);
3. US National Institutes of Health Ongoing Trials Register ClinicalTrials.gov (www.clinicaltrials.gov; searched 15 July 2022); and
4. World Health Organization International Clinical Trials Registry Platform (trialsearch.who.int/; searched 15 July 2022).

For details of our search strategies, please see [Appendix 1](#). The MEDLINE Ovid search was combined with the Cochrane Highly Sensitive Search Strategy for identifying randomised trials in MEDLINE: sensitivity- and precision-maximizing version (2008 revision) ([Lefebvre 2022](#)).

Searching other resources

The review authors checked the bibliographies of included trials and any relevant systematic reviews identified for further references to relevant trials.

The review authors also searched relevant resources, such as conference proceedings and books of conference abstracts (where accessible). This included the TSC International Research Conferences (2009 to 2019), 2012 International TSC Congress, World TSC Conference (2014, 2017, 2018) and the Human Genome Meeting (2010 to 2019). These resources were searched again to date on 15 July 2022 for the current review update. The review authors will continue to search resources from similar conferences for future updates of this review. Investigators were and will continue to be contacted whenever we identify eligible trials from these sources and if more detailed information is needed.

Data collection and analysis

Selection of studies

In order to select studies for inclusion in the review, two review authors independently applied the inclusion criteria. The review authors reached consensus by discussion between all four authors for any disagreements which arose on the suitability of a study for inclusion in the review.

Data extraction and management

One author (NFDI) extracted data using the standard acquisition forms and then a second author (THS) verified the data. The authors reached consensus by discussion upon any disagreements arise on the data extraction. For missing information as specified in other parts of this manuscript, the authors will contact study investigators for future update of this review. The eligible time period for endpoint analysis is at least one month after rapamycin administration. We excluded studies from the analysis when the only time period reported was less than one month. While we planned to analyse data in three blocks of time (one to three months; over three months to six months; and longer than six months), none of the included studies reported enough information for such analyses. We aim, if possible, to undertake this for an update of this review, pending communication with study authors.

Assessment of risk of bias in included studies

One review author (JTXY) assessed the risk of bias for each study and a second review author (KK) verified the decisions. Where needed, the review authors reached agreement on the decisions on the risk of bias assessment through discussion. The review authors generated a risk of bias table for each study as described in the *Cochrane Handbook of Systematic Reviews of Interventions* (Higgins 2017). In particular, they examined the details of the following components:

1. sequence generation (e.g. whether randomisation was adequate);
2. allocation concealment (e.g. whether the allocation was adequately concealed);
3. blinding of participants, personnel and outcome assessors (e.g. whether the participants, personnel and outcome assessors were blinded);
4. incomplete outcome data (e.g. whether attrition and exclusion were reported);
5. selective outcome reporting (e.g. whether the study was free from selective outcome reporting);
6. other sources of bias (will be specified during the assessment).

The review authors assessed all the components using the methods as described in the *Cochrane Handbook for Systematic Reviews of Interventions*. For each item, the table provides a description of what was reported in the study and the subjective judgement regarding possible bias (low, high or unclear risk of bias) (Higgins 2017).

Measures of treatment effect

We pooled the analysis of different types of the different drugs types and plan to perform subgroup analysis for rapamycin and rapalogs if in future versions of the review we identify more studies. We separated the analysis of different modes of applications into systemic or topical administration.

The review authors reported dichotomous outcomes (number of participants having 50% reduction in tumour size and skin lesions, presence or absence of adverse effects and treatment response to creatinine level). We calculated the risk ratios (RRs) and 95% confidence intervals (95% CIs) based on the ratio of an outcome among treatment-allocated participants to that among controls.

We calculated the pooled estimate of the treatment effect for each outcome across studies by determining the RR.

We could not obtain tumour size in the form of continuous data. Instead, review authors obtained response of treatment to tumour size (Bissler 2013 (EXIST 2); Franz 2013 (EXIST 1)) and skin lesions (Bissler 2013 (EXIST 2); Franz 2013 (EXIST 1); Koenig 2012) and we analysed data as dichotomous. The studies reported creatinine levels as the number of participants with increased creatinine levels in both treatment or placebo arms; again we analysed these data as dichotomous. Two studies reported one outcome each as median change only, which we could not analyse in the review; lung function (Bissler 2013 (EXIST 2)) and frequency of seizures (Franz 2013 (EXIST 1)).

In future updates of this review, if continuous data of these outcomes become available, we will record them as either mean change from baseline for each group or mean post-treatment values and standard deviation (SD) for each group. Currently we have assessed continuous outcome data by the calculation of the mean difference (MD) and 95% CIs. Where studies report multiple measures for the same outcome (e.g. absolute change FEV₁ % predicted, percentage change in FEV₁ % predicted, or percentage change of absolute FEV₁ volumes), we will calculate the standardised MDs (SMD). We will consider absolute changes in FEV₁ in the context of comparable data being available for each participant before and after the intervention so that a calculation of the effect size is possible.

Unit of analysis issues

We did not include any cross-over studies in the review. In future updates, we will only include cross-over studies if we consider there to be a sufficient washout period between the treatment arms. We will analyse any data from such studies using paired analyses as described by Elbourne (Elbourne 2002).

For cluster-randomised studies, if we regard these as not being analysed correctly by study authors, we will calculate the effective sample size and monitor and analyse them based on the method described by Donner (Donner 2002). We plan to analyse any such studies separately. We aim to address the risk of unit of analysis error caused by repeated observations on participants and whether the individual or the tumours are randomised based on the information provided in the *Cochrane Handbook of Systematic Reviews of Interventions* (Higgins 2022). If the individual is randomised, we will include all tumours observed in the studies.

Dealing with missing data

Where any included studies were only reported as abstracts, presented at meetings, or reported to the review authors, we sought full reports from the study investigators. For missing information as specified in other parts of this manuscript, we plan to contact study investigators for future updates of this review. In order to allow an intention-to-treat (ITT) analysis, we grouped data by allocated treatment groups, irrespective of later exclusion (regardless of cause) or loss to follow-up.

Assessment of heterogeneity

We tested for heterogeneity between studies using a standard Chi² test and the I² statistic (Higgins 2003).

The Chi² test is a statistical test for heterogeneity, whereas I² assesses the quantity of inconsistency across studies in the meta-analysis. The authors used a cut-off P value of 0.1 to determine significance. This is because of the generally anticipated low power of the reported studies due to the disease being rare. The review authors used the following I² ranges to interpret heterogeneity:

1. 0% to 40%: might not be important;
2. 30% to 60%: may represent moderate heterogeneity;
3. 50% to 90%: may represent substantial heterogeneity; and
4. 75% to 100%: considerable heterogeneity.

The review authors visually assessed the forest plot to see if CIs overlapped.

Assessment of reporting biases

For all studies, we compared the 'Methods' section of the full published paper with the 'Results' section to ensure that all measured outcomes were reported.

This review included fewer than 10 studies for any outcome, so we did not construct any funnel plots for assessment, either visually or statistically (small-study effects analyses). However, in future updates, provided that we include at least 10 studies, we will carry out a visual assessment of funnel plots and statistical tests for funnel plot asymmetry (small-study effects analyses). If we identify any evidence of small-study effects, we will attempt to understand the source, including the possibility of reporting bias.

Data synthesis

We employed a fixed-effect analysis in this review. In future updates, if there is evidence of heterogeneity (I² statistic more than 40%), we plan to use a random-effects analysis.

We compared rapamycin or rapalogs versus placebo or other standard treatment. We determined the exact time points that we reported after analysing the included studies, but they were at least six months apart.

Subgroup analysis and investigation of heterogeneity

In future versions of this review, if we identify heterogeneity between the studies (I² statistic more than 40%), we will examine subgroups, such as: age of participants (0 years to 10 years, over 10 years to 20 years, over 20 years) and the gene affected (*TSC1* or *TSC2*).

In addition to this, where appropriate, we plan to perform subgroup analyses of different medications (rapamycin or rapalogs), different locations and types of tumour, and if rapamycin or rapalogs were administered in conjunction with other agent(s).

Sensitivity analysis

The review authors plan to test the robustness of the results with the following sensitivity analyses:

1. studies where quasi-randomisation methods are used;
2. studies where there are variations among one or more inclusion criteria; and
3. studies of different designs (e.g. cross-over studies).

In addition, we plan to undertake a sensitivity analysis to investigate the effects of combining endpoint analysis across all time periods.

Summary of findings and assessment of the certainty of the evidence

We created a summary of findings table for each comparison we report with the following outcomes, depending on data available in the included studies:

1. 50% reduction of tumour size;
2. response to skin lesions;
3. frequency of seizures (times);
4. creatinine level (mg/dL);
5. FEV₁/FVC ratio;
6. other outcomes relevant to participants' well-being; and
7. any reported adverse effect or toxicity.

We assessed the certainty of the evidence for each outcome using the GRADE approach, based on the risk of bias within the trials, relevance to our population of interest (indirectness), unexplained heterogeneity or inconsistency, imprecision of the results or high risk of publication bias. We downgraded the certainty of the evidence once if the risk was serious and twice if we deemed the risk to be very serious.

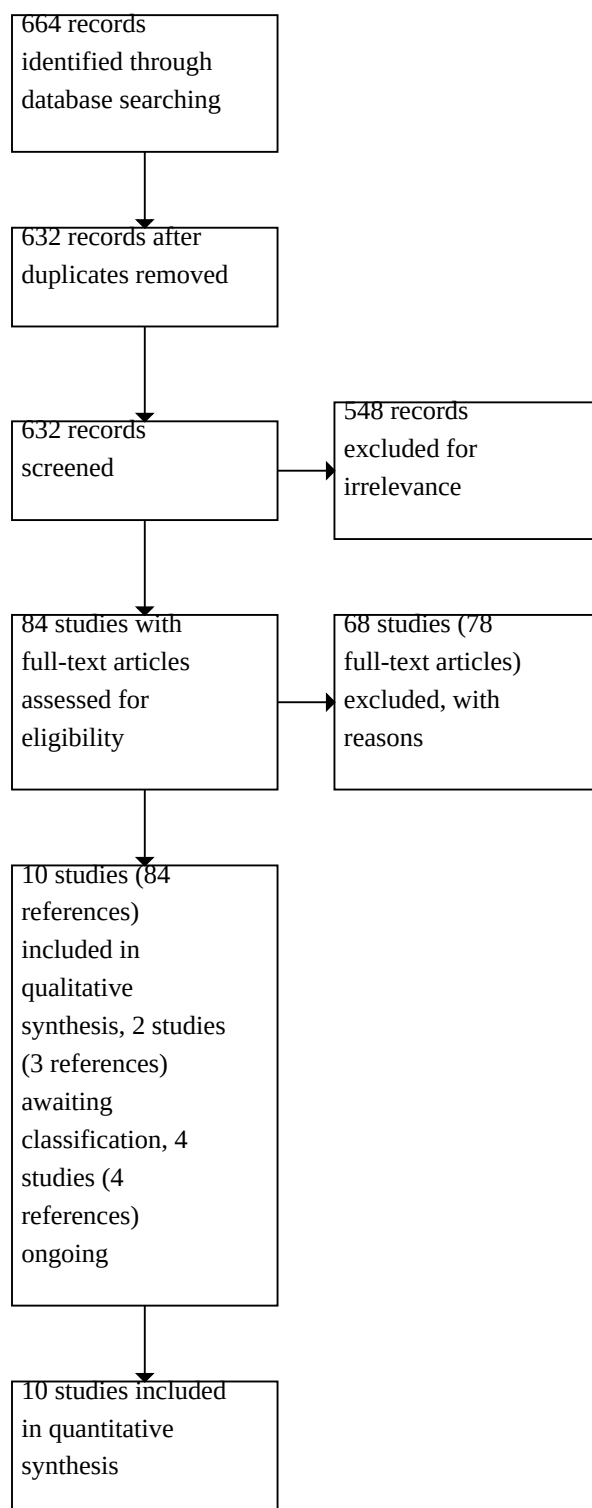
RESULTS

Description of studies

Results of the search

Please refer to the study flow diagram ([Figure 1](#)).

Figure 1.



The search identified 84 relevant studies and we excluded 68 studies. In the original version of the review we included three studies (Bissler 2013 (EXIST 2); Franz 2013 (EXIST 1); Koenig 2012); at this update(2022), we included a further seven studies (French 2016 (EXIST 3); Koenig 2018; Krueger 2017; Overwater 2016; Overwater 2019; Wataya-Kaneda 2017; Wataya-Kaneda 2018). We have listed two studies as awaiting classification in a future update of this review (Randell 2016; NCT03140449), and four studies are ongoing (EUCTR2011-006308-12-ES; NCT02860494; NCT03363763; NCT03826628).

Included studies

Refer to [Characteristics of included studies](#).

Trial characteristics

Nine out of 10 studies were described as double-blind and one study was open-label (Overwater 2016), all studies were randomised, placebo-controlled studies (Bissler 2013 (EXIST 2); Franz 2013 (EXIST 1); Koenig 2012; French 2016 (EXIST 3); Koenig 2018; Krueger 2017; Overwater 2016; Overwater 2019; Wataya-Kaneda 2017; Wataya-Kaneda 2018). Five studies were described as phase 3 studies (Bissler 2013 (EXIST 2); Franz 2013 (EXIST 1); French 2016 (EXIST 3); Overwater 2019; Wataya-Kaneda 2018), while five were described as phase 2 studies (Koenig 2012; Koenig 2018; Krueger 2017; Overwater 2016; Wataya-Kaneda 2017).

Six studies were multicentre studies including Bissler study (24 centres in 11 countries) (Bissler 2013 (EXIST 2)), Franz (24 centres in 10 countries) (Franz 2013 (EXIST 1)), French 2016 (99 sites in 25 countries) (French 2016 (EXIST 3)), Koenig 2018 (nine sites in USA and one in Australia) (Koenig 2018), Krueger study (Massachusetts and Ohio in USA) (Krueger 2017), Wataya Kaneda (nine sites in Japan) (Wataya-Kaneda 2018). The Koenig study was carried out in a single centre in Texas, USA (Koenig 2012), Both Overwater studies were run in a single centre in the Netherlands (Overwater 2016; Overwater 2019), and the earlier Wataya Kaneda study took place in a single centre in Osaka Japan (Wataya-Kaneda 2017).

Participants

The total number of participants for all 10 included studies was 1008 (484 males and 524 females), and the number of participants randomised in each study ranged from 23 (Overwater 2016) to 366 (French 2016 (EXIST 3)). Three studies recruited only children and adolescents (Krueger 2017; Overwater 2016; Overwater 2019), two studies specified participants should be over the age of 13 (Koenig 2012) or over the age of 18 (Bissler 2013 (EXIST 2)), while the remaining studies recruited a mix of children and adults with ages ranging from two years to 65 years. All participants were diagnosed with: tuberous sclerosis complex (TSC), but different studies used different criteria. Four studies used the older criteria (Bissler 2013 (EXIST 2); Franz 2013 (EXIST 1); Koenig 2012; Wataya-Kaneda 2017) and five studies used the new criteria (Koenig 2018; Krueger 2017; Overwater 2016; Overwater 2019; Wataya-Kaneda 2018), while the criteria were not clear in one study and we have contacted the study investigators for clarification (French 2016 (EXIST 3)). Two studies additionally required at least one angiomyolipoma (3 cm or larger in diameter) (Bissler 2013 (EXIST 2); Franz 2013 (EXIST 1);), two specified at least three isolated facial angiofibroma (Wataya-Kaneda 2017; Wataya-Kaneda 2018) and one required visible facial angiofibroma (Koenig 2018). One study stated participants had treatment-resistant epilepsy (French 2016 (EXIST

3)) while in another participants were experiencing one epileptic seizure per week and were resistant to at least two antiepileptic drugs (Overwater 2016). In one study participants had an intelligent quotient (IQ) below 80 or learning disability or special schooling or autism spectrum disorder (Overwater 2019).

Interventions

Systemic intervention

Five studies compared oral everolimus to a placebo (Bissler 2013 (EXIST 2); Franz 2013 (EXIST 1); French 2016 (EXIST 3); Krueger 2017; Overwater 2019) and one study compared oral sirolimus to a placebo (Overwater 2016). One study compared treatments over a 18-week core phase which was continued for 48 weeks in an extension phase (French 2016 (EXIST 3)). Three studies compared treatment periods of six months (Bissler 2013 (EXIST 2); Franz 2013 (EXIST 1); Krueger 2017)), and one of these had an extension phase of up to four years, if the results of the core phase favoured everolimus (Franz 2013 (EXIST 1)). One study administered treatment for 12 months (Overwater 2019). In one adult study the starting dose was 10 mg per day (Bissler 2013 (EXIST 2)), while one paediatric study started with a dose of 2.5 mg per day (Overwater 2019). Three studies calculated doses in mg/m² body surface area per day and depending on age, with starting doses of 4.5 mg/m² in two studies (Franz 2013 (EXIST 1); Krueger 2017), and ranging from 3 mg/m² to 9 mg/m² per day in the third study (French 2016 (EXIST 3)). One study administered an oral solution of sirolimus 1 mg/mL once daily for 12 months, with dose adjustments made to attain a trough concentration of 5 ng/mL to - 10 ng/mL (Overwater 2016). In all studies doses were modified either on the basis of safety findings (Bissler 2013 (EXIST 2)) or to attain specific blood trough concentrations; in one study the targeted trough concentration was 5 ng/mL to 15 ng/mL (Franz 2013 (EXIST 1)), in one study it was 5 ng/mL to 10 ng/mL (Overwater 2019), in one study it was 5 mcg/mL and 15 mcg/mL (Krueger 2017), and in the final study the trough concentrations ranged from 3 ng/mL to 7 ng/mL (low trough) to 9 ng/mL to 15 ng/mL (high trough) (French 2016 (EXIST 3)).

Topical intervention

Two studies compared sirolimus with a placebo (Wataya-Kaneda 2017; Wataya-Kaneda 2018). Two studies used sirolimus gel twice daily for a period of 12 weeks; in the earlier study, three different concentrations of sirolimus gel (0.05%, 0.1%, 0.2%) were compared with placebo (Wataya-Kaneda 2017), while in the later study, just 0.2% sirolimus gel was used (Wataya-Kaneda 2018).

Two studies compared topical rapamycin with a placebo for a period of six months (Koenig 2012; Koenig 2018). In one study treatment was given at a dose of either 1 mg per 30 cc (0.003%) or 5 mg per 30 cc (0.015%) every morning (Koenig 2012); while in the second study 1 mL (1 pump) of rapamycin 0.1% (0.03g per 30g) or 1% (0.3g per 30g) was administered topically every day at bedtime (Koenig 2018).

Outcomes

Two studies reported effects of everolimus on tumour volume (Bissler 2013 (EXIST 2), Franz 2013 (EXIST 1)). These studies also reported on tumour response and time for tumour progression. The types of tumours reported include angiomyolipoma and subependymal giant cell astrocytoma (SEGA). Six studies reported on skin lesion response (Bissler 2013 (EXIST 2), Franz 2013 (EXIST

1), Koenig 2012, Koenig 2018, Wataya-Kaneda 2017, Wataya-Kaneda 2018). Two studies (Bissler 2013 (EXIST 2), Franz 2013 (EXIST 1)) did not specify the type of skin lesions, while the rest specifically looked into facial angiofibroma. None of the included studies reported aneurysm size of angiomyolipoma. Three studies reported on seizure frequency (Franz 2013 (EXIST 1); French 2016 (EXIST 3); Overwater 2016). The French study also reported on change from baseline in seizure frequency, frequency of seizure-free days, and proportion of patients achieving 25% reduction in seizure frequency from baseline (French 2016 (EXIST 3)). Overwater 2016 also reported on number of patients who responded with 50% reduction in seizure frequency, seizure severity, and number of status epilepticus episodes. No studies reported on the ratio of FEV₁/FVC, although the Bissler study reported FEV₁ level (Bissler 2013 (EXIST 2)). No studies reported creatinine level (mg/dL), however, one study reported creatinine levels as a dichotomous data in terms of number of participants with increased or decreased levels (Bissler 2013 (EXIST 2)). Four studies reported on other outcomes relevant to patients' well-being such as global intellectual ability, language, learning and memory, attention, executive function, processing speed, academic skills, cognitive development, adaptive behaviour, sensory processing, autistic features, emotional and behaviour problems, change in full-scale IQ, performance IQ, verbal IQ, change in autistic features, visual motor integration, fine motor skills, behavioural and emotional problems, social and communication skills, sleep quality, sensory processing, quality of life, as well as behavioural and emotional problems at school (Krueger 2017, Overwater 2016, Overwater 2019, Wataya-Kaneda 2018). All studies reported adverse events (Bissler 2013 (EXIST 2), Franz 2013 (EXIST 1), French 2016 (EXIST 3), Koenig 2012, Koenig 2018, Krueger 2017, Overwater 2016, Overwater 2019, Wataya-Kaneda 2017, Wataya-Kaneda 2018).

Excluded studies

Please refer to the [Characteristics of excluded studies](#) table.

A total of 68 studies were excluded from this review. Of these, 46 were either non-randomised or non-controlled (Bissler 2019; Cabrera 2011; ChiCTR-OPC-14005488; ChiCTR-OPN-16008236; Cuevas 2012; Dabora 2011; Davies 2008; Davies 2011; DRKS00005584; EUCTR2007-005978-30-ES; EUCTR2010-022583-13-DE; Franz 2018; JPRN-UMIN000002844; JPRN-UMIN000006108; JPRN-UMIN000015114; JPRN-UMIN000011559; JPRN-UMIN000024270; Kenerson 2002; Krueger 2010; Krueger 2013a; Krueger 2013b; NCT00457808; NCT00457964; NCT00490789; NCT00552955; NCT00792766; NCT01092208; NCT01217125; NCT01266291; NCT01767779; NCT01780441; NCT01929642; NCT02104011; NCT02201212; NCT02325505; NCT02451696; NCT02461459; NCT02634931; NCT02654340; NCT03047213; NCT03213678; NCT03525834; NCT03649919; Tanaka 2013; Wataya-Kaneda 2015; Zhang 2018). Two studies mixed people with TSC with people who had other diagnoses without clear information on how many people with TSC were included (Gupta 2019; Young 2013). One study was pharmacokinetic-pharmacodynamic modelling article (Sallas 2015). A further 19 studies were either case series, case reports or observational case control (Birca 2010; Foster 2012; Franz 2006; Herry 2007; Hofbauer 2008; Koenig 2008; Lam 2010; NCT00001465; NCT00001532; NCT00001975; Pressey 2010; Salido 2012; Sparagana 2010; Staehler 2012; Wataya-Kaneda 2011; Wataya-Kaneda 2012; Wheless 2013; Wienecke 2006; Yalon 2011).

Risk of bias in included studies

Please refer to [Figure 2](#) and [Figure 3](#).

Figure 2. Risk of bias graph illustrating percentages of each risk of bias item for all ten included studies (Bissler 2013 (EXIST 2), Franz 2013 (EXIST 1), French 2016 (EXIST 3); Koenig 2012; Koenig 2018; Krueger 2017; Overwater 2016; Overwater 2019; Wataya-Kaneda 2017; Wataya-Kaneda 2018).

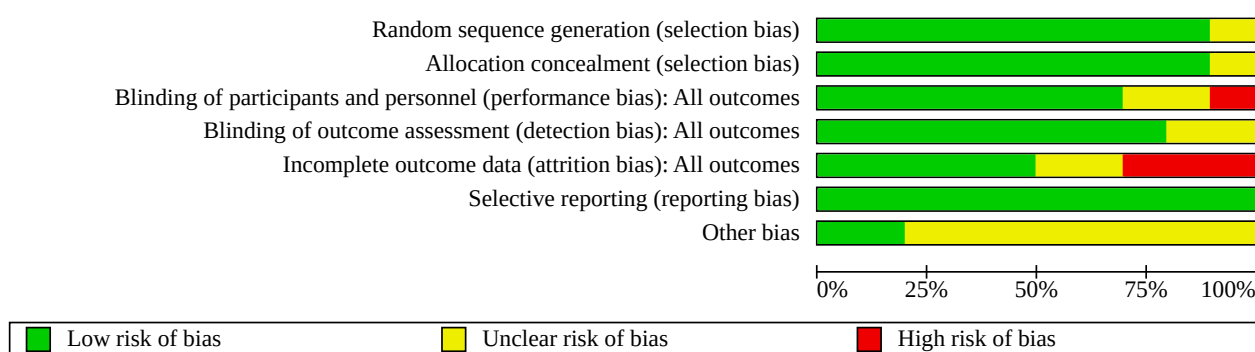


Figure 3. Risk of bias graph summarizing review authors' judgements about each risk of bias item for each included studies (Bissler 2013 (EXIST 2); Franz 2013 (EXIST 1); French 2016 (EXIST 3); Koenig 2012; Koenig 2018; Krueger 2017; Overwater 2016; Overwater 2019; Wataya-Kaneda 2017; Wataya-Kaneda 2018).

	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias): All outcomes	Blinding of outcome assessment (detection bias): All outcomes	Incomplete outcome data (attrition bias): All outcomes	Selective reporting (reporting bias)	Other bias
Bissler 2013 (EXIST 2)	+	?	+	+	+	+	?
Franz 2013 (EXIST 1)	+	+	+	+	+	+	?
French 2016 (EXIST 3)	+	+	+	+	?	+	?
Koenig 2012	?	+	+	+	-	+	+
Koenig 2018	+	+	?	+	-	+	?
Krueger 2017	+	+	+	?	-	+	?
Overwater 2016	+	+	-	+	?	+	?
Overwater 2019	+	+	+	+	+	+	?
Wataya-Kaneda 2017	+	+	?	?	+	+	+
Wataya-Kaneda 2018	+	+	+	+	+	+	?

Allocation

Sequence generation

Nine studies described the use of a computer-generated random sequence, and we judged these to have a low risk of bias (Bissler 2013 (EXIST 2); Franz 2013 (EXIST 1); French 2016 (EXIST 3); Koenig 2018; Krueger 2017; Overwater 2016; Overwater 2019; Wataya-Kaneda 2017; Wataya-Kaneda 2018). We judged the remaining study to have unclear risk of bias as no process of generating allocation sequence was described (Koenig 2012).

Allocation concealment

Three studies described the use of interactive internet-response systems for allocation concealment and therefore, we judged these as having low risk of bias for allocation concealment (Franz 2013 (EXIST 1); Overwater 2019; Wataya-Kaneda 2018). A further six studies described central allocation, and we judged these studies to also have a low risk of bias for allocation concealment (French 2016 (EXIST 3); Koenig 2012; Koenig 2018; Krueger 2017; Overwater 2016; Wataya-Kaneda 2017).

One study did not describe allocation concealment, and we therefore graded this as having an unclear risk (Bissler 2013 (EXIST 2)).

Blinding

Six studies gave details on the level of participants and all study personnel (caregiver, investigator and outcomes assessor) and we assessed these as having a low risk of both performance bias and detection bias (Bissler 2013 (EXIST 2); Franz 2013 (EXIST 1); French 2016 (EXIST 3); Koenig 2012; Overwater 2019; Wataya-Kaneda 2018).

The Koenig 2018 study did not describe the blinding of participants and study personnel in detail, but clearly described the blinding of outcome assessors. Therefore, we assessed this as having an unclear risk of performance bias but a low risk of detection bias (Koenig 2018).

Conversely, the Krueger 2017 study clearly described the blinding of participants and study personnel, but did not describe the blinding of outcome assessors. Thus, we judged the study as having a low risk of performance bias, but an unclear risk of detection bias (Krueger 2017).

The Wataya-Kaneda 2017 study described neither the blinding of participants and study personnel nor of outcome assessors in detail. Thus, we assessed this study as having an unclear risk of both performance bias and detection bias (Wataya-Kaneda 2017).

The remaining study had an open-label design and thus the participants were not blinded. However, study neuropsychologist and neurophysiologist were masked to treatment. We therefore assessed this study as having a high risk of performance bias but a low risk of detection bias (Overwater 2016).

Incomplete outcome data

We judged five studies to have a low risk of attrition bias (Bissler 2013 (EXIST 2); Franz 2013 (EXIST 1); Overwater 2019; Wataya-Kaneda 2017; Wataya-Kaneda 2018). In the Bissler 2013 study, efficacy analyses were undertaken on all randomised participants,

and safety analyses were undertaken on all participants who received at least one dose of the study drug and had at least one post-baseline assessment. Participants who were not assessed (either because of dropout or other reasons) were considered as non-responders (Bissler 2013 (EXIST 2)). In two studies, efficacy and safety analyses were carried out on all participants (Franz 2013 (EXIST 1); Wataya-Kaneda 2018); and in the remaining two studies, intention-to-treat (ITT) analyses were performed for both safety and efficacy analyses (Overwater 2019; Wataya-Kaneda 2017).

We judged three studies to have a high risk of attrition bias (Koenig 2012; Koenig 2018; Krueger 2017). In the earliest study, only 23 out of 28 participants were analysed upon completion of the intervention; there was no information pertaining to which arms the dropouts came from and outcomes were only reported as percentages of participants reporting improvements of their skin lesions on each arm (Koenig 2012). In the Koenig 2018 study, all randomised participants were included in the safety analyses while efficacy analyses were undertaken only on randomised participants with evaluable photos at both baseline and at least one post-baseline visit; only 159 out of 179 participants had evaluable photos and were included in the efficacy analyses. (Koenig 2018). In the third study only 42 out of 47 participants were analysed even though the study claimed to have performed an ITT analysis (Krueger 2017).

We judged two studies to have an unclear risk of attrition bias (Overwater 2016; French 2016 (EXIST 3)). In the Overwater study, all participants were included in ITT analyses for efficacy outcomes; however, the number of participants analysed in the safety analyses were not stated clearly and ITT analyses were not mentioned. The French 2016 study did not state the total number of participants analysed for several outcomes (Overwater 2016; French 2016 (EXIST 3)).

Selective reporting

Overall, there is a low risk of bias for selective reporting for all 10 studies as in each study all outcomes recorded in the 'Methods' section were reported in 'Results' section (Bissler 2013 (EXIST 2); Franz 2013 (EXIST 1); French 2016 (EXIST 3); Koenig 2012; Koenig 2018; Krueger 2017; Overwater 2016; Overwater 2019; Wataya-Kaneda 2017; Wataya-Kaneda 2018).

Other potential sources of bias

We judged seven studies to have an unclear risk of bias as the study authors involved in the study design, discussion, research, oversight of data collection and data analysis and interpretation, were employees, stock owners or consultants of the funder (Novartis) (Bissler 2013 (EXIST 2); Franz 2013 (EXIST 1); French 2016 (EXIST 3); Koenig 2018; Krueger 2017; Overwater 2019; Wataya-Kaneda 2018).

One study used a cross-over design, which required additional considerations on potential risk of bias. The choice of cross-over design may be suitable given the condition (epilepsy) which is relatively stable. Although it was mentioned that the study lasted for 12 months, it was unclear how long the follow-up was for each period. Carry-over effect is unknown as there is no information about a washout period before the cross-over occurred. We are currently awaiting confirmation from the author regarding this information. Data from each period of cross-over

were not available. We are therefore unable to perform paired analysis and compare the results with the other parallel-group trials, until further information from the trial authors ([Overwater 2016](#)).

No other potential source of bias was identified from the remaining two studies, and we judged these to have a low risk of bias ([Koenig 2012](#); [Wataya-Kaneda 2017](#)).

Effects of interventions

See: [Summary of findings 1](#) Summary of findings for systemic administration of rapalogs; [Summary of findings 2](#) Summary of findings for topical administration of rapamycin

The certainty of the evidence has been graded for those outcomes included in the summary of findings tables. For the definitions of these gradings, please refer to the summary of findings tables ([Summary of findings 1](#); [Summary of findings 2](#)). We included a total of 10 studies (1008 participants) ([Bissler 2013 \(EXIST 2\)](#); [Franz 2013 \(EXIST 1\)](#); [French 2016 \(EXIST 3\)](#); [Koenig 2012](#); [Koenig 2018](#); [Krueger 2017](#); [Overwater 2016](#); [Overwater 2019](#); [Wataya-Kaneda 2017](#); [Wataya-Kaneda 2018](#)).

Systemic administration of rapamycin or rapalogs versus placebo

Six studies (703 participants) reported on this comparison ([Bissler 2013 \(EXIST 2\)](#); [Franz 2013 \(EXIST 1\)](#); [French 2016 \(EXIST 3\)](#); [Krueger 2017](#); [Overwater 2016](#); [Overwater 2019](#)).

Primary outcome

1. Tumour size

Two studies reported the response of tumour size to oral administration of everolimus in terms of a reduction in the total volume of tumours to 50% or more relative to baseline ([Bissler 2013 \(EXIST 2\)](#); [Franz 2013 \(EXIST 1\)](#)). The results were presented as dichotomous data as the number of participants experiencing this event in both the treatment and placebo groups.

1.1. Angiomyolipoma

Bissler reported that 33 out of 79 participants showed at least a 50% reduction in the size of angiomyolipoma in the treatment group versus none out of 39 in the placebo group ([Bissler 2013 \(EXIST 2\)](#)). Franz reported response to the size of renal angiomyolipomas; 16 out of 30 participants in treatment group versus none of 14 in placebo group showed at least a 50% reduction in angiomyolipoma size ([Franz 2013 \(EXIST 1\)](#)). Our meta-analysis showed that a significant proportion of participants achieved a 50% reduction in angiomyolipoma size within the treatment arm (risk ratio (RR) 24.69, 95% confidence interval (CI) 3.51 to 173.41; 2 studies, 162 participants; high-certainty evidence; [Analysis 1.1](#)).

Given both studies followed intention-to-treat (ITT) principle, it should be noted that 5 of 79 participants in the Bissler study had sporadic lymphangioleiomyomatosis (without: tuberous sclerosis complex (TSC)). We remain unable to separate them from the analysis after an attempt to contact the trial author ([Bissler 2013 \(EXIST 2\)](#)).

1.2. Subependymal giant cell astrocytoma (SEGA)

One study reported a 50% reduction of SEGA volume in, a statistically significant, 27 out of 78 participants in the treatment group versus none out of 38 participants in the placebo group (RR 27.85, 95% CI 1.74 to 444.82; 1 study, 117 participants; moderate-certainty evidence; [Analysis 1.1](#)) ([Franz 2013 \(EXIST 1\)](#)).

Secondary outcomes

1. Response to skin lesions

Two studies administered everolimus orally and reported the response to skin lesions. However, the trial authors have not responded to our queries regarding their definition of 'response' ([Bissler 2013 \(EXIST 2\)](#); [Franz 2013 \(EXIST 1\)](#)).

Bissler reported 20 participants with a response to skin lesions out of 77 participants in the treatment group as compared to no participants among 37 participants in the placebo group ([Bissler 2013 \(EXIST 2\)](#)). Franz reported 30 participants with a response to skin lesions out of 72 participants in the treatment group as compared to four out of 38 participants in placebo group ([Franz 2013 \(EXIST 1\)](#)). Of note, all skin lesion responses were reported as incomplete during the core phase period in [Franz 2013 \(EXIST 1\)](#) study. We analysed the data from these two studies and found that the proportion of participants who showed a skin response was significantly increased in the treatment arms (RR 5.78, 95% CI 2.30 to 14.52; 2 studies, 224 participants; high-certainty evidence; [Analysis 1.2](#)).

2. Creatinine level (mg/dL)

One study reported creatinine levels in terms of number of participants with increased or decreased levels (dichotomous data) ([Bissler 2013 \(EXIST 2\)](#)). One out of 79 participants in treatment group versus three out of 39 participants in placebo group had increased blood creatinine levels. This study showed no difference between treatment groups (RR 0.16, 95% CI 0.02 to 1.53; 1 study, 118 participants; moderate-certainty evidence; [Analysis 1.3](#)).

3. Frequency of seizure

Three studies report on seizure frequency ([Franz 2013 \(EXIST 1\)](#); [French 2016 \(EXIST 3\)](#); [Overwater 2016](#)), but we were only able to analyse data from one of these studies ([French 2016 \(EXIST 3\)](#)). One study had a cross-over study design and we can not include data in the analysis until we obtain data from the first period prior to crossing the arms ([Overwater 2016](#)). Another study reported the median change of seizure frequency between baseline and week 24; however, a large proportion of participants did not experience seizures at baseline ([Franz 2013 \(EXIST 1\)](#)).

Using an ITT analysis, French reported that 11 out of 247 participants showed seizure-free (100% reduction) rate in the treatment group versus 1 out of 119 in the placebo group. ([French 2016 \(EXIST 3\)](#)). This study showed there was no difference shown between treatment and placebo (RR 5.30, 95% CI 0.69 to 40.57; 1 study, 366 participants; moderate-certainty evidence; [Analysis 1.4](#)).

Nevertheless, when considering a 50% reduction of seizure frequency, French reported that 85 out of 247 participants showed over 50% reduction in seizure frequency in the intervention group versus 18 out of 119 in the placebo group which is significant (RR 2.28 (95% CI 1.44 to 3.60) study, 366 participants; moderate-certainty evidence; [Analysis 1.4](#)) ([French 2016 \(EXIST 3\)](#)).

Conversely, results reported at the end of the cross-over study for this outcome show no difference between groups, RR 1.50 (95% CI 0.64 to 3.53), with nine out of 23 participants experiencing a greater than 50% reduction during the sirolimus period as compared to six out of 23 participants during standard care only period ([Overwater 2016](#)) (moderate-certainty evidence).

Similarly, regarding a 25% reduction of seizure frequency, French reported that 152 out of 247 participants in intervention group showed over 25% decrease in seizure frequency versus 45 out of 119 participants from placebo group which is statistically significant (RR 1.63, 95% CI 1.27 to 2.09; 1 study, 366 participants; moderate-certainty evidence; [Analysis 1.4](#)) ([French 2016 \(EXIST 3\)](#)).

All three studies reported seizure frequency as continuous outcomes ([Franz 2013 \(EXIST 1\)](#); [French 2016 \(EXIST 3\)](#); [Overwater 2016](#)). French reported seizure frequency in terms of the median seizure frequency and higher percentage reduction of seizure frequency in treatment group as compared to placebo group ([French 2016 \(EXIST 3\)](#)). The authors reported that the median (range) seizure frequency of at 18 weeks (end of the core phase) was 8.5 seizures per week (0 to 217.7 seizures) in the placebo group, 6.8 seizures per week (0 to 193.5 seizures) in the low-exposure group (everolimus trough level 3 ng/mL to 7 ng/mL), and 4.9 seizures per week (0 – 133.7 seizures) in high-exposure group (everolimus trough level 9 ng/mL to 15 ng/mL). However, we are unable to analyse this result until we obtain data on the mean seizure frequency in each arm. To date we have received no response to our request for data. [Franz](#) reported the median change of seizure frequency between baseline and at week 24; however, a large proportion of participants did not experience seizures at baseline ([Franz 2013 \(EXIST 1\)](#)). The study reported sensitivity analyses on the subset of participants who had at least one seizure at baseline. Investigators reported that the placebo group had a higher median in the baseline of seizure frequency at baseline (11 in 24 hours) compared to the treatment group (5.9 in 24 hours). The median change at 24 weeks was -4.1 in 24 hours (95% CI -10.9 to 5.8) in the placebo group versus -2.9 in 24 hours (95% CI -4.0 to 1.0) in the treatment group (moderate-certainty evidence) ([Franz 2013 \(EXIST 1\)](#)). To date we have received no response to our attempt to obtain data from the study investigators on mean seizure frequency in each treatment arm.

[Overwater](#) reported 41% less frequent seizure during the sirolimus period as compared to standard care period (95% CI -69% to 14%; $P = 0.11$) in the ITT analysis. However, we are unable to analyse these results until we obtain data on the mean seizure frequency in of each treatment arm from both study periods ([Overwater 2016](#)). We have had no response to our request for data on the mean seizure frequency and the number of responders in each arm from both study periods.

4. Aneurysm size for angiomyolipomas (any unit of analysis found)

None of the included studies reported aneurysm size for angiomyolipomas ([Bissler 2013 \(EXIST 2\)](#); [Franz 2013 \(EXIST 1\)](#); [French 2016 \(EXIST 3\)](#); [Krueger 2017](#); [Overwater 2016](#); [Overwater 2019](#)).

5. FEV₁/FVC ratio

No study reported analysable data for FEV₁ / FVC ratio. [Bissler](#) reported only median values at baseline and week 24 for FEV₁. At

baseline, the median values of FEV₁ were 2.9 L in the treatment group versus 2.7 L in the placebo group, while at week 24, the median values for both groups were 2.7 L. For the subset of participants with lymphangioleiomyomatosis and sporadic lymphangioleiomyomatosis, the median percentage change of FEV₁ in the treatment arm (in 24 out of 79 participants) was -1%, while in the placebo arm (10 out of 39 participants) this was -4% ([Bissler 2013 \(EXIST 2\)](#)).

6. Other outcomes relevant to participants' well-being

Three studies reported multiple assessments on the category of outcomes related to neurocognitive, neuropsychiatry, behavioural, sensory and motor development ([Krueger 2017](#); [Overwater 2016](#); [Overwater 2019](#)).

[Krueger](#) reported that no assessment showed an effect of treatment, except for attention and executive function (based on Stockings of Cambridge - Cambridge Neuropsychological Test Automated Battery (CANTAB)) and social cognition (based on Social Responsiveness Scale (SRS)). Attention and executive function was reported to improve in the placebo group, while social cognition was reported to improve in the everolimus group ([Krueger 2017](#)). The study reported that the placebo group did indeed have a better score for attention and executive function at six months compared to the everolimus group (mean difference (MD) -1.26, 95%CI -2.03 to -0.49; $P = 0.001$; 1 study, 42 participants; low-certainty evidence; [Analysis 1.5](#)). However, the study failed to show difference in social cognition at six months in the everolimus group compared to the placebo group (MD -4.98, 95%CI -17.34 to 7.38; $P = 0.43$; 1 study, 42 participants; low-certainty evidence; [Analysis 1.6](#)).

The earlier [Overwater](#) study reported no significant differences in cognitive or motor development, behavioral problems, adaptive behaviour, or sensory processing were identified between oral sirolimus and standard care ([Overwater 2016](#)). We have received no response to our request for mean score of the scales from each arm in both study periods.

The later [Overwater](#) study reported IQ, autistic features, visual motor integration, behavioural and emotional problems (in and out of school), social dysfunctioning and autistic features, communication skills, sleep quality, sensory processing, quality of life, as well as memory and executive functioning where the investigators found no differences between the placebo and everolimus groups ([Overwater 2019](#)).

7. Adverse events or toxicity

We analysed adverse events in three subgroups: any adverse event; adverse events leading to dose reduction, interruption or withdrawal; and severe adverse events. It is of note that these three subgroups might overlap. All included studies reported adverse events during the course of each study but none were definitely related to treatment ([Bissler 2013 \(EXIST 2\)](#); [Franz 2013 \(EXIST 1\)](#); [French 2016 \(EXIST 3\)](#); [Krueger 2017](#); [Overwater 2016](#); [Overwater 2019](#)).

7.1. Any adverse event

Five studies reported any adverse events regardless of severity. They used the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0 (CTCAE v3.0) ([Bissler 2013 \(EXIST 2\)](#), [Franz 2013 \(EXIST 1\)](#), [Krueger 2017](#), [Overwater 2019](#)) or

version 4.03 (French 2016 (EXIST 3)). The 2019 Overwater 2019 also used the WHO Adverse Reaction Terminology (Overwater 2019).

Most adverse events were grade 1 or 2. The most common adverse events in the Franz study were mouth ulceration, stomatitis, convulsion and pyrexia (Franz 2013 (EXIST 1)), and in the Bissler study were nasopharyngitis, acne-skin lesions, headache, cough and hypercholesterolaemia (Bissler 2013 (EXIST 2)). For French 2016 (EXIST 3), common adverse events include stomatitis, diarrhoea, nasopharyngitis, pyrexia, and upper respiratory tract infection. Krueger 2017 reported gastrointestinal complaints, primarily stomatitis, infections, neurological complaints (headache) and respiratory adverse events (cough). Lastly Overwater 2019 outlined gastrointestinal adverse events, upper respiratory tract infection, aphthous ulcers, acne-like skin lesions, headache, eczema, haemorrhagic disorders, aphthous ulcers were the common adverse events. Krueger 2017 categorised adverse events into those of cardiac, dermatologic, gastrointestinal, general, genitourinary, haematological, infectious, metabolic, musculoskeletal, neurologic, psychiatric, respiratory, and unspecified.

Only one of the studies reporting the number of participants experiencing at least one adverse event showed a difference between groups and demonstrated that more participants in the placebo group experienced more adverse events than in the treatment group (RR 1.21, 95% CI 1.09 to 1.34) (French 2016 (EXIST 3)); however when combined with the other studies, the pooled data showed no difference between treatment and placebo groups (RR 1.09, 95% CI 0.97 to 1.22; 5 studies, 680 participants; moderate-certainty evidence; Analysis 1.7).

Table 1 summarises adverse events from 404 participants in the treatment arm and 197 participants in the placebo arm from three studies (Bissler 2013 (EXIST 2); Franz 2013 (EXIST 1); French 2016 (EXIST 3)). There were no reports on particular adverse events from two studies (Krueger 2017; Overwater 2019).

7.2 Adverse events leading to dose reduction, interruption or withdrawal

Five studies using systemic administration reported adverse events leading to a clinical decision of dose reduction, interruption or withdrawal (Bissler 2013 (EXIST 2); Franz 2013 (EXIST 1); French 2016 (EXIST 3); Overwater 2016; Overwater 2019). However, due to cross-over study design, we can not include data from one study until we obtain data from the first period prior to crossing the arms (Overwater 2016).

Two out of the four studies reported that significantly more participants in the intervention arm experienced more adverse events leading to dose reduction, interruption or withdrawal (Bissler 2013 (EXIST 2); Franz 2013 (EXIST 1)), while in two studies there was no difference between groups (French 2016 (EXIST 3); Overwater 2019). When we pooled the data from all four studies, we observed more participants experiencing adverse events leading to a clinical decision of dose reduction, interruption or withdrawal in the treatment group than the placebo group (RR 2.61, 95% CI 1.58 to 4.33; 4 studies, 633 participants; high-certainty evidence; Analysis 1.7).

The cross-over study reported that five participants discontinued sirolimus due to adverse events by the end of the study (Overwater 2016). These included aphthous ulcers in two children,

pneumonia requiring hospitalisation, upper respiratory tract infection, and increased seizure frequency. Excluding the increased seizure frequency, all adverse events subsided after sirolimus was discontinued.

7.3. Severe adverse events

Severe adverse events were reported by three studies which administered everolimus or sirolimus orally (French 2016 (EXIST 3); Krueger 2017; Overwater 2016). As already stated above, due to the cross-over study design, we can not include data from one study until we obtain data from the first period prior to crossing the arms (Overwater 2016).

The larger study reported that 52 out of 247 participants in the treatment group experienced severe adverse events versus 13 out of 119 participants from the placebo group (French 2016 (EXIST 3)). Severe adverse events (AEs) included stomatitis, neutropenia, pneumonia and irregular menstruation; but no deaths were reported during the core phase (French 2016 (EXIST 3)). The smaller study reported that outlined that 13 out of 32 participants in the intervention group and one out of 15 participants in the placebo group experienced severe adverse events such as pneumonia, pyelonephritis and behavioural or personality changes (Krueger 2017). When pooled, data from the two studies showed that while there is a tendency towards a higher risk of severe adverse events in the treatment group, there is no statistical difference in risk between groups (RR 2.35, 95% CI 0.99 to 5.58; 2 studies, 413 participants; high-certainty evidence; Analysis 1.7).

The cross-over study stated that five out of 23 participants during the sirolimus period reported serious adverse events compared to two out of 23 participants during the placebo period; the severe adverse events included upper respiratory tract infection, pneumonia, otitis media and anorexia (Overwater 2016).

Topical administration of rapamycin or rapalogs versus placebo

Four studies with a total of 305 participants are included in this comparison (Koenig 2012; Koenig 2018; Wataya-Kaneda 2017; Wataya-Kaneda 2018).

Primary outcome

1. Tumour size

No studies reported on tumour size.

Secondary outcomes

1. Response to skin lesions

1.2.1 Improvement in any skin lesion

One study which administered rapamycin topically (skin application) reported a defined response to skin lesions as measured based on the participants' perception of their skin condition (Koenig 2012). The study author provided data showing that 11 out of 15 participants in the treatment group reported improved skin lesions after treatment versus three out of eight participants in the placebo group (Koenig 2012). A second study which also administered topical rapamycin assessed the intervention effects on skin lesions via photo readers' rating of paired baseline and end-of-trial photographs for each participant. Investigators reported that 83 out of 113 participants from the treatment group showed improvement on skin lesion (facial

angiofibroma) compared to 13 out of 51 participants from the placebo group (Koenig 2018). Our meta-analysis showed a greater number of participants in the intervention group reporting an improvement in skin lesions (RR at 2.72, 95% CI 1.76 to 4.18; $P < 0.00001$; 2 studies, 187 participants; high-certainty evidence; Analysis 2.1).

1.2.2 Deterioration in any skin lesion

One study which administered topical rapamycin reported on whether skin lesions were worse after the intervention (Koenig 2018). Investigators found that 14 out of 113 participants in the treatment group experienced a deterioration in skin lesions compared to 23 out of 51 participants in the placebo group, with the result still favouring the use of rapamycin (RR 0.27, 95% CI 0.15 to 0.49; 1 study, 164 participants; high-certainty evidence; Analysis 2.2).

1.2.3 Angiofibroma

Three studies reported angiofibroma response to treatment (Koenig 2018; Wataya-Kaneda 2017; Wataya-Kaneda 2018).

The three-arm study by Koenig applied the Angiofibroma Grading Scale (AGS) and investigators reported that at six months the mean improvement for the 1% rapamycin group (54 participants analysed) was 16.7 points compared with 11.0 points for the 0.1% rapamycin (54 participants analysed) and 2.1 points for vehicle only (placebo) group (49 participants analysed); $P < 0.001$ for 1% and 0.1% compared to vehicle only (Koenig 2018). However, we are unable to analyse the results (hence certainty of the evidence) until we obtain the corresponding standard deviations (SDs). To date we have not received a response from the authors to our request for the data.

The Wataya-Kaneda 2018 study administered topical sirolimus and reported response to facial angiofibroma. At both time points, data showed an improvement with treatment, despite wide CIs; at over one and up to three months investigators reported an improvement in facial angiofibroma in 13 participants out of 30 in the treatment group compared to no participants out of 32 in the placebo group, RR 28.74 (95% CI 1.78 to 463.19); and at over three and up to six months, investigators reported an improvement in facial angiofibroma in 18 out of 30 participants in the treatment group compared to none of the 32 participants in the placebo group (RR 39.39, 95% CI 2.48 to 626.00; 1 study, 62 participants; low-certainty evidence; Analysis 2.1).

The third study reported the mean change in treatment response (with a score of 1 showing remarkable improvement up to 5 showing worse condition, such that a decrease in numerical values shows an improvement) (Wataya-Kaneda 2017). In the paediatric group, investigators reported a significant improvement with sirolimus compared to placebo (MD -1.50, 95% CI -2.64 to -0.36; 1 study, 18 participants; moderate-certainty evidence; Analysis 2.3); but in the adult group, there was no difference between groups (MD -0.75, 95% CI -1.58 to 0.08; 1 study, 18 participants; moderate-certainty evidence; Analysis 2.3). The combined result, showed a significant improvement with treatment (MD -1.01, 95% CI -1.68 to -0.34; 1 study, 36 participants; moderate-certainty evidence; Analysis 2.3).

1.2.4 Cephalic plaques

The Wataya-Kaneda 2018 study reported the response in cephalic plaques at the same time points as for the previous outcome measures. At over one and up to three months, the study reported an improvement in cephalic plaques in four out of 13 participants in the intervention group versus none of the 16 participants in the placebo group; but this result did not show a difference between groups when analysed (RR 10.93, 95% CI 0.64 to 186.08; 1 study, 29 participants; low-certainty evidence; Analysis 2.1). At over three and up to six months, the study reported an improvement in cephalic plaques in six out of 13 participants in the treatment group compared to one out of 16 participants from placebo group; showing a borderline improvement with treatment (RR 7.38, 95% CI 1.01 to 53.83; 1 study, 29 participants; low-certainty evidence; Analysis 2.1).

2. Creatinine level (mg/dL)

No study reported creatinine levels (Koenig 2012; Koenig 2018; Wataya-Kaneda 2017; Wataya-Kaneda 2018).

3. Frequency of seizure

No study reported on the frequency of seizure (Koenig 2012; Koenig 2018; Wataya-Kaneda 2017; Wataya-Kaneda 2018).

4. Aneurysm size for angiomyolipomas (any unit of analysis found)

None of the included studies reported aneurysm size for angiomyolipomas (Koenig 2012; Koenig 2018; Wataya-Kaneda 2017; Wataya-Kaneda 2018).

5. FEV₁ / FVC ratio

No study reported on FEV₁/FVC ratio (Koenig 2012; Koenig 2018; Wataya-Kaneda 2017; Wataya-Kaneda 2018).

6. Other outcomes

6.1. Quality of Life

Quality of life was reported by two studies (Koenig 2018; Wataya-Kaneda 2018).

Koenig reported that there was no treatment effect on quality of life; however, we are unable to analyse these results (hence certainty of the evidence) ourselves until we obtain detailed data on the mean scores of each treatment arm for each of the questionnaires used Dermatology Life Quality Index (DLQI), Child Dermatology Life Quality Index (CDLQI) and Family Dermatology Life Quality Index (FDLQI) (Koenig 2018). To date we have received no response to our request for the data.

The second study also used two of the same questionnaires (DLQI and CDLQI) and combined them to assess quality of life (Wataya-Kaneda 2018). Investigators reported no difference in the mean change in score between the sirolimus and placebo groups (MD 0.30, 95%CI -1.01 to 1.61; 1 study, 62 participants; low-certainty evidence; Analysis 2.4).

6.2. Satisfaction

Wataya-Kaneda 2017 reported the mean of satisfaction amongst participants at 12 weeks (with a score of 1 showing extreme satisfaction up to 5 showing extreme dissatisfaction, such that a decrease in numerical values shows better satisfaction). In the child group, participants were more satisfied with sirolimus (MD 1.50,

95% CI 2.69 to 0.31; 1 study, 18 participants; low-certainty evidence; [Analysis 2.5](#)). In adult group, however, difference in satisfaction was not observed (MD 0.25, 95% CI 1.52 to -1.02; 1 study, 18 participants; low-certainty evidence; [Analysis 2.5](#)). Overall results for satisfaction were significant (MD 0.92, 95%CI 1.79 to 0.05; 1 study, 36 participants; low-certainty evidence; [Analysis 2.5](#)).

7. Adverse events or toxicity

Our review analysed adverse events in three subgroups i.e. any adverse event, adverse events leading to dose reduction, interruption or withdrawal and severe adverse events. It is of note that the three subgroups might overlap. Three included studies reported adverse events during the course of each study but none were definitely related to treatment ([Koenig 2018](#); [Wataya-Kaneda 2017](#); [Wataya-Kaneda 2018](#)). However, one study showed 29 out of 122 participants in treatment group experienced at least one drug-related adverse event compared to only 6 out of 57 participants in the placebo group ([Koenig 2018](#)). We analysed these data by grouping them under any adverse event data which is not necessarily confined to drug-related adverse events.

7.1. Any adverse event

Three studies of topical administration reported adverse events ([Wataya-Kaneda 2017](#); [Wataya-Kaneda 2018](#); [Koenig 2018](#)). Our meta-analysis showed a higher risk of participants in the treatment arm experiencing an adverse event (RR 1.72, 95% CI 1.10 to 2.67; 3 studies, 277 participants; moderate-certainty evidence; [Analysis 2.6](#)).

One study reported 72 out of 122 participants in the treatment group and 17 out of 57 in the placebo group experienced an adverse event, these included pain, pruritus and acne at application sites ([Koenig 2018](#)). Authors clarified that nearly all adverse events were mild, and there were no drug-related serious events or drug-related moderate or severe events. No apparent hypersensitivity or allergic-type adverse events were reported.

The [Wataya-Kaneda 2017](#) study reported adverse events, including mild to moderate dryness without sign of inflammation and irritation, in 20 out of 24 participants in the treatment group and 4 out of 12 participants in the control group; it is of note that investigators detected low-level systemic absorption of rapamycin in some of the participants ([Wataya-Kaneda 2017](#)).

The third study also reported mild to moderate skin dryness, application site irritation, and pruritus, which occurred in 27 out of 30 participants in the treatment group versus 22 out of 32 participants in the placebo group ([Wataya-Kaneda 2018](#)).

7.2 Adverse events leading to dose reduction, interruption or withdrawal

None of the studies in this comparison reported adverse events that led to dose reduction, interruption or withdrawal from the study ([Koenig 2012](#); [Koenig 2018](#); [Wataya-Kaneda 2017](#); [Wataya-Kaneda 2018](#)).

7.3. Severe adverse events

The [Koenig 2012](#) study reported that one participant from the treatment group experienced a serious adverse event, albeit unrelated to the study treatment. The participant aspirated during a seizure and developed pneumonia, which progressed to septic shock. The rapamycin concentrations were undetectable at the

time of hospital admission, and the participant was immediately withdrawn from the study. This study did not list specific adverse events in the participants; however, the authors reported that there were no serious adverse events related to the study product and there was no detectable systemic absorption of the rapamycin during the study period ([Koenig 2012](#)).

The later study by the same investigators reported severe adverse events in five out of 122 participants in the treatment group and 3 out of 57 participants in the placebo group ([Koenig 2018](#)). Two participants were withdrawn from this study owing to an adverse event, only one of which was a suspected drug reaction (mild application site cutaneous eruption). However, when analysed there is no difference between groups in the risk of severe adverse events (RR 0.78, 95% CI 0.19 to 3.15; 1 study, 179 participants; moderate-certainty evidence; [Analysis 2.6](#)).

DISCUSSION

Summary of main results

For this 2022 update of the review update, we have added seven new included studies which brings in additional 737 participants ([French 2016 \(EXIST 3\)](#); [Koenig 2018](#); [Krueger 2017](#), [Overwater 2016](#); [Overwater 2019](#); [Wataya-Kaneda 2017](#); [Wataya-Kaneda 2018](#)). In this update we have additionally reported on neuropsychiatric outcomes as well as collecting and analysing data on seizure frequency, which were not reported in the previous review version. We have analysed results for systemic and topical administration of treatment separately.

Systemic administration

Six studies (703 participants) reported on this comparison ([Bissler 2013 \(EXIST 2\)](#); [Franz 2013 \(EXIST 1\)](#); [French 2016 \(EXIST 3\)](#); [Krueger 2017](#); [Overwater 2016](#); [Overwater 2019](#)). We have summarised our findings [Summary of findings 1](#). In the current update, we are now able to present data on response to tumour size, response to skin lesions, creatinine level, and FEV₁/FVC ratio.

More participants with angiomyolipoma who received oral everolimus (a rapalog) had at least a 50% reduction in tumour size compared to those receiving placebo at six months (risk ratio (RR) 24.69, 95% confidence interval *(CI) 3.51 to 173.41; P = 0.001; 2 studies, 162 participants; high-certainty evidence; [Analysis 1.1](#)) ([Bissler 2013 \(EXIST 2\)](#); [Franz 2013 \(EXIST 1\)](#)). Similarly, more participants with subependymal giant cell astrocytoma (SEGA) who received oral everolimus (rapalog) had at least a 50% reduction in tumour volume compared to those receiving placebo at six months (RR 27.85, 95% CI 1.74 to 444.82; P = 0.94; 1 study, 117; moderate-certainty evidence; [Analysis 1.1](#)) ([Franz 2013 \(EXIST 1\)](#)).

These two studies also reported an increased chance of showing a response to skin lesions with oral everolimus at six months (RR 5.78, 95% CI 2.30 to 14.52; P = 0.0002; 2 studies, 224 participants; high-certainty evidence; [Analysis 1.2](#)) ([Bissler 2013 \(EXIST 2\)](#); [Franz 2013 \(EXIST 1\)](#)).

One study reported an effect of oral everolimus on serum creatinine, where results showed no difference in levels of serum creatinine at six months (RR 0.16, 95% CI 0.02 to 1.53; P = 0.11; 1 study, 118 participants; moderate-certainty evidence; [Analysis 1.3](#)) ([Bissler 2013 \(EXIST 2\)](#)).

Three studies reported on seizure frequency (Franz 2013 (EXIST 1); French 2016 (EXIST 3); Overwater 2016), but only one study provided data for analysis (French 2016 (EXIST 3)). This study did not show any difference in the number of participants who were seizure-free at 18 weeks (RR 5.30, 95% CI 0.69 to 40.57; $P = 0.11$; 1 study, 366 participants; high-certainty evidence; Analysis 1.4). One of the other studies reported the median change of seizure frequency between baseline and week 24; investigators may have reported results in this way because the data for this outcome were skewed (i.e. most participants might have had experienced a few seizures but a small number of participants may have experienced many) which will change the distribution of the data (the median would be a more appropriate statistic to use in this case) (Franz 2013 (EXIST 1)). Reporting showed a large proportion of participants did not experience seizures at baseline. Moreover, participants were selected for the study based on the need for treatment of subependymal giant cell astrocytomas rather than presence of seizures (Franz 2013 (EXIST 1)). One study had a cross-over design and did not report separate data from the first period (Overwater 2016). We identified differences in the number of participants with a 25% and 50% reduction in seizure frequency from one study (French 2016 (EXIST 3)). This study showed that participants had at least a 25% decrease in seizure frequency with the intervention compared to placebo (RR 1.63, 95% CI 1.27 to 2.09; $P = 0.0001$; 1 study, 366 participants; high-certainty evidence; Analysis 1.4) and at least a 50% reduction with the intervention compared to placebo (RR 2.28, 95% CI 1.44 to 3.60; $P = 0.0004$; 1 study, 366 participants; high-certainty evidence; Analysis 1.4) (French 2016 (EXIST 3)).

None of the included studies reported aneurysm size for angiomyolipomas.

One study reported on FEV₁ / FVC ratio, but with no analysable data (Bissler 2013 (EXIST 2)). For a subset of participants with lymphangioleiomyomatosis and sporadic lymphangioleiomyomatosis, investigators reported that the median percentage reduction in FEV₁ was greater in the placebo group than in the oral everolimus group after 24 weeks (Bissler 2013 (EXIST 2)).

Three studies reported multiple assessments of outcomes related to neurocognitive, neuropsychiatry, behavioural, sensory and motor development, but none showed any effect of oral everolimus (Krueger 2017; Overwater 2016; Overwater 2019).

Five studies reported adverse events (Bissler 2013 (EXIST 2); Franz 2013 (EXIST 1); French 2016 (EXIST 3); Krueger 2017; Overwater 2019). We found no difference in the risk of having any adverse event between participants receiving oral everolimus and those receiving placebo (RR 1.09, 95% CI 0.97 to 1.22; $P = 0.16$; 5 studies, 680 participants; high-certainty evidence; Analysis 1.7). However, one large study comprising more than half of the participants in this analysis clearly showed a higher risk of at least one adverse event among participants receiving oral everolimus (RR 1.21, 95% CI 1.09 to 1.34; 1 study, 366 participants; Analysis 1.7) (French 2016 (EXIST 3)).

Four studies reported on adverse events resulting in withdrawal, an interruption in treatment, or a reduction in dose level and showed that these events were higher in the oral everolimus group (RR 2.61, 95% CI 1.58 to 4.33; 4 studies, 633 participants; $P = 0.0002$;

high-certainty evidence; Analysis 1.7) (Bissler 2013 (EXIST 2), Franz 2013 (EXIST 1), French 2016 (EXIST 3), Overwater 2019). Two studies reported on severe adverse events (French 2016 (EXIST 3), Krueger 2017). Data showed no difference between groups, but there were more severe adverse events among participants receiving oral everolimus compared to those receiving placebo, which almost reached statistical significance (RR 2.35, 95% CI 0.99 to 5.58; 2 studies, 413 participants; high-certainty evidence; Analysis 1.7).

Nevertheless, these are adverse events of which correlation to the treatment *per se* is not certain.

Topical administration

Four studies with a total of 305 participants are included in this comparison (Koenig 2012; Koenig 2018; Wataya-Kaneda 2017; Wataya-Kaneda 2018). We have summarized the findings Summary of findings 2.

No studies reported on tumour size. However, we observed that participants receiving topical rapamycin compared placebo showed improvements in any skin lesions (RR 2.72, 95% CI 1.76 to 4.18; $P < 0.00001$; 2 studies, 187 participants; high-certainty evidence; Analysis 2.1) as well as facial angiofibroma at one to three months (RR 28.74, 95% CI 1.78 to 463.19; $P = 0.02$; 1 study, 62 participants; Analysis 2.1) and at three to six months (RR 39.39, 95% CI 2.48 to 626.00; $P = 0.009$; 1 study, 62 participants; moderate-certainty evidence; Analysis 2.1). Three studies reported similar tendencies in cephalic plaques (Koenig 2012; Koenig 2018; Wataya-Kaneda 2018), but the results were not statistically significant at three months (RR 10.93, 95% CI 0.64 to 186.08; $P = 0.10$; 1 study, 29 participants; moderate-certainty evidence; Analysis 2.1) and barely so at three to six months (RR 7.38, 95% CI 1.01 to 53.83; $P = 0.05$; 1 study, 29 participants; moderate-certainty evidence; Analysis 2.1). Similarly, we also observed that participants receiving placebo experienced a deterioration in skin lesions at six months (RR 0.27, 95% CI 0.15 to 0.49; $P < 0.0001$; 1 study, 164 participants; high-certainty evidence; Analysis 2.2) (Koenig 2018).

Participants receiving topical rapamycin experienced significantly better general improvement in terms of their facial angiofibromas, compared to those receiving placebo (RR -1.01, 95% CI -1.68 to -0.34; $P = 0.003$; 1 study, 36 participants; moderate-certainty evidence; Analysis 2.3), although such tendency was barely significant for the adult group.

No study in this comparison reported on creatinine levels, frequency of seizure, aneurysm size, or FEV₁/FVC ratio.

Two studies reported on quality of life (Koenig 2018; Wataya-Kaneda 2018), but only one provided analysable data (Wataya-Kaneda 2018). We found no difference in the mean change in score between the sirolimus and placebo groups at six months (MD 0.30, 95%CI -1.01 to 1.61; $P = 0.65$; 1 study, 62 participants; low-certainty evidence; Analysis 2.4). Only children receiving topical rapamycin reported better satisfaction at 12 weeks and not the adult participants (Wataya-Kaneda 2017); however, combined data showed better satisfaction with topical rapamycin (MD -0.92, 95% CI -1.79 to -0.05; $P = 0.04$; 1 study, 36 participants; high-certainty evidence; Analysis 2.5).

Three studies reported on adverse events (Koenig 2018; Wataya-Kaneda 2017; Wataya-Kaneda 2018). We observed that participants receiving topical rapamycin showed higher risks of having any

adverse events compared to those receiving placebo (RR 1.72, 95% CI 1.10 to 2.67; $P = 0.02$; 3 studies, 277 participants; moderate-certainty evidence; [Analysis 2.6](#)). None of the studies in this comparison reported adverse events that led to dose reduction, interruption or withdrawal from the study ([Koenig 2012](#); [Koenig 2018](#); [Wataya-Kaneda 2017](#); [Wataya-Kaneda 2018](#)). There was no difference between groups in the risk of having severe adverse events (RR 0.78, 95% CI 0.19 to 3.15; $P = 0.73$; 1 study, 179 participants; moderate-certainty evidence; [Analysis 2.6](#)).

Overall completeness and applicability of evidence

We identified 10 studies meeting our inclusion criteria and including outcomes of interest to this review. Seven of these studies were newly included in the current review update.

However, the objective of correlating intervention to adverse effects was not satisfactorily met, as most of the manifestations were observed as adverse events and not specifically treatment-related adverse effects. It should be noted that small proportions of the participants (five of 162) analysed for renal angiomyolipoma have sporadic lymphangioliomyomatosis (without TSC).

Quality of the evidence

We judged all 10 included studies to generally have a low risk of bias. One study had a high risk of performance bias with no blinding of participants ([Overwater 2016](#)), and three studies had a high risk of attrition bias with 17.9% participant attrition ([Koenig 2012](#)), and exclusion of 11.2% ([Koenig 2018](#)) and 10.6% ([Krueger 2017](#)) participants from their final analyses. Some information is lacking from the study authors to judge the level of potential bias for: generation of sequence ([Koenig 2012](#)); allocation concealment ([Bissler 2013 \(EXIST 2\)](#)); blinding of participants and personnel ([Koenig 2018](#); [Wataya-Kaneda 2017](#)); blinding of outcome assessment ([Krueger 2017](#); [Wataya-Kaneda 2017](#)); and incomplete outcome data ([French 2016 \(EXIST 3\)](#); [Overwater 2016](#)). We also need more information to judge the risk from other potential sources of bias in three studies ([Bissler 2013 \(EXIST 2\)](#); [Franz 2013 \(EXIST 1\)](#); [Overwater 2016](#)). In two of these studies, some authors who are employees, stock owners or consultants of the funder (Novartis) were involved in the study design, discussion, research, overseeing of data collection and data analysis and interpretation leading to an unclear risk of other bias ([Bissler 2013 \(EXIST 2\)](#); [Franz 2013 \(EXIST 1\)](#)). In the third study, we had some concerns related to the cross-over design ([Overwater 2016](#)).

The evidence from studies using systemic administration was generally of high certainty, except for the outcome of any adverse events which was downgraded to moderate certainty because of substantial heterogeneity ([Bissler 2013 \(EXIST 2\)](#); [Franz 2013 \(EXIST 1\)](#); [French 2016 \(EXIST 3\)](#); [Krueger 2017](#); [Overwater 2016](#); [Overwater 2019](#)).

The evidence from studies using topical administration showed mixed certainty levels ([Koenig 2012](#); [Koenig 2018](#); [Wataya-Kaneda 2017](#); [Wataya-Kaneda 2018](#)). We found high-certainty evidence for outcomes related to mean general improvements in facial

angiofibroma (continuous outcome), improvement as well as worsening in any skin lesions, quality of life, satisfaction, and severe adverse events. We downgraded the level of certainty for angiofibroma and cephalic plaques (dichotomous outcomes) because of large CIs, while we downgraded the level of certainty for adverse events because of substantial heterogeneity.

Potential biases in the review process

The strengths of this review are based on the careful selection of randomised studies of a rare disease. We have noted that not all relevant data could be obtained, especially those data relating to adverse effects.

Agreements and disagreements with other studies or reviews

A systematic review on non-randomised studies carried out by this author team showed similar findings, where absolute tumour size reduction was noted in people treated with rapamycin or rapalogs ([Sasongko 2015](#)).

AUTHORS' CONCLUSIONS

Implications for practice

Convincing evidence of a reduction of tumour size after 24 weeks of treatment with oral everolimus (rapalogs) in both renal angiomyolipoma and subependymal giant cell astrocytoma (SEGA) would provide sufficient evidence for its use in clinical practice as the benefits outweigh the risks. With this in mind, and with the positive effects on the size reduction in renal angiomyolipoma and SEGA, this review concurs with the decision of the U.S. Food and Drug Administration (FDA) and European Medicine Agency (EMA) on the use of everolimus for both types of tumours. Rapamycin or rapalogs may also have a beneficial effect on skin lesions.

Implications for research

More research is needed to further define the effects of rapamycin or rapalogs on other manifestations of tuberous sclerosis complex, such as seizures and neurocognitive problems. Beneficial effects noted in this review on skin lesions need to be further established. This research should take the form of a randomised study design, involving participants diagnosed according to the latest consensus tuberous sclerosis complex diagnostic criteria. Future research should also consider rapalog use in children due to the age-dependent nature of manifestations and complications in tuberous sclerosis (TSC).

ACKNOWLEDGEMENTS

The review authors wish to thank Tracey Remington, Nikki Jahnke and Jacqueline Ho for their helpful comments and guidance throughout the manuscript writing. The review authors wish to thank Nik Mohd. Ariff Nik Abdul Malik for his great assistance during study searching and selection as well as data extraction and analyses and Nur Farrah Dila Ismail for her contribution to the original version of this review.

REFERENCES

References to studies included in this review

Bissler 2013 (EXIST 2) {published data only}

Bissler J, Kingswood C, Zonnenberg BA, Frost M, Belousova E, Radzikowska E, et al. Updated safety results from EXIST-2: everolimus therapy for angiomyolipoma (AML) associated with tuberous sclerosis complex (TSC) or sporadic lymphangioleiomyomatosis (sLAM). *Journal of Clinical Oncology* 2012;**30**(15 Suppl 1):4632. [CN-01028275]

Bissler J, Kingswood JC, Radzikowska E, Zonnenberg BA, Frost M, Sauter M, et al. Everolimus for renal angiomyolipoma associated with tuberous sclerosis complex: exist-2 long-term efficacy and safety. *Journal of Urology* 2014;**191**(4 Suppl 1):e540. [DOI: [10.1016/j.juro.2014.02.1508](https://doi.org/10.1016/j.juro.2014.02.1508)] [CN-01058878]

Bissler J, Kingswood JC, Zonnenberg B, Frost M, Belousova E, Radzikowska E, et al. Exist-2: effect of everolimus on angiomyolipoma in patients with tuberous sclerosis complex or sporadic lymphangioleiomyomatosis. *Journal of Urology* 2012;**187**(4 Suppl 1):e528. [DOI: [10.1016/j.juro.2012.02.1636](https://doi.org/10.1016/j.juro.2012.02.1636)] [CN-01033818]

Bissler JJ, Budde K, Sauter M, Franz DN, Zonnenberg BA, Frost MD, et al. Effect of everolimus on renal function in patients with tuberous sclerosis complex: evidence from EXIST-1 and EXIST-2. *Nephrology, Dialysis, Transplantation* 2019;**34**(6):1000-8. [DOI: <https://doi.org/10.1093/ndt/gfy132>] [PMID: 30053159]

Bissler JJ, Kingswood C, Zonnenberg B, Frost M, Belousova E, Radzikowska E, et al. Examining everolimus in a study of tuberous sclerosis complex (TSC) trial (EXIST-2): updated safety results. *BJU International* 2012;**110**:7. [CN-01030577]

Bissler JJ, Kingswood JC, Radzikowska E, Zonnenberg BA, Belousova E, Frost MD, et al. Everolimus long-term use in patients with tuberous sclerosis complex: Four-year update of the EXIST-2 study. *PloS one* 2017;**12**(8):e0180939. [DOI: [10.1371/journal.pone.0180939](https://doi.org/10.1371/journal.pone.0180939)] [PMID: 28792952]

* Bissler JJ, Kingswood JC, Radzikowska E, Zonnenberg BA, Frost M, Belousova E, et al. Everolimus for angiomyolipoma associated with tuberous sclerosis complex or sporadic lymphangioleiomyomatosis (EXIST-2): a multicentre, randomised, double-blind, placebo-controlled trial. *Lancet* 2013;**381**(9869):817-24. [DOI: [https://doi.org/10.1016/S0140-6736\(12\)61767-X](https://doi.org/10.1016/S0140-6736(12)61767-X)] [PMID: 23312829]

Bissler JJ, Kingswood JC, Radzikowska E, Zonnenberg BA, Frost M, Belousova E, et al. Everolimus for renal angiomyolipoma associated with tuberous sclerosis complex (TSC) from EXIST-2: continued efficacy and diminishing adverse events after ~3.5 years of treatment. *European Urology Supplements* 2015;**14**(2):e1. [CN-01078023]

Bissler JJ, Kingswood JC, Radzikowska E, Zonnenberg BA, Frost M, Belousova E, et al. Everolimus for renal angiomyolipoma associated with tuberous sclerosis complex (TSC): EXIST-2 3-year follow-up. *European Urology Supplements* 2014;**13**(1):e1139. [CN-01063730]

Bissler JJ, Kingswood JC, Radzikowska E, Zonnenberg BA, Frost M, Belousova E, et al. Everolimus for renal angiomyolipoma in patients with tuberous sclerosis complex or sporadic lymphangioleiomyomatosis: extension of a randomized controlled trial. *Nephrology, Dialysis, Transplantation* 2016;**31**(1):111-9. [DOI: [10.1093/ndt/gfv249](https://doi.org/10.1093/ndt/gfv249)] [PMID: 26156073]

Bissler JJ, Kingswood JC, Zonnenberg BA, Frost M, Belousova E, Radzikowska E, et al. Everolimus therapy for angiomyolipoma in patients with tuberous sclerosis complex or sporadic lymphangioleiomyomatosis: results from EXIST-2. *Journal of Clinical Oncology* 2012;**30**(5 Suppl 1):356. [CN-01028108]

Bissler JJ, Nonomura N, Budde K, Zonnenberg BA, Fischereder M, Voi M, et al. Angiomyolipoma rebound tumor growth after discontinuation of everolimus in patients with tuberous sclerosis complex or sporadic lymphangioleiomyomatosis. *PLOS One* 2018;**13**(9):e0201005. [DOI: [10.1371/journal.pone.0201005](https://doi.org/10.1371/journal.pone.0201005)] [PMID: 30192751]

Bissler JJ, Zonnenberg B, Frost M, Radzikowska E, Sauter M, Nonomura N, et al. Everolimus therapy for angiomyolipoma (AML) associated with tuberous sclerosis complex (TSC) or sporadic lymphangioleiomyomatosis (LAM): exist-2 update. *Nephrology, Dialysis, Transplantation* 2013;**28**:i28-9. [DOI: [10.1093/ndt/gft158](https://doi.org/10.1093/ndt/gft158)] [CN-01027043]

Franz DN, Budde K, Kingswood JC, Belousova E, Sparagana S, de Vries PJ, et al. Effect of everolimus on skin lesions in patients treated for subependymal giant cell astrocytoma and renal angiomyolipoma: final 4-year results from the randomized EXIST-1 and EXIST-2 studies. *Journal of the European Academy of Dermatology and Venereology* 2018;**32**(10):1796-803. [DOI: [10.1111/jdv.14964](https://doi.org/10.1111/jdv.14964)] [PMID: 29569806]

Franz DN, Jozwiak S, Belousova E, Frost M, Kuperman R, Bebin EM, et al. Effect of everolimus on renal angiomyolipoma in pediatric patients with tuberous sclerosis complex (TSC) being treated for subependymal giant cell astrocytoma (SEGA). *BJU International* 2014;**114**:7-8. [DOI: [10.1111/bju.12941/abstract](https://doi.org/10.1111/bju.12941/abstract)] [CN-01074195]

Kwiatkowski DJ, Palmer MR, Jozwiak S, Bissler J, Franz D, Segal S, et al. Response to everolimus is seen in TSC-associated SEGAs and angiomyolipomas independent of mutation type and site in TSC1 and TSC2. *European Journal of Human Genetics* 2015;**23**(12):1665-72. [PMID: 25782670]

NCT00790400. Efficacy and safety of RAD001 in patients aged 18 and over with Angiomyolipoma associated with either tuberous sclerosis complex (TSC) or sporadic lymphangioleiomyomatosis (LAM) (EXIST-2). clinicaltrials.gov/ct2/show/NCT00790400 (first posted November 13 2008).

Franz 2013 (EXIST 1) {published data only}

Bissler JJ, Budde K, Sauter M, Franz DN, Zonnenberg BA, Frost MD, et al. Effect of everolimus on renal function in patients with tuberous sclerosis complex: evidence from EXIST-1 and EXIST-2. *Nephrology, Dialysis, Transplantation*

2019;**34**(6):1000-8. [DOI: <https://doi.org/10.1093/ndt/gfy132>] [PMID: 30053159]

Bissler JJ, Franz DN, Frost MD, Belousova E, Bebin EM, Sparagana S, et al. The effect of everolimus on renal angiomyolipoma in pediatric patients with tuberous sclerosis being treated for subependymal giant cell astrocytoma. *Pediatric Nephrology* 2018;**33**(1):101-9. [DOI: [10.1007/s00467-017-3806-1](https://doi.org/10.1007/s00467-017-3806-1)] [PMID: 28993887]

Franz D, Belousova E, Frost M, Kuperman R, Bebin M, Korf B, et al. Everolimus for the treatment of renal angiomyolipoma in patients with tuberous sclerosis complex being treated for subependymal giant cell astrocytoma. *Neurology* 2012;**78**(1 Meeting Abstracts):P04.188. [DOI: [10.1212/WNL.78.1](https://doi.org/10.1212/WNL.78.1)] [CN-01033658]

Franz D, Belousova E, Sparagana S, Bebin E, Frost M, Kuperman R, et al. Long-term efficacy and safety of everolimus for the treatment of subependymal giant cell astrocytoma (SEGA) associated with tuberous sclerosis complex (TSC) in exist-1: approximately 3.5 years of exposure. *Neurology* 2015;**84**:P2.235. [CN-01102987]

Franz D, Lam D, Cauwel H, Jozwiak S. Effect of everolimus on subependymal nodules (SENs) and tubers in patients with subependymal giant cell astrocytoma (SEGA) associated with tuberous sclerosis complex (TSC): Results from the exist-1 trial. In: 65th American Academy of Neurology Annual Meeting San Diego, CA United States. Lippincott Williams and Wilkins, 2013.

Franz D, Lam D, Cauwel H, Jozwiak S. Effect of everolimus on subependymal nodules (SENs) and tubers in patients with subependymal giant cell astrocytoma (SEGA) associated with tuberous sclerosis complex (TSC): results from the exist-1 trial. *Neurology* 2013;**80**(7 Suppl):P01.049. [CN-01025881]

* Franz DN, Belousova E, Sparagana S, Bebin EM, Frost M, Kuperman R, et al. Efficacy and safety of everolimus for subependymal giant cell astrocytomas associated with tuberous sclerosis complex (EXIST-1): a multicentre, randomised, placebo-controlled phase 3 trial. *Lancet* 2013;**381**(9861):125-32. [DOI: [10.1016/S0140-6736\(12\)61134-9](https://doi.org/10.1016/S0140-6736(12)61134-9)] [PMID: 23158522]

Franz DN, Belousova E, Sparagana S, Bebin EM, Frost M, Kuperman R, et al. Everolimus for subependymal giant cell astrocytoma associated with tuberous sclerosis complex: final 4-year long-term results from EXIST-1. *Annals of Neurology* 2015;**78**:S70-1. [DOI: [10.1002/ana.24498](https://doi.org/10.1002/ana.24498)] [CN-01476466]

Franz DN, Belousova E, Sparagana S, Bebin EM, Frost M, Kuperman R, et al. Everolimus for subependymal giant cell astrocytoma associated with tuberous sclerosis complex: final long-term results from approximately 4 years of treatment in EXIST-1. *Annals of Neurology* 2015;**78**:S158. [DOI: [10.1002/ana.24477](https://doi.org/10.1002/ana.24477)] [CN-01136066]

Franz DN, Belousova E, Sparagana S, Bebin EM, Frost M, Kuperman R, et al. Everolimus for subependymal giant cell astrocytoma in patients with tuberous sclerosis complex: 2-year open-label extension of the randomised EXIST-1 study. *Lancet. Oncology* 2014;**15**(13):1513-20. [PMID: 25456370]

Franz DN, Belousova E, Sparagana S, Bebin EM, Frost MD, Kuperman R, et al. Long-term use of everolimus in patients with tuberous sclerosis complex: final results from the EXIST-1 study. *PloS One* 2016;**11**(6):e0158476. [DOI: [10.1371/journal.pone.0158476](https://doi.org/10.1371/journal.pone.0158476)] [CN-01401990]

Franz DN, Belousova E, Sparagana S, De Vries PJ, Berkowitz N, Brechenmacher T, et al. Effect of everolimus on skin lesions in patients being treated for subependymal giant cell astrocytoma: final 4-year results from EXIST-1. *Pediatric Dermatology* 2016;**33**(6):704. [CN-01620080]

Franz DN, Brechenmacher T, Segal S, Jozwiak S. Safety of everolimus by age category for subependymal giant cell astrocytoma (SEGA) associated with tuberous sclerosis complex (TSC): results from the exist-1 trial. *Annals of Neurology* 2014;**76**:S179. [DOI: [10.1002/ana.24226](https://doi.org/10.1002/ana.24226)] [CN-01055344]

Franz DN, Budde K, Kingswood JC, Belousova E, Sparagana S, de Vries PJ, et al. Effect of everolimus on skin lesions in patients treated for subependymal giant cell astrocytoma and renal angiomyolipoma: final 4-year results from the randomized EXIST-1 and EXIST-2 studies. *Journal of the European Academy of Dermatology and Venereology* 2018;**32**(10):1796-803. [DOI: [10.1111/jdv.14964](https://doi.org/10.1111/jdv.14964)] [PMID: 29569806]

Franz DN, Jozwiak S, Belousova E, Frost M, Kuperman R, Bebin EM, et al. Effect of everolimus on renal angiomyolipoma in pediatric patients with tuberous sclerosis complex (TSC) being treated for subependymal giant cell astrocytoma (SEGA). *BJU International* 2014;**114**:7-8. [DOI: [10.1111/bju.12941/abstract](https://doi.org/10.1111/bju.12941/abstract)] [CN-01074195]

Franz DN, Kuperman R, Flamini JR, Wu JY, Berkowitz N, Niolat J, et al. The effect of everolimus on growth and sexual maturation in patients treated for subependymal giant cell astrocytoma associated with tuberous sclerosis complex: results from the 4-year final analysis of EXIST-1. *Annals of Neurology* 2015;**78**:S219. [DOI: [10.1002/ana.24477](https://doi.org/10.1002/ana.24477)] [CN-01136059]

Jansen A, Jozwiak S, Belousova E, Frost M, Kuperman R, Bebin M, et al. Effect of everolimus therapy on renal angiomyolipoma response in patients with tuberous sclerosis complex being treated for subependymal giant cell astrocytoma: exploratory results from EXIST-1. *Nephrology, Dialysis, Transplantation* 2012;**27**:ii325. [DOI: [10.1093/ndt/gfs232](https://doi.org/10.1093/ndt/gfs232)] [CN-01033165]

Jóźwiak S, Belousova E, Kingswood C, Frost M, Kuperman R, Bebin M, et al. The effect of everolimus on renal angiomyolipoma in patients with tuberous sclerosis complex being treated for subependymal giant cell astrocytoma. *European Urology, Supplements* 2012;**11**(1):e74-74a. [CN-01031221]

Jóźwiak S, Belousova E, Sparagana S, Bebin M, Frost M, Shah G, et al. Everolimus in the treatment of subependymal giant cell astrocytomas (SEGAs) associated with tuberous sclerosis complex (TSC): the EXIST-1 trial. *Developmental Medicine and Child Neurology* 2012;**54**:109. [DOI: [10.1111/j.1469-8749.2012.04283.x](https://doi.org/10.1111/j.1469-8749.2012.04283.x)] [CN-01031868]

Jóźwiak S, Brechenmacher T, Segal S, Franz DN. Everolimus for subependymal giant cell astrocytomas (SEGAs) associated

with tuberous sclerosis complex (TSC): results from the EXIST-1 trial by age category. *European Journal of Paediatric Neurology* 2013;**17**:S44. [DOI: [10.1016/S1090-3798%2813%2970150-8](https://doi.org/10.1016/S1090-3798%2813%2970150-8)] [CN-01025113]

Jóźwiak S, Brechenmacher T, Segal S, Franz DN. Safety of everolimus by age category for subependymal giant cell astrocytomas (SEGAs) associated with tuberous sclerosis complex (TSC): results from the EXIST-1 trial. *European Journal of Paediatric Neurology* 2013;**17**:S119. [DOI: [10.1016/S1090-3798%2813%2970418-5](https://doi.org/10.1016/S1090-3798%2813%2970418-5)] [CN-01025111]

Jóźwiak S, Kotulska K, Berkowitz N, Brechenmacher T, Franz DN. Safety of everolimus in patients younger than 3 years of age: results from EXIST-1, a randomized, controlled clinical trial. *Journal of Pediatrics* 2016;**172**:151-155.e1. [DOI: [10.1016/j.jpeds.2016.01.027](https://doi.org/10.1016/j.jpeds.2016.01.027)] [PMID: 26858193]

Jóźwiak S, Stein K, Kotulska K. Everolimus (RAD001): first systemic treatment for subependymal giant cell astrocytoma associated with tuberous sclerosis complex. *Future Oncology* 2012;**8**(12):1515-23. [PMID: 23231513]

Karaoğlu M. Adjunctive everolimus therapy for treatment-resistant focal-onset seizures associated with tuberous sclerosis: a phase 3, randomised, double-blind, placebo-controlled study [Tüberoskleroz ile ilişkili, Tedaviye Dirençli Fokal Başlangıçlı Nöbetler için Yardımcı Everolimus Tedavisi: 3 Aşamalı, Randomize, Çift Kör, Plasebo Kontrollü Çalışma]. *Frontiers in Neurology / Nörolojide Öne Çıkanlar, Türk nöroloji dergisi [turkish journal of neurology]* 2018;**24**(2):193-4. [DOI: [10.4274/tnd.78466](https://doi.org/10.4274/tnd.78466)]

Kingswood C, Franz D, Belousova E, Berkowitz N, Brechenmacher T, Jóźwiak S, et al. Effect of everolimus on renal angiomyolipoma in pediatric patients from the final analysis of exist-1. *Pediatric Nephrology* 2015;**30**(9):1549-50. [DOI: [10.1007/s00467-015-3158-7](https://doi.org/10.1007/s00467-015-3158-7)] [CN-01131253]

Kingswood J, Jóźwiak S, Belousova E, Frost M, Kuperman R, Bebin M, et al. Effect of everolimus on renal angiomyolipoma (AML) in patients with tuberous sclerosis complex (TSC) being treated for subependymal giant cell astrocytoma (SEGA). *Nephrology, Dialysis, Transplantation* 2013;**28**:i316. [DOI: [10.1093/ndt/gft126](https://doi.org/10.1093/ndt/gft126)] [CN-01027030]

Kingswood J, Zonnenberg B, Frost M, Cheung W, Wang J, Brechenmacher T, et al. Pharmacokinetics and exposure-safety relationship of everolimus in patients with renal angiomyolipoma (AML) associated with tuberous sclerosis complex (TSC) or sporadic lymphangioleiomyomatosis. *Nephrology, Dialysis, Transplantation* 2013;**28**:i316. [DOI: [10.1093/ndt/gft126](https://doi.org/10.1093/ndt/gft126)] [CN-01027029]

Kingswood JC, Jozwiak S, Belousova ED, Frost MD, Kuperman RA, Bebin EM, et al. The effect of everolimus on renal angiomyolipoma in patients with tuberous sclerosis complex being treated for subependymal giant cell astrocytoma: subgroup results from the randomized, placebo-controlled, phase 3 trial EXIST-1. *Nephrology, Dialysis, Transplantation* 2014;**29**(6):1203-10. [DOI: [10.1093/ndt/gfu013](https://doi.org/10.1093/ndt/gfu013)] [PMID: 24729041]

Kingswood JC, Jóźwiak S, Belousova ED, Frost MD, Kuperman RA, Bebin EM, et al. The effect of everolimus on renal

angiomyolipoma in patients with tuberous sclerosis complex being treated for subependymal giant cell astrocytoma: subgroup results from the randomized, placebo-controlled, Phase 3 trial EXIST-1. *Nephrology, Dialysis, Transplantation* 2014;**29**(6):1203-10. [PMID: 24729041]

Kwiatkowski DJ, Palmer MR, Jóźwiak S, Bissler J, Franz D, Segal S, et al. Response to everolimus is seen in TSC-associated SEGAs and angiomyolipomas independent of mutation type and site in TSC1 and TSC2. *European Journal of Human Genetics* 2015;**23**(12):1665-72. [PMID: 25782670]

NCT00789828. Efficacy and safety of Everolimus (RAD001) in patients of all ages with subependymal giant cell astrocytoma associated with tuberous sclerosis complex (TSC)(EXIST-1). www.clinicaltrials.gov/ct2/show/NCT00789828 (first posted 13 November 2008).

Sparagana S, Belousova E, Jozwiak S, Korf B, Frost M, Kuperman R, et al. Everolimus treatment f subependymal giant cell astrocytomas (SEGAs) associated with tuberous sclerosis complex (TSC): the EXIST-1 trial. *Neuro-Oncology* 2011;**13**(Suppl 3):Medical and Neuro-oncology. [ABSTRACT NO: 41] [DOI: [10.1093/neuonc/nor152](https://doi.org/10.1093/neuonc/nor152)]

Sparagana S, Flamini JR, Wu JY, Kuperman R, Berkowitz N, Niolat J, et al. Everolimus exposure and overall tolerability in patients treated for subependymal giant cell astrocytoma associated with tuberous sclerosis complex: results from the 4-year final analysis of EXIST-1. *Annals of Neurology* 2015;**78**(S19):S159. [DOI: [10.1002/ana.24477](https://doi.org/10.1002/ana.24477)]

French 2016 (EXIST 3) {published data only}

Curatolo P, Franz DN, Lawson JA, Yapici Z, Ikeda H, Polster T, et al. Adjunctive everolimus for children and adolescents with treatment-refractory seizures associated with tuberous sclerosis complex: post-hoc analysis of the phase 3 EXIST-3 trial. *Lancet. Child & Adolescent Health* 2018;**2**(7):495-504. [PMID: 30169322]

De Vries PJ, Franz DN, Lawson JA, Yapici Z, Polster T, Nabbout R, et al. Adjunctive everolimus therapy for the treatment of refractory seizures in people with tuberous sclerosis complex. *Journal of Intellectual Disability Research* 2016;**60**(9):839. [DOI: [10.1111/jir.12324](https://doi.org/10.1111/jir.12324)]

Franz D, De Vries P, Curatolo P, Nabbout R, Fan J, Neary M, et al. Psychometric evaluation of quality of life in a three-arm, randomized, double-blind, placebo-controlled phase 3 study of everolimus as adjunctive therapy in patients with tuberous sclerosis complex (TSC) who have refractory partial-onset seizures (EXIST-3). *Annals of Neurology* 2017;**82**:S282. [DOI: [10.1002/ana.25022](https://doi.org/10.1002/ana.25022)] [CN-01612522]

Franz D, Lawson J, Yapici Z, Ikeda H, Polster T, Nabbout R, et al. Efficacy and safety of everolimus based on prior and concomitant antiepileptic drugs in patients with tuberous sclerosis complex (TSC)-associated treatment-refractory seizures: a subanalysis of the phase 3 EXIST-3 study. *Neurology* 2018;**90**(15 Suppl):S9.007. [CN-01608776]

French J, Lawson J, Yapici Z, Polster T, Nabbout R, Curatolo P, et al. Exposure-response analysis of adjunctive everolimus therapy

in patients with refractory partial-onset seizures associated with tuberous sclerosis complex (TSC). *European Journal of Neurology* 2016;**23**:54. [DOI: [10.1111/ene.13091](https://doi.org/10.1111/ene.13091)] [CN-01267457]

French JA, Curatolo P, Lawson JA, Yapici Z, Ikeda H, Polster T, et al. Adjunctive everolimus in patients with treatment-refractory seizures associated with tuberous sclerosis complex (TSC): analysis of exposure-efficacy and exposure-safety relationships in the randomized, phase 3, exist-3 trial. *Epilepsia* 2017;**58**:S14-5. [DOI: [10.1111/epi.13944](https://doi.org/10.1111/epi.13944)] [CN-01452778]

* French JA, Lawson JA, Yapici Z, Ikeda H, Polster T, Nabbout R, et al. Adjunctive everolimus therapy for treatment-resistant focal-onset seizures associated with tuberous sclerosis (EXIST-3): a phase 3, randomised, double-blind, placebo-controlled study. *Lancet* 2016;**388**(10056):2153-63. [DOI: [10.1016/S0140-6736\(16\)31419-2](https://doi.org/10.1016/S0140-6736(16)31419-2)] [PMID: 27613521]

French JA, Lawson JA, Yapici Z, Ikeda H, Polster T, Nabbout R, et al. Outcomes in patients with tuberous sclerosis complex-associated treatment-refractory seizures, transitioning from placebo in core phase to everolimus during extension phase of the EXIST-3 trial. *Journal of the Neurological Sciences* 2017;**381**:87. [DOI: [10.1016/j.jns.2017.08.293](https://doi.org/10.1016/j.jns.2017.08.293)] [CN-01451315]

French JA, Lawson JA, Yapici Z, Polster T, Nabbout R, Curatolo P, et al. Adjunctive everolimus therapy for the treatment of refractory seizures associated with tuberous sclerosis complex: results from a randomized, placebo-controlled, phase 3 trial. *Annals of Neurology* 2016;**80**:S32. [DOI: [10.1002/ana.24759](https://doi.org/10.1002/ana.24759)] [CN-01743601]

Goyer I, Dahdah N, Major P. Use of mTOR inhibitor everolimus in three neonates for treatment of tumors associated with tuberous sclerosis complex. *Pediatric Neurology* 2015;**52**(4):450-3. [PMID: 25682485]

Mizuguchi M, Ikeda H, Kagitani-Shimono K, Yoshinaga H, Suzuki Y, Aoki M, et al. Everolimus for epilepsy and autism spectrum disorder in tuberous sclerosis complex: EXIST-3 substudy in Japan. *Brain & Development* 2019;**41**(1):1-10. [PMID: 30060984]

Mizuguchi M, Ikeda H, Kagitani-Shimono K, Yoshinaga H, Suzuki Y, Aoki M, et al. Efficacy and safety of everolimus in Japanese patients with refractory seizures associated with TSC. *No to hattatsu [Brain and development]* 2017;**49**:S283. [CN-01406813]

NCT01713946. A placebo-controlled study of efficacy & safety of 2 trough-ranges of everolimus as adjunctive therapy in patients with tuberous sclerosis complex (TSC) & refractory partial-onset seizures [A three-arm, randomized, double-blind, placebo-controlled study of the efficacy and safety of two trough-ranges of everolimus as adjunctive therapy in patients with tuberous sclerosis complex (TSC) & refractory partial-onset seizures]. clinicaltrials.gov/ct2/show/record/NCT01713946 (first posted 25 October 2012). [2011-000860-90 (EudraCT Number)] [CRAD001M2304]

Nabbout R, Franz DN, Lawson J, Curatolo P, Neary M, Fan J, et al. Impact of everolimus treatment on quality of life (QOL) in patients with tuberous sclerosis complex (TSC) who have refractory partial-onset seizures in the context of an

interventional clinical trial (EXIST-3). *Epilepsia* 2017;**58**:S102-3. [DOI: [10.1111/epi.13944](https://doi.org/10.1111/epi.13944)] [CN-01452769]

Polster T, Franz DN, Lawson JA, Yapici Z, Ikeda H, Nabbout R, et al. Long-term efficacy and safety of everolimus as adjunctive therapy in patients with tuberous sclerosis complex (TSC)-associated treatment-refractory seizures: final analysis of the exist-3 study. *Epilepsia* 2018;**59**(S3):S14. [ABSTRACT NO.: 24] [DOI: [10.1111/epi.14612](https://doi.org/10.1111/epi.14612)]

Roberts C, Kuperman R, Koenig MK, Wu JY, Kohrman M, Curatolo P, et al. Efficacy and safety of adjunctive everolimus for treatment resistant focal-onset seizures in tuberous sclerosis according to age: insights from EXIST-3. *American Academy of Neurology* 2017;**88**(16 Suppl):P3.146.

Wiemer-Kruel A, Nabbout R, Fan P-C, Ruiz Falco ML, Polster T, Curatolo P, et al. Outcomes among adult patients with tuberous sclerosis complex (TSC)-associated treatment-refractory seizures treated with adjunctive everolimus: final analysis of the exist-3 study. *Epilepsia* 2018;**59**(S3):S5. [ABSTRACT NO.: 005] [DOI: [10.1111/epi.14612](https://doi.org/10.1111/epi.14612)]

Yapici Z, Fan P-C, Lawson JA, Belousova E, Gazri HE, Ridolfi A, et al. Long-term safety, rollover study of adjunctive everolimus in patients with tuberous sclerosis complex (TSC)-associated treatment-refractory seizures who continue to benefit from everolimus treatment after completion of exist-3. *Epilepsia* 2018;**59**(S3):S296. [ABSTRACT NO.: p784] [DOI: [10.1111/epi.14612](https://doi.org/10.1111/epi.14612)] [0013-9580]

de Vries P, Franz D, Curatolo P, Nabbout R, Berkowitz N, Neary M, et al. Psychometric evaluation of quality of life measures in a three-arm, randomized, double-blind, placebo-controlled phase III study of everolimus as adjunctive therapy in patients with tuberous sclerosis complex (TSC) who have refractory partial-onset seizures (EXIST-3). *American Academy of Neurology* 2017;**88**(16 Suppl):P2.057.

de Vries PJ, Franz DN, Curatolo P, Nabbout R, Neary M, Herbst F, et al. Measuring health-related quality of life in tuberous sclerosis complex - psychometric evaluation of three instruments in individuals with refractory epilepsy. *Frontiers in Pharmacology* 2018;**9**:964. [PMID: 30214408]

Koenig 2012 {published data only}

* Koenig MK, Hebert AA, Roberson J, Samuels J, Slopis J, Woerner A, et al. Topical rapamycin therapy to alleviate the cutaneous manifestations of tuberous sclerosis complex: a double-blind, randomized, controlled trial to evaluate the safety and efficacy of topically applied rapamycin. *Drugs in R&D* 2012;**12**(3):121-6. [DOI: [10.2165/11634580-000000000-00000](https://doi.org/10.2165/11634580-000000000-00000)] [PMID: 22934754]

NCT01031901. Topical rapamycin therapy to alleviate cutaneous manifestations of tuberous sclerosis complex (TSC) and neurofibromatosis I (NF1). clinicaltrials.gov/ct2/show/NCT01031901 (first posted 15 December 2009).

Koenig 2018 {published data only}

* Koenig MK, Bell CS, Hebert AA, Roberson J, Samuels JA, Slopis JM, et al. Efficacy and safety of topical rapamycin in patients with facial angiofibromas secondary to tuberous

sclerosis complex: the TREATMENT randomized clinical trial. *JAMA Dermatology* 2018;**154**(7):773-80. [DOI: [10.1001/jamadermatol.2018.0464](https://doi.org/10.1001/jamadermatol.2018.0464)] [PMID: 29800048]

NCT01526356. Topical rapamycin to erase angiofibromas in TSC [Phase II study of topical rapamycin to erase angiofibromas in TSC-multicenter evaluation of a novel therapy]. clinicaltrials.gov/ct2/show/record/NCT01526356 (first posted 3 February 2012). [DEPARTMENT OF DEFENSE USAMRMC (OTHER GRANT/FUNDING NUMBER: W81XWH-11-1-0240) [HSC-MS-11-0501]

Krueger 2017 {published data only}

* Krueger DA, Sadhwani A, Byars AW, de Vries PJ, Franz DN, Whittemore VH, et al. Everolimus for treatment of tuberous sclerosis complex-associated neuropsychiatric disorders. *Annals of Clinical and Translational Neurology* 2017;**4**(12):877-87. [DOI: [10.1002/acn3.494](https://doi.org/10.1002/acn3.494)] [PMID: 29296616]

NCT01289912. Trial of RAD001 and neurocognition in tuberous sclerosis complex (TSC) [Randomized double-blind phase 2 trial of RAD001 for neurocognition in individuals with tuberous sclerosis complex]. clinicaltrials.gov/ct2/show/record/NCT01289912 (first posted 4 February 2011). [10-06-0247]

Overwater 2016 {published data only}

EUCTR2010-022655-29-NL. Randomised open label cross-over study into the efficacy of Rapamycin in children with Tuberous sclerosis complex with intractable Epilepsy (RATE-trial). trialsearch.who.int/Trial2.aspx?TrialID=EUCTR2010-022655-29-NL (date of registration 22 October 2010).

NTR3178. Study into treatment of children with difficult to treat epilepsy due to Tuberous sclerosis complex with rapamycin [Randomised open label cross-over study into the efficacy of rapamycin in children with tuberous sclerosis complex with intractable epilepsy]. trialsearch.who.int/Trial2.aspx?TrialID=NTR3178 (date of registration 2 December 2011). [MEC-2010-362]

* Overwater IE, Rietman AB, Bindels-de Heus K, Looman CW, Rizopoulos D, Sibindi TM, et al. Sirolimus for epilepsy in children with tuberous sclerosis complex: a randomized controlled trial. *Neurology* 2016;**87**(10):1011-8. [DOI: [10.1212/WNL.0000000000003077](https://doi.org/10.1212/WNL.0000000000003077)]

Overwater 2019 {published data only}

NCT01730209. Efficacy of RAD001/everolimus in autism and neuropsychological deficits in children with tuberous sclerosis complex. clinicaltrials.gov/ct2/show/NCT01730209 (first posted 21 November 2012). [NL38619.078.11]

* Overwater IE, Rietman AB, Mous SE, Bindels-de Heus K, Rizopoulos D, ten Hoopen LW, et al. A randomized controlled trial with everolimus for IQ and autism in tuberous sclerosis complex. *Neurology* 2019;**93**(2):e200-9. [DOI: [10.1212/WNL.0000000000007749](https://doi.org/10.1212/WNL.0000000000007749)] [PMID: 31217257]

Wataya-Kaneda 2017 {published data only}

JPRN-UMIN000012420. Randomized, double-blind, placebo-controlled, clinical trial with OSD-001 for skin lesions due to tuberous sclerosis complex. upload.umin.ac.jp/cgi-open-bin/

[ctr_e/ctr_view.cgi?recptno=R000014541](https://clinicaltrials.gov/ct2/show/record/NCT000014541) (date of disclosure 27 November 2013).

* Wataya-Kaneda M, Nakamura A, Tanaka M, Hayashi M, Matsumoto S, Yamamoto K, et al. Efficacy and safety of topical sirolimus therapy for facial angiofibromas in the tuberous sclerosis complex: a randomized clinical trial. *JAMA dermatology* 2017;**153**(1):39-48. [DOI: [10.1001/jamadermatol.2016.3545](https://doi.org/10.1001/jamadermatol.2016.3545)] [PMID: 27837201]

Wataya-Kaneda 2018 {published data only}

NCT02635789. Phase III trial of topical formulation of sirolimus to skin lesions in patients with tuberous sclerosis complex (TSC) [A double-blind, randomized, placebo-controlled phase III trial to investigate the efficacy and safety of NPC-12G gel (topical formulation of sirolimus) to angiofibroma and other skin lesions in patients with tuberous sclerosis complex (TSC)]. <https://clinicaltrials.gov/ct2/show/NCT02635789> (first posted 21 December 2015). [NPC-12G-1]

Wataya-Kaneda M, Ohno Y, Fujita Y, Yokozeki H, Niizeki H, Ogai M, et al. Sirolimus gel treatment for tuberous sclerosis complex involving facial angiofibromas and cephalic plaques: a multicenter randomized controlled trial. *Journal of Investigative Dermatology* 2018;**138**(5):S179. [DOI: [10.1016/j.jid.2018.03.1067](https://doi.org/10.1016/j.jid.2018.03.1067)]

* Wataya-Kaneda M, Ohno Y, Fujita Y, Yokozeki H, Niizeki H, Ogai M, et al. Sirolimus gel treatment vs placebo for facial angiofibromas in patients with tuberous sclerosis complex: a randomized clinical trial. *JAMA Dermatology* 2018;**154**(7):781-8. [DOI: [10.1001/jamadermatol.2018.1408](https://doi.org/10.1001/jamadermatol.2018.1408)] [PMID: 29800026]

References to studies excluded from this review

Birca 2010 {published data only}

Birca A, Mercier C, Major P. Rapamycin as an alternative to surgical treatment of subependymal giant cell astrocytomas in a patient with tuberous sclerosis complex. *Journal of Neurosurgery. Pediatrics* 2010;**6**(4):381-4. [PMID: 20887114]

Bissler 2019 {published data only}

Bissler JJ, Budde K, Sauter M, Franz DN, Zonnenberg BA, Frost MD, et al. Effect of everolimus on renal function in patients with tuberous sclerosis complex: evidence from EXIST-1 and EXIST-2 [Bissler JJ, Budde K, Sauter M, Franz DN, Zonnenberg BA, Frost MD, Belousova E, Berkowitz N, Ridolfi A, Christopher Kingswood J. Effect of everolimus on renal function in patients with tuberous sclerosis complex: evidence from EXIST-1 and EXIST-2. *Nephrol Dial Transplant*. 2019 Jun 1;**34**(6):1000-1008. doi: 10.1093/ndt/gfy132. PMID: 30053159;]. *Nephrology, Dialysis Transplantation* 1 June 2019;**34**(6):1000-8. [PUBMED CENTRAL: PMC6545468] [PMID: (PMID) 30053159]

Cabrera 2011 {published data only}

Cabrera Lopez C, Marti T, Catala V, Torres F, Mateu S, Ballarin Castan J, et al. Effects of rapamycin on angiomyolipomas in patients with tuberous sclerosis. *Nefrologia : publicacion oficial de la Sociedad Espanola Nefrologia* 2011;**31**(3):292-8. [PMID: 21629335]

ChiCTR-OPC-14005488 {published data only}

Li Han-zhong, Zhang Yu-shi, Li Shu-qiang. Efficacy of everolimus for TSC-associated renal angiomyolipoma. www.chictr.org.cn/showprojen.aspx?proj=9856 (first registered 7 November 2014).

ChiCTR-OPN-16008236 {published data only}

ChiCTR-OPN-16008236. TSC1/2 gene detection and everolimus treatment for renal angiomyolipoma associated with tuberous sclerosis complex. www.chictr.org.cn/showprojen.aspx?proj=13935 (first posted 7 April 2016).

Cuevas 2012 {published data only}

Cuevas Asencio I, Alborno Lapez R, Salido Vallejo R, Reyes Malia M. Topical sirolimus 0.4% formulation for treatment of facial angiofibromas [Formulación de sirolimus 0.4% para el tratamiento de angiofibromas faciales]. *Farmacia Hospitalaria* 2012;**36**(5):433-4. [DOI: [10.1016/j.farma.2011.09.006](https://doi.org/10.1016/j.farma.2011.09.006)] [PMID: 22858090]

Dabora 2011 {published data only}

Dabora SL, Franz DN, Ashwal S, Sagalowsky A, DiMario FJ Jr, Miles D, et al. Multicenter phase 2 trial of sirolimus for tuberous sclerosis: kidney angiomyolipomas and other tumors regress and VEGF-D levels decrease. *PLoS One* 2011;**6**(9):e23379. [PMID: 21915260]

Davies 2008 {published data only}

Davies DM, Johnson SR, Tattersfield AE, Kingswood JC, Cox JA, McCartney DL, et al. Sirolimus therapy in tuberous sclerosis or sporadic lymphangioleiomyomatosis. *New England Journal of Medicine* 2008;**358**(2):200-3. [DOI: [10.1056/NEJMc072500](https://doi.org/10.1056/NEJMc072500)] [PMID: 18184971]

Davies 2011 {published data only}

Davies DM, de Vries PJ, Johnson SR, McCartney DL, Cox JA, Serra AL, et al. Sirolimus therapy for angiomyolipoma in tuberous sclerosis and sporadic lymphangioleiomyomatosis: a phase 2 trial. *Clinical Cancer Research* 2011;**17**(12):4071-81. [PMID: 21525172]

DRKS00005584 {published data only}

DRKS00005584. EMINENTS (Everolimus Maintenance Therapy in SEGAs) [Maintenance therapy with everolimus in patients with tuberous sclerosis successfully treated for subependymal giant cell astrocytoma]. www.drks.de/drks_web/navigate.do?navigationId=trial.HTML&TRIAL_ID=DRKS00005584 (first registered 3 March 2015). [DRKS-ID: DRKS00005584] [EUDRACT-NO: EudraCT-No. (for studies acc. to Drug Law): 2015-000941-22] [UNIVERSAL TRIAL NUMBER (UTN): U1111-1152-1134]

EUCTR2007-005978-30-ES {published data only}

EUCTR2007-005978-30-ES. Clinical trial to determine the efficacy and safety of rapamycin in angiomyolipomas in patients with tuberous sclerosis [Ensayo clínico para evaluar la eficacia y seguridad de la rapamicina en los angiomiolipomas en pacientes con esclerosis tuberosa]. trialsearch.who.int/Trial2.aspx?TrialID=EUCTR2007-005978-30-ES (first registered 3 December 2007).

EUCTR2010-022583-13-DE {published data only}

EUCTR2010-022583-13-DE. The EFFECTS STUDY: Everolimus For Fast Expanded Access in TSC SEGAs [An open-label, multi-center, expanded access study of RAD001 in patients with subependymal giant cell astrocytomas (SEGAs) associated with tuberous sclerosis complex (TSC). The EFFECTS STUDY: everolimus For Fast Expanded Access in TSC SEGAs - EFFECTS STUDY]. trialsearch.who.int/Trial2.aspx?TrialID=EUCTR2010-022583-13-DE (first posted 23 December 2010). [ID NUMBER: CN-01814546]

Foster 2012 {published data only}

Foster RS, Bint LJ, Halbert AR. Topical 0.1% rapamycin for angiofibromas in paediatric patients with tuberous sclerosis: a pilot study of four patients. *Australasian Journal of Dermatology* 2012;**53**(1):52-6. [PMID: 22309333]

Franz 2006 {published data only}

Franz DN, Leonard J, Tudor C, Chuck G, Care M, Sethuraman G, et al. Rapamycin causes regression of astrocytomas in tuberous sclerosis complex. *Annals of Neurology* 2006;**59**(3):490-8. [PMID: 16453317]

Franz 2018 {published data only}

Franz DN, Lawson JA, Yapici Z, Ikeda H, Polster T, Nabbout R, et al. Everolimus for treatment-refractory seizures in TSC: Extension of a randomized controlled trial. *Neurology. Clinical Practice* 2018;**8**(5):412-20. [DOI: [10.1212/CPJ.0000000000000514](https://doi.org/10.1212/CPJ.0000000000000514)] [PMID: 30564495]

Gupta 2019 {published data only}

Gupta N, Lee HS, Young LR, Strange C, Moss J, Singer LG, et al. Analysis of the MILES cohort reveals determinants of disease progression and treatment response in lymphangioleiomyomatosis. *European Respiratory Journal* 2019;**53**(4):1802066. [PMID: 30846465]

Herry 2007 {published data only}

Herry I, Neukirch C, Debray MP, Mignon F, Crestani B. Dramatic effect of sirolimus on renal angiomyolipomas in a patient with tuberous sclerosis complex. *European Journal of Internal Medicine* 2007;**18**(1):76-7. [PMID: 17223050]

Hofbauer 2008 {published data only}

Hofbauer GF, Marcollo-Pini A, Corsenca A, Kistler AD, French LE, Wuthrich RP, et al. The mTOR inhibitor rapamycin significantly improves facial angiofibroma lesions in a patient with tuberous sclerosis. *British Journal of Dermatology* 2008;**159**(2):473-5. [PMID: 18547304]

JPRN-UMIN000002844 {published data only}

JPRN-UMIN000002844. Development of topical treatment with rapamycin for skin lesions of tuberous sclerosis complex. upload.umin.ac.jp/cgi-open-bin/ctr_e/ctr_view.cgi?recptno=R000003457 (first registered 3 December 2009).

JPRN-UMIN000006108 {published data only}

JPRN-UMIN000006108. Clinical trial for development of topical rapamycin treatment for white macules due to tuberous sclerosis complex, vitiligo vulgaris, and other congenital diseases. upload.umin.ac.jp/cgi-open-bin/ctr_e/ctr_view.cgi?

recptno=R000007206 (first registered 3 August 2011). [ID NO.: CN-01836103]

JPRN-UMIN000011559 {published data only}

JPRN-UMIN000011559. Therapeutic drug monitoring of Everolimus in patients with renal cell carcinoma and tuberous sclerosis. upload.umin.ac.jp/cgi-open-bin/ctr_e/ctr_view.cgi?recptno=R000013524 (first registered 01 September 2013).

JPRN-UMIN000015114 {published data only}

JPRN-UMIN000015114. Clinical study of safety and efficacy of rapamycin topical medication for skin lesions due to tuberous sclerosis complex. upload.umin.ac.jp/cgi-open-bin/ctr_e/ctr_view.cgi?recptno=R000017546 (first registered 10 September 2014).

JPRN-UMIN000024270 {published data only}

JPRN-UMIN000024270. Study of everolimus for autistic spectrum disorder in tuberous sclerosis. upload.umin.ac.jp/cgi-open-bin/ctr_e/ctr_view.cgi?recptno=R000027932 (first registered 03 October 2016).

Kenerson 2002 {published data only}

Kenerson HL, Aicher LD, True LD, Yeung RS. Activated mammalian target of rapamycin pathway in the pathogenesis of tuberous sclerosis complex renal tumors. *Cancer Research* 2002;**62**(20):5645-50. [PMID: 12384518] [WEB ADDRESS: <https://cancerres.aacrjournals.org/content/62/20/5645.long>]

Koenig 2008 {published data only}

Koenig MK, Butler IJ, Northrup H. Regression of subependymal giant cell astrocytoma with rapamycin in tuberous sclerosis complex. *Journal of Child Neurology* 2008;**23**(10):1238-9. [PMID: 18952591]

Krueger 2010 {published data only}

Krueger DA, Care MM, Holland K, Agricola K, Tudor C, Mangeshkar P, et al. Everolimus for subependymal giant-cell astrocytomas in tuberous sclerosis. *New England Journal of Medicine* 2010;**363**(19):1801-11. [DOI: [10.1056/NEJMoa1001671](https://doi.org/10.1056/NEJMoa1001671)] [PMID: 21047224]

Krueger 2013a {published data only}

Ess KC, Roach ES. New therapies for tuber-less sclerosis: White matter matters? *American Academy of Neurology* 2012;**78**(8):520-1. [DOI: [10.1212/WNL.0b013e318248a232](https://doi.org/10.1212/WNL.0b013e318248a232)]

* Krueger DA, Care MM, Agricola K, Tudor C, Mays M, Franz DN. Everolimus long-term safety and efficacy in subependymal giant cell astrocytoma. *Neurology* 2013;**80**(6):574-80. [DOI: [10.1212/WNL.0b013e3182815428](https://doi.org/10.1212/WNL.0b013e3182815428)] [PMID: 23325902]

NCT00411619. Everolimus (RAD001) therapy of giant cell astrocytomas in patients with tuberous sclerosis complex. www.clinicaltrials.gov/ct2/show/NCT00411619 (first registered 14 December 2006). [CCHMC IRB# 06-07-50]

Tillema JM, Leach JL, Krueger DA, Franz DN. Everolimus alters white matter diffusion in tuberous sclerosis complex. *Neurology* 2012;**78**(8):526-31. [PMID: 22262746]

Krueger 2013b {published data only}

* Krueger DA, Wilfong AA, Holland-Bouley K, Anderson AE, Agricola K, Tudor C, et al. Everolimus treatment of refractory epilepsy in tuberous sclerosis complex. *Annals of Neurology* 2013;**74**(5):679-87. [DOI: [10.1002/ana.23960](https://doi.org/10.1002/ana.23960)] [PMID: 23798472]

NCT01070316. Everolimus (RAD001) therapy for epilepsy in patients with tuberous sclerosis complex (TSC). clinicaltrials.gov/ct2/show/record/NCT01070316 (first posted 18 February 2010);NA:NA. [2009-0998]

Lam 2010 {published data only}

Lam C, Bouffet E, Tabori U, Mabbott D, Taylor M, Bartels U. Rapamycin (sirolimus) in tuberous sclerosis associated pediatric central nervous system tumors. *Pediatric Blood & Cancer* 2010;**54**(3):476-9. [DOI: [10.1002/pbc.22298](https://doi.org/10.1002/pbc.22298)] [PMID: 19856393]

NCT00001465 {published data only}

NCT00001465. Study of the disease process of lymphangioleiomyomatosis [Characterization of the Pathogenesis of Lymphangioleiomyomatosis (LAM)]. clinicaltrials.gov/ct2/show/NCT00001465 (first posted 03 November 1999).

NCT00001532 {published data only}

NCT00001532. Role of genetic factors in the development of lung disease. clinicaltrials.gov/ct2/show/NCT00001532 (first posted 04 November 1999).

NCT00001975 {published data only}

NCT00001975. Study of skin tumors in tuberous sclerosis [Cutaneous tumorigenesis in patients with tuberous sclerosis]. clinicaltrials.gov/ct2/show/NCT00001975 (first posted 21 January 2000).

NCT00457808 {published data only}

NCT00457808. Rapamycin therapy for patients with tuberous sclerosis complex and sporadic LAM [Rapamycin therapy of angiomyolipomas in patients with tuberous sclerosis complex and sporadic lymphangioleiomyomatosis]. clinicaltrials.gov/ct2/show/NCT00457808 (first posted 09 April 2007). [CCHMC IRB # 02-10-16] [R21CA103486 (U.S. NIH Grant/Contract)]

NCT00457964 {published data only}

NCT00457964. RAD001 therapy of angiomyolipomata in patients with TS complex and sporadic LAM [RAD001 therapy of angiomyolipomata in patients with tuberous sclerosis complex and sporadic lymphangioleiomyomatosis]. clinicaltrials.gov/ct2/show/NCT00457964 (first posted 09 April 2007). [CCHMC IRB #2008-0812(04-07-22)]

NCT00490789 {published data only}

NCT00490789. Trial of efficacy and safety of sirolimus in tuberous sclerosis and LAM [A trial of the efficacy and safety of sirolimus (rapamycin) therapy for renal angiomyolipomas in patients with tuberous sclerosis complex and sporadic lymphangioleiomyomatosis]. clinicaltrials.gov/ct2/show/NCT00490789 (first posted 25 June 2007). [TESSTAL]

NCT00552955 {published data only}

NCT00552955. Effect of fasting on the size of abdominal lymphatic tumors in women [Effect of fasting on the size of lymphangioleiomyomas in patients with lymphangioleiomyomatosis]. clinicaltrials.gov/ct2/show/NCT00552955 (first posted 02 November 2007). [08-H-0016] [080016]

NCT00792766 {published data only}

NCT00792766. Long term follow up for RAD001 therapy of angiomyolipomata in patients with tuberous sclerosis (TSC) and sporadic lymphangioleiomyomatosis (LAM) [Long term follow up for RAD001 therapy of angiomyolipomata in patients with tuberous sclerosis (TSC) and sporadic lymphangioleiomyomatosis]. clinicaltrials.gov/ct2/show/NCT00792766 (first posted 18 November 2008). [CCHMC IRB# 2008-0333]

NCT01092208 {published data only}

NCT01092208. Studies of autistic patients: gene networks and clinical subtypes. clinicaltrials.gov/ct2/show/NCT01092208 (first posted 24 March 2010). [10-CH-0084] [100084]

NCT01217125 {published data only}

NCT01217125. Rapamycin in angiomyolipomas in patients with tuberous sclerosis [Clinical trial to determine the efficacy and safety of rapamycin in angiomyolipomas in patients with tuberous sclerosis]. clinicaltrials.gov/ct2/show/NCT01217125 (first posted 08 October 2010). [EudraCT 2007-005978-30]

NCT01266291 {published data only}

NCT01266291. Sabril for complex partial seizures in adult tolerability study (TS) patients [Sabril for treatment of adult refractory partial seizures symptomatic of tuberous sclerosis: an open label, phase IV prospective safety and tolerability study]. clinicaltrials.gov/ct2/show/NCT01266291 (first posted 24 December 2010). [811542]

NCT01767779 {published data only}

NCT01767779. Potential EEG biomarkers and antiepileptogenic strategies for epilepsy in TSC [Potential EEG biomarkers and antiepileptogenic strategies for epilepsy in TSC]. clinicaltrials.gov/ct2/show/NCT01767779 (first posted 14 January 2013). [1P20NS080199-01 (U.S. NIH Grant/Contract)]

NCT01780441 {published data only}

NCT01780441. Early biomarkers of autism in infants with tuberous sclerosis complex (TSC) [Longitudinal study to identify early biomarkers of autism spectrum disorder (ASD) in infants with tuberous sclerosis complex (TSC)]. clinicaltrials.gov/ct2/show/NCT01780441 (first posted 31 January 2013). [1U01NS082320-01 (U.S. NIH Grant/Contract)] [IRB-P00005074]

NCT01929642 {published data only}

NCT01929642. Rapalogues for autism phenotype in TSC: a feasibility study. clinicaltrials.gov/ct2/show/NCT01929642 (first posted 28 August 2013). [AM00037881]

NCT02104011 {published data only}

NCT02104011. Treatment of renal angiomyolipomas in tuberous sclerosis by beta-blockers [Treatment of renal

angiomyolipomas in tuberous sclerosis by beta-blockers: pilot trial]. clinicaltrials.gov/ct2/show/NCT02104011 (first posted 04 April 2014). [CHUBX 2011/35]

NCT02201212 {published data only}

NCT02201212. Everolimus for cancer with TSC1 or TSC2 mutation [A phase II trial of everolimus for cancer patients with inactivating mutations in TSC1 or TSC2 or activating MTOR mutations]. clinicaltrials.gov/ct2/show/NCT02201212 (first posted 28 July 2014). [14-229] [CRAD001MUS217T]

NCT02325505 {published data only}

NCT02325505. Characterization of patients with tuberous sclerosis complex, lymphangioleiomyomatosis and angiomyolipoma [Clinical Profile Characterization of Patients With Tuberous Sclerosis Complex, Lymphangioleiomyomatosis and Angiomyolipoma Followed at Hospital Das Clínicas, University of Sao Paulo Medical School]. clinicaltrials.gov/ct2/show/NCT02325505 (first posted 25 December 2014). [4147/14/127]

NCT02451696 {published data only}

NCT02451696. A pilot study to evaluate the effects of everolimus on brain mTOR activity and cortical hyperexcitability in TSC and FCD. clinicaltrials.gov/ct2/show/NCT02451696 (first posted 22 May 2015). [14-00245]

NCT02461459 {published data only}

NCT02461459. Autism spectrum disorder (ASD) and intellectual disability (ID) determinants in tuberous sclerosis complex (TSC). clinicaltrials.gov/ct2/show/NCT02461459 (first posted 03 June 2015). [1U54NS092090 (U.S. NIH Grant/Contract)] [IRB-P00013585]

NCT02634931 {published data only}

NCT02634931. Long-term trial of topical sirolimus to angiofibroma in patient with tuberous sclerosis complex [A long-term, single-arm, open-label trial of NPC-12G (topical formulation of sirolimus) to angiofibroma and other skin lesions in patients with tuberous sclerosis complex]. clinicaltrials.gov/ct2/show/NCT02634931 (first posted 18 December 2015). [NPC-12G-2]

NCT02654340 {published data only}

NCT02654340. Biomarkers for tuberous sclerosis complex (BioTuScCom) [Biomarkers for tuberous sclerosis complex: an international multicenter observational longitudinal protocol]. clinicaltrials.gov/ct2/show/NCT02654340 (first posted 13 January 2016). [TSC 08-2018]

NCT03047213 {published data only}

NCT03047213. Sapanisertib in treating patients with locally advanced or metastatic bladder cancer with TSC1 and/or TSC2 mutations [An open label, multicenter, single arm phase II study to evaluate the activity and tolerability of the novel mTOR inhibitor, MLN0128 (TAK-228), in patients with locally advanced or metastatic transitional cell carcinoma of the urothelial tract whose tumors harbor a TSC1 and/or a TSC2 mutation]. clinicaltrials.gov/ct2/show/NCT03047213 (first posted 08 February 2017). [1505015958] [2000021268] [9767 (Other Identifier: CTEP)] [9767 (Other Identifier: Yale University

Cancer Center LAO)) [NCI-2015-00121 (Registry Identifier: CTRP (Clinical Trial Reporting Program))) [UM1CA186689 (U.S. NIH Grant/Contract)]

NCT03213678 {published data only}

NCT03213678. PI3K/mTOR inhibitor LY3023414 in treating patients with relapsed or refractory advanced solid tumors, non-hodgkin lymphoma, or histiocytic disorders with TSC or PI3K/MTOR mutations (a pediatric MATCH treatment trial) [NCI-COG pediatric MATCH (Molecular Analysis For Therapy Choice)- phase 2 subprotocol of LY3023414 in patients with solid tumors]. clinicaltrials.gov/ct2/show/NCT03213678 (first posted 11 July 11 2017). [APEC1621D (Other Identifier: CTEP)] [APEC1621D (Other Identifier: Children's Oncology Group)] [NCI-2017-01249 (Registry Identifier: CTRP (Clinical Trial Reporting Program))) [U10CA180886 (U.S. NIH Grant/Contract)]

NCT03525834 {published data only}

NCT03525834. Safety and efficacy of everolimus (Afinitor®) in Chinese adult patients with angiomyolipoma associated with tuberous sclerosis complex [Phase IV, single arm study of safety and efficacy of everolimus in Chinese adults with tuberous sclerosis complex who have renal angiomyolipoma not requiring immediate surgery]. clinicaltrials.gov/ct2/show/NCT03525834 (first posted 16 May 2018). [CRAD001M2401]

NCT03649919 {published data only}

NCT03649919. Multi-center clinical study on the diagnosis and treatment management of rare neurological disease in children [Multi-center clinical study on the diagnosis and treatment management of rare neurological disease in children]. clinicaltrials.gov/ct2/show/NCT03649919 (first posted 28 August 2018). [EKYY-MCSDTMRNDC]

Pressey 2010 {published data only}

Pressey JG, Wright JM, Geller JI, Joseph DB, Pressey CS, Kelly DR. Sirolimus therapy for fibromatosis and multifocal renal cell carcinoma in a child with tuberous sclerosis complex. *Pediatric Blood & Cancer* 2010;**54**(7):1035-7. [DOI: [10.1002/pbc.22401](https://doi.org/10.1002/pbc.22401)] [PMID: 20108343]

Salido 2012 {published data only}

Salido R, Garnacho-Saucedo G, Cuevas-Asencio I, Ruano J, Galan-Gutierrez M, Velez A, et al. Sustained clinical effectiveness and favorable safety profile of topical sirolimus for tuberous sclerosis - associated facial angiofibroma. *Journal of the European Academy of Dermatology and Venereology* 2012;**26**(10):1315-8. [PMID: 21834948]

Sallas 2015 {published data only}

Sallas W, Chiparus O, Cheung W, Urva S, Wang J, Cauwel H, et al. Population PK-PD modeling of everolimus in the treatment of patients with tuberous sclerosis complex (TSC) who have subependymal giant cell astrocytomas (SEGA). *Journal of Pharmacokinetics and Pharmacodynamics* 2013;**40**(1):S40. [ABSTRACT NO.: M-035] [DOI: [10.1007/s10928-013-9308-2](https://doi.org/10.1007/s10928-013-9308-2)]

Sparagana 2010 {published data only}

Sparagana SP, Wilkes DC, Thompson CE, Bowers DC. Optic nerve tumor in tuberous sclerosis complex is not responsive

to sirolimus. *Pediatric Neurology* 2010;**42**(6):443-6. [PMID: 20472200]

Staehler 2012 {published data only}

Staehler M, Sauter M, Helck A, Linsenmaier U, Weber L, Mayer K, et al. Nephron-sparing resection of angiomyolipoma after sirolimus pretreatment in patients with tuberous sclerosis. *International Urology and Nephrology* 2012;**44**(6):1657-61. [PMID: 23054313]

Tanaka 2013 {published data only}

Tanaka M, Wataya-Kaneda M, Nakamura A, Matsumoto S, Katayama I. First left-right comparative study of topical rapamycin vs. vehicle for facial angiofibromas in patients with tuberous sclerosis complex. *British Journal of Dermatology* 2013;**169**(6):1314-8. [PMID: 23909960]

Wataya-Kaneda 2011 {published data only}

Wataya-Kaneda M, Tanaka M, Nakamura A, Matsumoto S, Katayama I. A topical combination of rapamycin and tacrolimus for the treatment of angiofibroma due to tuberous sclerosis complex (TSC): a pilot study of nine Japanese patients with TSC of different disease severity. *British Journal of Dermatology* 2011;**165**(4):912-6. [PMID: 21692771]

Wataya-Kaneda 2012 {published data only}

Wataya-Kaneda M, Tanaka M, Nakamura A, Matsumoto S, Katayama I. A novel application of topical rapamycin formulation, an inhibitor of mTOR, for patients with hypomelanotic macules in tuberous sclerosis complex. *Archives of Dermatology* 2012;**148**(1):138-9. [DOI: [10.1001/archderm.148.1.138](https://doi.org/10.1001/archderm.148.1.138)] [PMID: 22250258]

Wataya-Kaneda 2015 {published data only}

Wataya-Kaneda M, Tanaka M, Yang L, Yang F, Tsuruta D, Nakamura A, et al. Clinical and histologic analysis of the efficacy of topical rapamycin therapy against hypomelanotic macules in tuberous sclerosis complex. *JAMA Dermatology* 2015;**151**(7):722-30. [DOI: [10.1001/jamadermatol.2014.4298](https://doi.org/10.1001/jamadermatol.2014.4298)] [PMID: 25692384]

Wheless 2013 {published data only}

Wheless JW, Almoazen H. A novel topical rapamycin cream for the treatment of facial angiofibromas in tuberous sclerosis complex. *Journal of Child Neurology* 2013;**28**(7):933-6. [DOI: [10.1177/0883073813488664](https://doi.org/10.1177/0883073813488664)] [PMID: 23680945]

Wienecke 2006 {published data only}

Wienecke R, Fackler I, Linsenmaier U, Mayer K, Licht T, Kretzler M. Antitumoral activity of rapamycin in renal angiomyolipoma associated with tuberous sclerosis complex. *American Journal of Kidney Diseases* 2006;**48**(3):e27-9. [PMID: 16931204]

Yalon 2011 {published data only}

Yalon M, Ben-Sira L, Constantini S, Toren A. Regression of subependymal giant cell astrocytomas with RAD001 (Everolimus) in tuberous sclerosis complex. *Child's Nervous System* 2011;**27**(1):179-81. [PMID: 20703486]

Young 2013 {published data only}

Kwiatkowski D. A biomarker for lymphangioleiomyomatosis. *Lancet Respiratory Medicine* 2013;**1**(6):424-5. [DOI: [10.1016/S2213-2600\(13\)70098-5](https://doi.org/10.1016/S2213-2600(13)70098-5)]

Wu H, Nikolaidis NM, Young LR, McCormack FX. Serum levels of vegf-c are elevated in sporadic but not tuberous sclerosis associated-lam, correlate with the need for supplemental oxygen, and impact lung function response to sirolimus. *American Journal of Respiratory and Critical Care Medicine* 2018;**197**:A1100. [DOI: [10.1164/ajrccm-conference.2018.197.1_MeetingAbstracts.A1100](https://doi.org/10.1164/ajrccm-conference.2018.197.1_MeetingAbstracts.A1100)]

* Young L, Lee HS, Inoue Y, Moss J, Singer LG, Strange C, et al. Serum VEGF-D a concentration as a biomarker of lymphangioleiomyomatosis severity and treatment response: a prospective analysis of the Multicenter International Lymphangioleiomyomatosis Efficacy of Sirolimus (MILES) trial. *Lancet. Respiratory Medicine* 2013;**1**(6):445-52. [PMID: 24159565]

Young LR, Inoue Y, Moss J, Singer L, Strange C, Nakata K, et al. Baseline serum VEGF-D levels are associated with disease severity and treatment response in lymphangioleiomyomatosis. *American Journal of Respiratory and Critical Care Medicine* 2011;**183**:A6351. [DOI: [10.1164/ajrccm-conference.2011.183.1_MeetingAbstracts.A6351](https://doi.org/10.1164/ajrccm-conference.2011.183.1_MeetingAbstracts.A6351)]

Zhang 2018 {published data only}

Fonseca dos Santos-Junior E, Ricardo Mendes de Oliveira J. The importance of the study of brain calcifications in patients with tuberous sclerosis complex. *Seizure - European Journal of Epilepsy* 2018;**61**:199. [DOI: [10.1016/j.seizure.2018.08.023](https://doi.org/10.1016/j.seizure.2018.08.023)]

* Zhang MN, Zou LP, Wang YY, Pang LY, Ma SF, Huang LL, et al. Calcification in cerebral parenchyma affects pharmacoresistant epilepsy in tuberous sclerosis. *Seizure* 2018;**60**:86-90. [PMID: 29929111]

References to studies awaiting assessment

NCT03140449 {published data only}

NCT03140449. Topical Rapamycin and calcitriol for angiofibroma of tuberous sclerosis [Study of combination therapy with topical rapamycin and calcitriol for cutaneous lesions of tuberous sclerosis: a double-blind randomized controlled trial]. *clinicaltrials.gov/ct2/show/NCT03140449* (first posted 04 May 2017). [201306009MINB]

Randell 2016 {published data only}

NCT01954693. A study of everolimus in the treatment of neurocognitive problems in tuberous sclerosis [TRON: a randomised, double blind, placebo-controlled study of RAD001 (everolimus) in the treatment of neurocognitive problems in tuberous sclerosis]. *clinicaltrials.gov/ct2/show/NCT01954693* (first posted 07 October 2013). [DOI: [10.1186/ISRCTN09739757](https://doi.org/10.1186/ISRCTN09739757)] [EUDRACT NUMBER: 2011-004854-25] [SPON803-10]

* Randell E, McNamara R, Davies DM, Owen-Jones E, Kirby N, Angel L, et al. The use of everolimus in the treatment of neurocognitive problems in tuberous sclerosis (TRON): study protocol for a randomised controlled trial. *Trials* 2016;**17**:398. [PMID: 27515417]

References to ongoing studies

EUCTR2011-006308-12-ES {published data only}

EUCTR2011-006308-12-ES. Clinical trial in patients with tuberous sclerosis for the study of the effects of topical rapamycin in reducing facial tumors associated with the disease [Phase II clinical trial, to study the efficacy and safety of topical rapamycin in reducing facial angiofibromas.]. *trialsearch.who.int/Trial2.aspx?TrialID=EUCTR2011-006308-12-ES* (date of registration 29 March 2012).

NCT02860494 {published data only}

NCT02860494. Topical everolimus in patients with tuberous sclerosis complex [Topical everolimus versus placebo for the treatment of facial angiofibromas in patients with tuberous sclerosis complex. A phase II/III, multicentre, randomized, double-blind, placebo-controlled study of 3 doses of topical everolimus]. *clinicaltrials.gov/ct2/show/NCT02860494* (first posted 09 August 2016). [EUDRACT NUMBER: 2018-002531-18] [69HCL16_0062]

NCT03363763 {published data only}

NCT03363763. Topical sirolimus ointment for cutaneous angiofibromas in subjects with tuberous sclerosis complex [Phase 2 multi center prospective rand. double blind placebo cont. parallel design study to evaluate safety & efficacy of topical sirolimus for cutaneous angiofibromas in subjects w/ tuberous sclerosis complex followed by opt. open label]. *clinicaltrials.gov/ct2/show/NCT03363763* (first posted 06 December 2017). [AUCTA-UAP006-PH2]

NCT03826628 {published data only}

NCT03826628. Dose-ranging efficacy and safety study of topical rapamycin cream for facial angiofibroma associated with tuberous sclerosis complex [A phase 2/3, multi-center, double-blind, placebo-controlled, randomized, parallel-group, dose-response comparison of the efficacy and safety study of topical rapamycin cream for the treatment of facial angiofibromas (FA) associated with tuberous sclerosis complex (TSC) in patients 6 years of age and over]. *clinicaltrials.gov/ct2/show/NCT03826628* (first posted 01 February 2019);NA:NA. [DOI: <https://clinicaltrials.gov/ct2/show/NCT03826628?id=NCT03826628&draw=2&rank=1>] [DSLIP-01]

Additional references

Annear 2019

Annear NM, Appleton RE, Bassi Z, Bhatt R, Bolton PF, Crawford P, et al. Tuberous sclerosis complex (TSC): expert recommendations for provision of coordinated care. *Frontiers in Neurology* 2019;**10**:1116. [DOI: [10.3389/fneur.2019.01116](https://doi.org/10.3389/fneur.2019.01116)] [PMID: 31781016]

Ballou 2008

Ballou LM, Lin RZ. Rapamycin and mTOR kinase inhibitors. *Journal of Chemical Biology* 2008;**1**(1-4):27-36. [PMID: 19568796]

Bhaskar 2007

Bhaskar PT, Hay N. The two TORCs and Akt. *Developmental Cell* 2007;**12**(4):487-502. [PMID: 17419990]

Bissler 2008

Bissler JJ, McCormack FX, Young LR, Elwing JM, Chuck G, Leonard JM, et al. Sirolimus for angiomyolipoma in tuberous sclerosis complex or lymphangioleiomyomatosis. *New England Journal of Medicine* 2008;**358**(2):140-51.

Brachmann 2009

Brachmann S, Fritsch C, Maira SM, Garcia-Echeverria C. PI3K and mTOR inhibitors: a new generation of targeted anticancer agents. *Current Opinion in Cell Biology* 2009;**21**(2):194-8. [PMID: 19201591]

Canpolat 2014

Canpolat M, Per H, Gumus H, Yikilmaz A, Unal E, Patiroglu T, et al. Rapamycin has a beneficial effect on controlling epilepsy in children with tuberous sclerosis complex: results of 7 children from a cohort of 86. *Child's Nervous System* 2014;**30**(2):227-40. [PMID: 23743820]

Cardamone 2014

Cardamone M, Flanagan D, Mowat D, Kennedy SE, Chopra M, Lawson JA. Mammalian target of rapamycin inhibitors for intractable epilepsy and subependymal giant cell astrocytomas in tuberous sclerosis complex. *Journal of Pediatrics* 2014;**164**(5):1195-200. [PMID: 24518170]

Cheadle 2000

Cheadle JP, Reeve MP, Sampson JR, Kwiatkowski DJ. Molecular genetic advances in tuberous sclerosis. *Human Genetics* 2000;**107**(2):97-114. [PMID: 11030407]

Chu-Shore 2010

Chu-Shore CJ, Major P, Camposano S, Muzykewicz D, Thiele EA. The natural history of epilepsy in tuberous sclerosis complex. *Epilepsia* 2010;**51**:1236-4. [DOI: [10.1111/j.1528-1167.2009.02474.x](https://doi.org/10.1111/j.1528-1167.2009.02474.x)] [PMID: 20041940]

CTCAE v3.0 [Computer program]

Common terminology criteria for adverse events (CTCAE). National Cancer Institute, Version 3. Bethesda, MD: Cancer Therapy Evaluation Program, 2006. Available at ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcae3.pdf.

Curatolo 2015a

Curatolo P, Moavero R, de Vries PJ. Neurological and neuropsychiatric aspects of tuberous sclerosis complex. *Lancet Neurology* 2015;**14**:733-45. [DOI: [10.1016/S1474-4422\(15\)00069-1](https://doi.org/10.1016/S1474-4422(15)00069-1)] [PMID: 26067126]

Curatolo 2015b

Curatolo P. Mechanistic target of rapamycin (mTOR) in tuberous sclerosis complex-associated epilepsy. *Pediatric Neurology* 2015;**52**:281-9. [DOI: [10.1016/j.pediatrneurol.2014.10.028](https://doi.org/10.1016/j.pediatrneurol.2014.10.028)] [PMID: 25591831]

Dabora 2001

Dabora SL, Jozwiak S, Franz DN, Roberts PS, Nieto A, Chung J, et al. Mutational analysis in a cohort of 224 tuberous sclerosis patients indicates increased severity of TSC2, compared with

TSC1, disease in multiple organs. *American Journal of Human Genetics* 2001;**68**(1):64-80. [PMID: 11112665]

Donner 2002

Donner A, Klar N. Issues in the meta-analysis of cluster randomised trials. *Statistics in Medicine* 2002;**21**(19):2971-80.

ECTSC 1993

European Chromosome 16 Tuberous Sclerosis Consortium. Identification and characterization of the tuberous sclerosis gene on chromosome 16. *Cell* 1993;**75**(7):1305-15. [PMID: 8269512]

Eden 2014

Eden KE, de Vries PJ, Moss J, Richards C, Oliver C. Self-injury and aggression in tuberous sclerosis complex: cross syndrome comparison and associated risk markers. *Journal of Neurodevelopmental Disorders* 2014;**6**(1):10. [PMID: 24822087]

Ehninger 2011

Ehninger D, Silva AJ. Rapamycin for treating tuberous sclerosis and autism spectrum disorders. *Trends in Molecular Medicine* 2011;**17**(2):78-87. [PMID: 21115397]

Elbourne 2002

Elbourne DR, Altman DG, Higgins PT, Curtin F, Worthington HV, Vail A. Meta-analyses involving cross-over trials: methodological issues. *International Journal of Epidemiology* 2002;**30**(1):140-9.

Faivre 2006

Faivre S, Kroemer G, Raymond E. Current development of mTOR inhibitors as anticancer agents. *Nature Reviews. Drug Discovery* 2006;**5**(8):671-88. [PMID: 16883305]

Falotico 2005

Falotico R. Are all sirolimus analogs alike? In: Angioplasty Summit; TCT Asia Pacific; 2005 Apr 28; Seoul, Korea. 2005.

Feliciano 2013

Feliciano DM, Lin TV, Hartman NW, Bartley CM, Kubera C, Hsieh L, et al. A circuitry and biochemical basis for tuberous sclerosis symptoms: from epilepsy to neurocognitive deficits. *International Journal of Developmental Neuroscience* 2013;**31**(7):667-78. [DOI: [10.1016/j.ijdevneu.2013.02.008](https://doi.org/10.1016/j.ijdevneu.2013.02.008)] [PMID: 23485365]

Gao 2001

Gao X, Pan D. TSC1 and TSC2 tumor suppressors antagonize insulin signaling in cell growth. *Genes & Development* 2001;**15**(11):1383-92. [PMID: 11390358]

Goncharova 2002

Goncharova EA, Goncharov DA, Eszterhas A, Hunter DS, Glassberg MK, Yeung RS, et al. Tuberin regulates p70 S6 kinase activation and ribosomal protein S6 phosphorylation. A role for the TSC2 tumor suppressor gene in pulmonary lymphangioleiomyomatosis (LAM). *Journal of Biological Chemistry* 2002;**277**(34):30958-67. [PMID: 12045200]

Haemel 2010

Haemel AK, O'Brian AL, Teng JM. Topical rapamycin: a novel approach to facial angiofibromas in tuberous sclerosis. *Archives of Dermatology* 2010;**146**(7):715-8. [PMID: 20644030]

Higgins 2003

Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003;**327**(7417):557-60.

Higgins 2017

Higgins JP, Altman DG, Sterne JA, editor(s). Chapter 8: Assessing risk of bias in included studies. In: Higgins JP, Churchill R, Chandler J, Cumpston MS, editor(s), *Cochrane Handbook for Systematic Reviews of Interventions* Version 5.2.0 (updated June 2017), Cochrane, 2017. Available from training.cochrane.org/handbook/archive/v5.2.

Higgins 2022

Higgins JP, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA, editor(s). *Cochrane Handbook for Systematic Reviews of Interventions* Version 6.3 (updated February 2022). Cochrane, 2022. Available from training.cochrane.org/handbook.

Hinton 2014

Hinton RB, Prakash A, Romp RL, Krueger DA, Knilans TK. Cardiovascular manifestations of tuberous sclerosis complex and summary of the revised diagnostic criteria and surveillance and management recommendations from the International Tuberous Sclerosis Consensus Group. *Journal of the American Heart Association* 2014;**3**(6):e001493. [PMID: 25424575]

Hoogeveen-Westerveld 2012

Hoogeveen-Westerveld M, Ekong R, Povey S, Karbassi I, Batish SD, den Dunnen JT, et al. Functional assessment of TSC1 missense variants identified in individuals with tuberous sclerosis complex. *Human Mutation* 2012;**33**(3):476-9. [PMID: 22161988]

Hoogeveen-Westerveld 2013

Hoogeveen-Westerveld M, Ekong R, Povey S, Mayer K, Lannoy N, Elmslie F, et al. Functional assessment of TSC2 variants identified in individuals with tuberous sclerosis complex. *Human Mutation* 2013;**34**(1):167-75. [PMID: 22903760]

Hyman 2000

Hyman MH, Whittemore VH. National Institutes of Health consensus conference: tuberous sclerosis complex. *Archives of Neurology* 2000;**57**(5):662-5. [PMID: 10815131]

Inoki 2002

Inoki K, Li Y, Zhu T, Wu J, Guan KL. TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. *Nature Cell Biology* 2002;**4**(9):648-57. [PMID: 12172553]

Johnson 2010

Johnson SR, Cordier JF, Lazor R, Cottin V, Costabel U, Harari S, et al. European Respiratory Society guidelines for the diagnosis and management of lymphangioleiomyomatosis. *European Respiratory Journal* 2010;**35**(1):14-26.

Joinson 2003

Joinson C, O'Callaghan FJ, Osborne JP, Martyn C, Harris T, Bolton PF. Learning disability and epilepsy in an epidemiological sample of individuals with tuberous sclerosis complex. *Psychological Medicine* 2003;**33**(2):335-44. [PMID: 12622312]

Krueger 2013

Krueger DA, Wilfong AA, Holland-Bouley K, Anderson AE, Agricola K, Tudor C, et al. Everolimus treatment of refractory epilepsy in tuberous sclerosis complex. *Annals of Neurology* 2013;**74**(5):679-87. [PMID: 23798472]

Kwiatkowski 2002

Kwiatkowski DJ, Zhang H, Bandura JL, Heiberger KM, Glogauer M, el-Hashemite N, et al. A mouse model of TSC1 reveals sex-dependent lethality from liver hemangiomas, and up-regulation of p70S6 kinase activity in Tsc1 null cells. *Human Molecular Genetics* 2002;**11**(5):525-34. [PMID: 11875047]

Kwiatkowski 2003

Kwiatkowski DJ. Tuberous sclerosis: from tubers to mTOR. *Annals of Human Genetics* 2003;**67**(Pt 1):87-96. [PMID: 12556239]

Lee 2005

Lee L, Sudentas P, Donohue B, Asrican K, Worku A, Walker V, et al. Efficacy of a rapamycin analog (CCI-779) and IFN-gamma in tuberous sclerosis mouse models. *Genes, Chromosomes & Cancer* 2005;**42**(3):213-27. [PMID: 15578690]

Lee 2009

Lee N, Woodrum CL, Nobil AM, Rautkys AE, Messina MP, Dabora SL. Rapamycin weekly maintenance dosing and the potential efficacy of combination sorafenib plus rapamycin but not atorvastatin or doxycycline in tuberous sclerosis preclinical models. *BMC Pharmacology* 2009;**9**:8. [PMID: 19368729]

Lefebvre 2022

Lefebvre C, Manheimer E, Briscoe S, Featherstone R, Littlewood A, Marshall C, et al. Chapter 4: Searching for and selecting studies. In Higgins JP, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA, editor(s). *Cochrane Handbook of Systematic Reviews of Interventions* Version 6.3 (updated February 2022). Cochrane, 2022. Available from training.cochrane.org/handbook.

LOVD TSC1

Povey S, Ekong R. Tuberous sclerosis database: Tuberous sclerosis 1 (TSC1). chromium.liacs.nl/LOVD2/TSC/home.php?select_db=TSC1 (accessed 14 August 2014).

LOVD TSC2

Povey S, Ekong R. Tuberous sclerosis database: Tuberous sclerosis 2 (TSC2). chromium.liacs.nl/LOVD2/TSC/home.php?select_db=TSC2 (accessed 14 August 2014).

Madke 2013

Madke B. Topical rapamycin (sirolimus) for facial angiofibromas. *Indian Dermatology Online Journal* 2013;**4**(1):54-7. [PMID: 23439391]

Manning 2002

Manning BD, Tee AR, Logsdon MN, Blenis J, Cantley LC. Identification of the tuberous sclerosis complex-2 tumor suppressor gene product tuberlin as a target of the phosphoinositide 3-kinase/akt pathway. *Molecular Cell* 2002;**10**(1):151-62. [PMID: 12150915]

McCormack 2010

McCormack F, Henske E. Lymphangioleiomyomatosis and pulmonary disease in TSC. In: Kwiatkowski D, Whittemore VH, Thiele EA, editors(s). *Tuberous Sclerosis Complex: Genes, Clinical Features, and Therapeutics*. Weinheim: Wiley-Blackwell, 2010.

McMahon 2012

McMahon J, Huang X, Yang J, Komatsu M, Yue Z, Qian J, Zhu X, Huang Y. Impaired autophagy in neurons after disinhibition of mammalian target of rapamycin and its contribution to epileptogenesis. *Journal of Neuroscience* 2012;**32**(45):15704-14. [DOI: [10.1523/JNEUROSCI.2392-12.2012](https://doi.org/10.1523/JNEUROSCI.2392-12.2012)] [PMID: 23136410]

Meikle 2008

Meikle L, Pollizzi K, Egnor A, Kramvis I, Lane H, Sahin M, Kwiatkowski DJ. Response of a neuronal model of tuberous sclerosis to mammalian target of rapamycin (mTOR) inhibitors: effects on mTORC1 and Akt signaling lead to improved survival and function. *Journal of Neuroscience* 2008;**28**(21):5422-32. [DOI: [10.1523/JNEUROSCI.0955-08.2008](https://doi.org/10.1523/JNEUROSCI.0955-08.2008)] [PMID: 18495876]

Muncy 2009

Muncy J, Butler IJ, Koenig MK. Rapamycin reduces seizure frequency in tuberous sclerosis complex. *Journal of Child Neurology* 2009;**24**(4):477. [PMID: 19151365]

Mutizwa 2011

Mutizwa MM, Berk DR, Anadkat MJ. Treatment of facial angiofibromas with topical application of oral rapamycin solution (1mg/mL(-1)) in two patients with tuberous sclerosis. *British Journal of Dermatology* 2011;**165**(4):922-3. [PMID: 21692776]

Napolioni 2008

Napolioni V, Curatolo P. Genetics and molecular biology of tuberous sclerosis complex. *Current Genomics* 2008;**9**(7):475-87. [PMID: 19506736]

NINDS 2013

National Institute of Neurological Disorders and Stroke (NINDS). Tuberous sclerosis fact sheet. www.ninds.nih.gov/disorders/tuberous_sclerosis/detail_tuberous_sclerosis.htm (accessed 1 August 2013).

Northrup 2013

Northrup H, Krueger DA, International Tuberous Sclerosis Complex Consensus Group. Tuberous sclerosis complex diagnostic criteria update: recommendations of the 2012 international tuberous sclerosis complex consensus conference. *Pediatric Neurology* 2013;**49**(4):234-54.

Northrup 2021

Northrup H, Aronow ME, Bebin EM, Bissler J, Darling TN, de Vries PJ, et al. Updated international tuberous sclerosis complex diagnostic criteria and surveillance and management recommendations. *Pediatric Neurology* 2021;**123**:50-66. [DOI: [10.1016/j.pediatrneurol.2021.07.011](https://doi.org/10.1016/j.pediatrneurol.2021.07.011)] [PMID: 34399110]

OMIM#191100 [Computer program]

TUBEROUS SCLEROSIS 1; TSC1. Online Mendelian Inheritance in Man® - National Center for Biotechnology Information, 12 February 2015 (last update). omim.org/entry/191100.

OMIM#613254 [Computer program]

TUBEROUS SCLEROSIS 2; TSC2. Online Mendelian Inheritance in Man® - National Center for Biotechnology Information, 12 February 2015 (last update). omim.org/entry/613254.

Osborne 1991

Osborne JP, Fryer A, Webb D. Epidemiology of tuberous sclerosis. *Annals of the New York Academy of Sciences* 1991;**615**:125-7. [PMID: 2039137]

Overwater 2014

Overwater IE, Rietman AB, Elgersma Y, de Wit MC. Treatment of intractable epilepsy in tuberous sclerosis complex with everolimus is not yet evidence-based. *Annals of Neurology* 2014;**75**(1):163-4. [PMID: 24243580]

Potter 2002

Potter CJ, Pedraza LG, Xu T. Akt regulates growth by directly phosphorylating Tsc2. *Nature Cell Biology* 2002;**4**(9):658-65. [PMID: 12172554]

Roach 1998

Roach ES, Gomez MR, Northrup H. Tuberous sclerosis complex consensus conference: revised clinical diagnostic criteria. *Journal of Child Neurology* 1998;**13**(12):624-8. [PMID: 9881533]

Roach 1999

Roach ES, DiMario FJ, Kandt RS, Northrup H. Tuberous Sclerosis Consensus Conference: recommendations for diagnostic evaluation. National Tuberous Sclerosis Association. *Journal of Child Neurology* 1999;**14**(6):401-7. [PMID: 10385849]

Rosner 2004

Rosner M, Freilinger A, Hengstschlager M. Proteins interacting with the tuberous sclerosis gene products. *Amino Acids* 2004;**27**(2):119-28. [PMID: 15351877]

RxList 2015a

John P Cunha, DO, FACOEP. Rapamune (Sirolimus). www.rxlist.com/rapamune-drug.htm 1 September 2022 (accessed 15 March 2023).

RxList 2015b

Torisel (Temsirolimus). www.rxlist.com/torisel-drug.htm 5 April 2018 (accessed 15 March 2023).

RxList 2016

Cunha JP. Afinitor (Everolimus). www.rxlist.com/afinitor-drug.htm 17 February 2022 (accessed 15 March 2023).

SAS 2013 [Computer program]

SAS®. SAS Institute Inc, Version 9.4. Cary, North Carolina, USA: SAS Institute Inc, 2013.

Sasongko 2015

Sasongko TH, Ismail NF, Nik Mohd Ariff NA, Zabidi-Hussin ZA. Rapamycin and its analogues (rapalogs) for tuberous sclerosis complex-associated tumors: a systematic review on non-randomized studies using meta-analysis. www.ojrd.com/content/10/1/95 (accessed 18 August 2015).

Sattler 1992

Sattler M, Guengerich FP, Yun CH, Christians U, Sewing KF. Cytochrome P-450 3A enzymes are responsible for biotransformation of FK506 and rapamycin in man and rat. *Drug Metabolism & Disposition* 1992;**20**(5):753-61.

Seibert 2011

Seibert D, Hong CH, Takeuchi F, Olsen C, Hathaway O, Moss J, et al. Recognition of tuberous sclerosis in adult women: delayed presentation with life-threatening consequences. *Annals of Internal Medicine* 2011;**154**(12):806-13. [PMID: 21690595]

Shepherd 1991

Shepherd C, Gomez M, Lie J, Crowson C. Causes of death in patients with tuberous sclerosis. *Mayo Clinic Proceedings* 1991;**66**(8):792-6.

Talos 2008

Talos DM, Kwiatkowski DJ, Cordero K, Black PM, Jensen FE. Cell-specific alterations of glutamate receptor expression in tuberous sclerosis complex cortical tubers. *Annals of Neurology* 2008;**63**(4):454-65. [DOI: [10.1002/ana.21342](https://doi.org/10.1002/ana.21342)] [PMID: 18350576]

Tanzi 2013

Tanzi MG. Juice interactions: What patients need to know. www.pharmacist.com/juice-interactions-what-patients-need-know (accessed 01 August 2014).

Tee 2003

Tee AR, Manning BD, Roux PP, Cantley LC, Blenis J. Tuberous sclerosis complex gene products, Tuberin and Hamartin, control mTOR signaling by acting as a GTPase-activating protein complex toward Rheb. *Current Biology* 2003;**13**(15):1259-68. [PMID: 12906785]

Tiberio 2011

Tiberio D, Franz DN, Phillips JR. Regression of a cardiac rhabdomyoma in a patient receiving everolimus. *Pediatrics* 2011;**127**(5):e1335-7. [PMID: 21464184]

Truchuelo 2012

Truchuelo T, Diaz-Ley B, Rios L, Alcantara J, Jaen P. Facial angiofibromas treated with topical rapamycin: an excellent choice with fast response. *Dermatology Online Journal* 2012;**18**(1):15. [PMID: 22301052]

Tyburczy 2015

Tyburczy ME, Dies KA, Glass J, Camposano S, Chekaluk Y, Thorner AR, et al. Mosaic and intronic mutations in TSC1/TSC2 explain the majority of TSC patients with no mutation identified

by conventional testing. *PLoS Genetics* 2015;**11**(11):e1005637. [PMID: 26540169]

van Slegtenhorst 1997

van Slegtenhorst M, de Hoogt R, Hermans C, Nellist M, Janssen B, Verhoef S, et al. Identification of the tuberous sclerosis gene TSC1 on chromosome 9q34. *Science* 1997;**277**(5327):805-8. [PMID: 9242607]

van Slegtenhorst 1999

van Slegtenhorst M, Verhoef S, Tempelaars A, Bakker L, Wang Q, Wessels M, et al. Mutational spectrum of the TSC1 gene in a cohort of 225 tuberous sclerosis complex patients: no evidence for genotype-phenotype correlation. *Journal of Medical Genetics* 1999;**36**(4):285-9. [PMID: 10227394]

Wesolowski 2014

Wesolowski R, Abdel-Rasoul M, Lustberg M, Paskell M, Shapiro CL, Macrae ER. Treatment-related mortality with everolimus in cancer patients. *Oncologist* 2014;**19**:661-8. [DOI: [10.1634/theoncologist.2013-0355](https://doi.org/10.1634/theoncologist.2013-0355)] [PMID: 24794158]

Weston 2012

Weston MC, Chen H, Swann JW. Multiple roles for mammalian target of rapamycin signaling in both glutamatergic and GABAergic synaptic transmission. *Journal of Neuroscience* 2012;**32**(33):11441-52. [PMID: 10.1523/JNEUROSCI.1283-12.2012] [PMID: 22895726]

Wiemer-Kruel 2014

Wiemer-Kruel A, Woerle H, Strobl K, Bast T. Everolimus for the treatment of subependymal giant cell astrocytoma probably causing seizure aggravation in a child with tuberous sclerosis complex: a case report. *Neuropediatrics* 2014;**45**(2):129-31. [PMID: 24293099]

Wong 2016

Wong M, Roper SN. Genetic animal models of malformations of cortical development and epilepsy. *Journal of Neuroscience Methods* 2016;**260**:73-82. [DOI: [10.1016/j.jneumeth.2015.04.007](https://doi.org/10.1016/j.jneumeth.2015.04.007)] [PMID: 25911067]

Yuan 2009

Yuan R, Kay A, Berg WJ, Lebowitz D. Targeting tumorigenesis: development and use of mTOR inhibitors in cancer therapy. *Journal of Hematology & Oncology* 2009;**2**:45. [PMID: 19860903]

Zeng 2008

Zeng LH, Xu L, Gutmann DH, Wong M. Rapamycin prevents epilepsy in a mouse model of tuberous sclerosis complex. *Annals of Neurology* 2008;**63**(4):444-53. [DOI: [10.1002/ana.21331](https://doi.org/10.1002/ana.21331)] [PMID: 18389497]

References to other published versions of this review

Sasongko 2014

Sasongko TH, Ismail NF, Malik NM, , Zabidi-Hussin ZA. Rapamycin and its analogues (rapalogs) for tuberous sclerosis complex. *Cochrane Database of Systematic Reviews* 2014, Issue 9. Art. No: CD011272. [DOI: [10.1002/14651858.CD011272](https://doi.org/10.1002/14651858.CD011272)]

Sasongko 2016

Sasongko TH, Ismail NF, Zabidi-Hussin ZA. Rapamycin and rapalogs for tuberous sclerosis complex. *Cochrane Database*

of Systematic Reviews 2016, Issue 7. Art. No: CD011272. [DOI: [10.1002/14651858.CD011272.pub2](https://doi.org/10.1002/14651858.CD011272.pub2)]

* Indicates the major publication for the study

CHARACTERISTICS OF STUDIES

Characteristics of included studies [ordered by study ID]

Bissler 2013 (EXIST 2)

Study characteristics

Methods	Double-blind, randomised, placebo-controlled, phase 3 study. Multicentre (24 centres in 11 countries).
Participants	118 participants (aged 18-61 years old, 40 males and 78 females) having at least one angiomyolipoma 3 cm or larger in diameter, and a diagnosis of tuberous sclerosis per consensus criteria (Roach 1998, Hyman 2000) were recruited; 79 participants were in the treatment group and 39 participants were in the placebo group. Participants were 18 years or older. Twenty-three participants in the placebo group withdrew, mainly due to disease progression. However, all participants were included in the analysis. There were 2/79 participants in intervention arm and 3/36 participants in the placebo arm with sporadic lymphangioleiomyomatosis (without TSC) which we could not separate from the analysis. For a future version of this review, we plan to contact study authors to obtain more detailed information so that we can analyse outcome from only people with TSC. We also plan to contact study authors to ascertain if the TSC participants meet the latest diagnostic criteria (Northrup 2013).
Interventions	Participants were randomised into 2 arms: 1. Oral everolimus 10 mg/day 2. Oral placebo Median exposure of intervention was 38 weeks for the treatment group and 34 weeks for placebo group. Kidney CT or MRI was carried out at baseline, 12, 24, 48 weeks and annually after start of treatment. Although it was stated that the core phase of the trial lasted until the last randomised participant had been treated for 6 months, there was no clear statement on the exact study duration, especially in view of another statement about a subsequent open-label phase. Study authors will be contacted for exact information about the timeline of randomisation.
Outcomes	1. Response rate of everolimus on angiomyolipomas (defined as the number of participants having 50% reduction in tumour size as compared to baseline) 2. Seizure frequency 3. Response to skin lesions 4. AEs
Notes	The study was funded by Novartis Pharmaceuticals.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Participants were randomly assigned in a 2:1 fashion to receive either everolimus or placebo, stratified by enzyme-inducing antiepileptic drug use at randomisation and by the presence of sporadic lymphangio leiomyomatosis. Random sequence was generated using interactive web response system.
Allocation concealment (selection bias)	Unclear risk	Not stated.

Bissler 2013 (EXIST 2) (Continued)

Blinding of participants and personnel (performance bias) All outcomes	Low risk	Participants received blinded study treatment until angiomyolipoma progression, occurrence of unacceptable toxicity, or participant withdrawal for any other reason. Double-blind (participant, caregiver, investigator, outcomes assessor).
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Kidney CT or MRI (same modality used throughout the study for each participant) was assessed by a blinded central radiology review. Adverse effects were monitored via participant-reported or caregiver-reported responses as well as investigator assessment.
Incomplete outcome data (attrition bias) All outcomes	Low risk	Efficacy analyses included all randomised participants, and safety analyses included all participants who received at least 1 dose of the study drug and had at least 1 post-baseline assessment. Participants not able to be assessed (either by dropout or other reasons) were considered as non responders.
Selective reporting (reporting bias)	Low risk	All outcomes that were mentioned in 'Methods' were reported in 'Results' section of the study report.
Other bias	Unclear risk	This study was funded by Novartis Pharmaceuticals, which is also manufacturer of the investigational product. Authors who are stock owners, employees or consultants of the funder (Novartis) were involved in the study design, discussion, research, overseeing of data collection and data analysis and interpretation. Concerns were acknowledged regarding conflict of interests among authors of this study, but due to limited number of studies we were unable to assess (through sensitivity analyses or subgroup analyses) if this has affected trial results.

Franz 2013 (EXIST 1)

Study characteristics

Methods	Double-blind, randomised, placebo-controlled, phase 3 trial. Multicentre (24 centres in 10 countries).
Participants	117 participants (aged 0.8 - 26.6 years old, 67 males and 50 females) having at least 1 angiomyolipoma 3 cm or larger in diameter, and a definite diagnosis of TSC per consensus criteria (Hyman 2000; Roach 1998) were recruited. There are 78 participants in the treatment group and 39 participants in the placebo group. In the treatment group, 1 participant was lost to follow-up and another participant withdrew consent. In the placebo group, 6 discontinued due to disease progression, 1 withdrew consent and another participant discontinued due to administrative problems. Data from all 117 recruited participants were analysed. For the future version of this review, we plan to contact study authors to ascertain if the people with TSC meet the latest diagnostic criteria (Northrup 2013).
Interventions	Participants were randomised into 2 arms: 1. Oral everolimus, from 4.5 mg/m² body surface area per day and subsequently adjusted to attain blood trough concentrations of 5 - 15 ng/mL with dose modifications allowed on for the purpose of treatment-related toxic effects. Starting dose for children with malignancies was chosen to be just less than the maximum tolerated dose (5 mg/m² per day) 2. Oral placebo Participants were treated for 6 months with an extension phase (up to 4 years after the last participant was randomly assigned to treatment) if the results of the core phase favoured everolimus. The median duration of study treatment was 41.9 weeks (range 24.0 - 78.9) for individuals in the everolimus group and 36.1 weeks (13.9 - 79.7) for those in the placebo group.

Franz 2013 (EXIST 1) (Continued)

Outcomes	1. Response rate (defined the number of participants having 50% reduction in tumour size as compared to baseline) of everolimus on SEGA 2. Angiomyolipomas 3. Seizure frequency 4. Response to skin lesions 5. AEs
Notes	The study was funded by Novartis Pharmaceuticals.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Participants were randomly assigned in a 2:1 ratio to everolimus or matching placebo, stratified according to the use of enzyme-inducing antiepileptic drugs. It is assumed that the researchers used computer-generated random sequence: Quote: "An interactive internet-response system was used for random assignment of patients..."
Allocation concealment (selection bias)	Low risk	The researchers concealed allocation according to the following statement: Quote: "An interactive internet-response system was used...for management of their treatment to maintain allocation concealment".
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Participants were blinded according to the following statement: Quote: "Patients were given masked study treatment (identical everolimus and placebo) unless discontinued..."
Blinding of outcome assessment (detection bias) All outcomes	Low risk	All study personnel were masked to treatment assignment, including outcome assessors.
Incomplete outcome data (attrition bias) All outcomes	Low risk	Efficacy and safety analyses included all participants.
Selective reporting (reporting bias)	Low risk	All outcomes that were mentioned in 'Methods' were reported in 'Results' sections of the study report.
Other bias	Unclear risk	This study was funded by Novartis Pharmaceuticals, which is also manufacturer of the investigational product. Authors who are employees or consultants of the funder (Novartis) were involved in the study design, discussion, research, overseeing of data collection and data analysis and interpretation. Concerns were acknowledged regarding conflict of interests among authors of this study, but due to limited number of studies we were unable to assess (through sensitivity analyses or subgroup analyses) if this has affected trial results.

French 2016 (EXIST 3)

Study characteristics

Methods	Double-blind, randomised, placebo-controlled, phase 3 trial. Multicentre (99 centres in 25 countries).
---------	--

French 2016 (EXIST 3) (Continued)

Participants	366 participants (190 males and 176 females) aged 2 – 65 years with a confirmed diagnosis of TSC and treatment-resistant epilepsy, with 16 or more seizures during the 8-week baseline phase (with no continuous 21-day seizure-free period) and receiving between 1 and 3 antiepileptic drugs at a stable dose for at least 12 weeks before randomisation were included. Our query to trial author regarding the diagnostic criteria used for the participants has not been responded.
Interventions	Participants randomised into 3 arms as follows: 1. Antiepileptic drugs plus placebo – 119 participants 2. Antiepileptic drugs plus everolimus 3–7 ng/mL – 117 participants 3. Antiepileptic drugs plus everolimus 9–15 ng/mL – 130 participants Duration of treatment was 18 weeks.
Outcomes	1. Frequency of seizure 2. AEs 3. Change from baseline in seizure frequency 4. Seizure frequency 5. Frequency of seizure-free days during the maintenance period 6. Seizure-free rate 7. Proportion of patients achieving at least a 25% reduction in seizure frequency from baseline 8. Exposure–response relationship analysis
Notes	The study was funded by Novartis Pharmaceuticals.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Patients were randomly assigned (1:1:1) via permuted-block randomisation (block size of 6) which was implemented by Interactive Response Technology software. Randomisation was stratified by age subgroup.
Allocation concealment (selection bias)	Low risk	Participants, investigators, site personnel, and the sponsor's study team were masked to treatment allocation, but allocation was not concealed from personnel in charge of drug supply, implementation of the randomisation list, and pharmacokinetic bioanalysis.
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Participants, investigators, site personnel, and the sponsor's study team were masked to treatment allocation. Everolimus pills were made identical to placebo pills to maintain blinding.
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Outcome assessors were blinded for most outcomes.
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Our query regarding the total number of participants included in the analysis for several outcomes has not been responded.
Selective reporting (reporting bias)	Low risk	All outcomes that were mentioned in 'Methods' were reported in 'Results' sections of the study report except for the outcome 'exposure-safety relationship'. We are still awaiting clarification from the author regarding this outcome.

French 2016 (EXIST 3) (Continued)

Other bias	Unclear risk	This study was funded by Novartis Pharmaceuticals, which is also manufacturer of the investigational product. Authors who are employee or consultants of the funder (Novartis) were involved in the study design, discussion, research, overseeing of data collection and data analysis and interpretation. Concerns were acknowledged regarding conflict of interests among authors of this study, but due to limited number of studies we were unable to assess (through sensitivity analyses or subgroup analyses) if this has affected trial results.
------------	--------------	---

Koenig 2012

Study characteristics

Methods	Double-blind, randomised, placebo-controlled study. Single centre in Texas, USA.
Participants	28 participants (mean age 23 years old, ranging from 13 to > 30 years old, 15 males and 13 females) diagnosed with TSC based on the consensus criteria (Roach 1999). 18 participants in the treatment group and 10 participants in the placebo group. Five participants were withdrawn from the study, but it is unclear if they come from the treatment arm or from placebo arm.
Interventions	Participants were randomised 3 arms. 1. topical (skin application) rapamycin 1 mg per 30 cc (0.003%) 2. topical (skin application) rapamycin 5 mg per 30 cc (0.015%) 3. topical (skin application) placebo A thin coating of rapamycin was applied on the skin directly over the treatment area every morning (topical administration). The product was allowed to air dry for 60 minutes following application and was removed by gentle washing in the morning. Participants were treated for 6 months.
Outcomes	1. Effect of rapamycin on participants' perception towards their skin appearance following the treatment (the participants were asked if they "got better on the treatment", "got worse on the treatment" or if "the treatment made no difference") 2. Rapamycin concentrations and complete blood counts (including haemoglobin level and platelet count) 3. AEs
Notes	The study was sponsored by the University of Texas Health Science Center, Houston.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Randomisation undertaken by the manufacturer of the investigational product: Quote: "The company randomized the investigational product". However, there was no statement as to how the allocation sequence was generated.
Allocation concealment (selection bias)	Low risk	Author clarified that central allocation was done to ensure concealment is maintained.
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Participants and study personnel were blinded: "Double Blind (Subject, Caregiver, Investigator, Outcomes Assessor)".

Koenig 2012 (Continued)

Blinding of outcome assessment (detection bias) All outcomes	Low risk	Outcome assessors were blinded.
Incomplete outcome data (attrition bias) All outcomes	High risk	Out of 28 participants included into the study, 23 participants completed the intervention. Outcome data was reported only as percentages. While there are dropouts, no reports as to how many dropouts in each treatment arm.
Selective reporting (reporting bias)	Low risk	All outcomes that were mentioned in 'Methods' were reported in 'Results' sections of the study report except for outcomes 'Total Cholesterol' and 'Dermatologic sensitivity at site of application (pain, erythema, edema, pruritis)'.
Other bias	Low risk	The authors reported no conflict of interest.

Koenig 2018

Study characteristics

Methods	Double-blind, randomised, placebo-controlled, Phase 2, multicentre study (9 sites in USA and 1 in Australia).
Participants	Participants had a diagnosis of TSC as per new criteria Northrup 2013 , visible facial angiofibromas, and the ability to comply with study procedures. Females of child-bearing potential were tested for pregnancy prior to enrolment and required to use appropriate contraceptive methods during the study. 179 participants (80 males and 99 females) aged 3 - 61 years were involved in the study, 149 participants received intended treatment; 157 participants were analysed, 22 participants were excluded on the basis of lacking either baseline or at least 1 post-baseline evaluable photograph.
Interventions	Topical application over the designated area daily at bedtime. 1. Dispensed 1% rapamycin (59 participants) 2. Dispensed 0.1% rapamycin (63 participants) 3. Dispensed vehicle only (57 participants) Received treatment for 6 months.
Outcomes	1. Change from baseline in the AGS 2. Photo readers' rating (better, same, or worse) of paired baseline and end-of-trial photographs for each participant 3. QoL questionnaire scores change from baseline 4. Safety analyses including AEs and assessment of serum rapamycin levels (MedDRA-coded)
Notes	The study was supported in part by the United States Department of Defense grant DOD TSCRP CDMRP W81XWH-11-1-0240, and Tuberous Sclerosis Australia.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Enrolled participants were randomised (1:1:1) to receive rapamycin (1% or 0.1%) or vehicle-only using a computerised random number generator. Stratification by site was performed and each site's randomisation was performed in block sizes of 6.

Koenig 2018 (Continued)

Allocation concealment (selection bias)	Low risk	Central allocation undertaken.
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Blinding of study personnel is not clearly stated. We are awaiting clarification from author on the blinding status.
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Blinding of outcome assessors were clearly mentioned. However, we are still awaiting clarification from author regarding the blinding status of the outcomes assessors for 2 outcomes (dermatologic sensitivity at site of application (pain, erythema, pruritis) and DLQI/CDLQI/FDLQI).
Incomplete outcome data (attrition bias) All outcomes	High risk	Participants who lack either baseline or at least 1 post-baseline evaluable photograph were excluded from analysis. Only 159 out of 179 participants were analysed for the AGS outcomes.
Selective reporting (reporting bias)	Low risk	All outcomes that were mentioned in 'Methods' were reported in 'Results' sections of the study report.
Other bias	Unclear risk	Three authors reported grants and personal fees from Novartis Pharmaceutical (manufacturer of the investigational product). Concerns were acknowledged regarding conflict of interests among authors of these studies, but due to limited number of studies we were unable to assess (through sensitivity analyses or subgroup analyses) if this has affected trial results.

Krueger 2017
Study characteristics

Methods	Double-blind, randomised, placebo-controlled, two centres (Massachusetts and Ohio, USA).
Participants	Participants were diagnosed with Northrup 2013 new criteria, aged between 6 - 21 years. 47 participants (24 males and 23 females) were randomised, 42 participants received intended treatment.
Interventions	2 treatment arms. 1. Everolimus (32 participants): dose of 4.5 mg/m ² per day, oral, once daily 2. Placebo (15 participants) Received treatment for 6 months.
Outcomes	1. AEs 2. Intellectual function at baseline 3. Language (Receptive – PPTV4; Expressive – EVT2) 4. WRAML2 (Learning and Memory, Verbal Learning, Verbal Recall) 5. CANTAB (Pattern Recognition Memory, Spatial Recognition Memory, Spatial Span, Spatial Working Memory, Attention and Executive Function (Reaction Time), Rapid Visual Processing, Stockings of Cambridge, Intraextra Dimensional Set Shift) 6. BRIEF (Global Executive Composite, Behavioral Regulation Index, Metacognition Index) 7. SRS (Socialization and behaviour (Total Social Responsiveness), Social Awareness, Social Cognition, Social Communication, Social Motivation, Autism Mannerisms) 8. BASC2 (Externalizing Problems, Internalizing Problems, Behavioral Symptoms Index, Adaptive Skills) 9. Adaptive Behavior Composite, 10. VABS2 (Communication, Socialization, Daily Living Skills, Motor Skills)

Rapamycin and rapalogs for tuberous sclerosis complex (Review)

Krueger 2017 (Continued)

- 11.Total Difficulties Scale (SDQ),
- 12.Motor skills (Dominant Hand Speed – Pegboard)
- 13.Nondominant Hand Speed – Pegboard)
- 14.Academic skills (Word Reading – WRAT4; Math Computation – WRAT4)

Notes	The study was funded by financial support of Novartis Pharmaceuticals, Autism Speaks (Grant #5735) and Tuberous Sclerosis Alliance.
-------	---

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Randomisation was 2:1 everolimus versus matching placebo, using the SciRan (Scientific Randomisation) program developed at BCH. Randomisation was stratified by age and IQ with two levels of each factor.
Allocation concealment (selection bias)	Low risk	Allocation performed centrally.
Blinding of participants and personnel (performance bias) All outcomes	Low risk	All participants and personnel were blinded; used a matching placebo.
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Blinding of outcome assessors were not clearly stated. We are still awaiting confirmation from author regarding the blinding status.
Incomplete outcome data (attrition bias) All outcomes	High risk	Only 42 participants were analysed even though ITT population was mentioned to be n = 47.
Selective reporting (reporting bias)	Low risk	All outcomes that were mentioned in 'Methods' were reported in 'Results' sections of the study report.
Other bias	Unclear risk	This study was funded by Novartis Pharmaceuticals, which is also manufacturer of the investigational product. Authors who are consultants of and/or, employee of, and/or receive funding from the funder (Novartis Pharmaceuticals, Tuberous Sclerosis Alliance, and Autism Speaks) were involved in the study concept and design, acquisition of data, analysis and interpretation of data, and drafting and revision of manuscript for intellectual content. Concerns were acknowledged regarding conflict of interests among authors of this study, but due to limited number of studies we were unable to assess (through sensitivity analyses or subgroup analyses) if this has affected trial results.

Overwater 2016
Study characteristics

Methods	Age-stratified randomisation. Open-label. Cross-over, single centre (the Netherlands)
Participants	Children aged between 3 months and 12 years with definite clinical diagnosis of TSC as per new criteria Northrup 2013 and at least 1 epileptic seizure per week and were resistant to at least 2 antiepileptic drugs. 23 participants (11 males and 12 females) were randomised and 22 participants received intended treatment.

Overwater 2016 (Continued)

Interventions	Titrated to trough level of 5-10 ng/mL Mean daily sirolimus dose in the last month of sirolimus was 3.65 mg and ranged from 0.9 to 8.0 mg. Administered once a day. 1. Early Sirolimus (12 participants) 2. Late Sirolimus (11 participants) Participants received treatment for 12 months.
Outcomes	1. Frequency of seizure 2. AEs 3. Proportion of responders (> 50% reduction of seizure frequency in the last month of either study period relative to baseline) 4. Seizure severity 5. Number of status epilepticus episodes 6. EEG 7. Cognitive development (Bayley Scales of Infant and Toddler Development or Wechsler Preschool and Primary Scale of Intelligence) 8. Adaptive behavior (Vineland Screener 2008) 9. Sensory processing (Short Sensory Profile-NL, 2006) 10. Autistic features (SRS 2007) 11. Emotional and behavioural problems (Child Behavior Checklist 2000)
Notes	This study was funded by a research grant from the Dutch Epilepsy Foundation.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Participants were randomly allocated by computer generation with permuted block design (block size 4) and stratified by age.
Allocation concealment (selection bias)	Low risk	Allocation concealment was centrally computerised.
Blinding of participants and personnel (performance bias) All outcomes	High risk	Participants were not blinded. Study neuropsychologist and neurophysiologist were masked to treatment.
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Blinding of outcome assessors were clearly mentioned.
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Total number of participants analysed were not listed for all outcomes. We are still waiting for author to provide us with more details on the data.
Selective reporting (reporting bias)	Low risk	All outcomes that were mentioned in 'Methods' were reported in 'Results' sections of the study report.
Other bias	Unclear risk	This study used cross-over design which require additional considerations on potential risk of bias. The choice of cross-over design may be suitable given the condition (epilepsy) is relatively stable. Although it was mentioned that the study lasted for 12 months, it was unclear how long the follow-up was for each period.

Overwater 2016 (Continued)

Carry-over effect is unknown as there is no information about a washout period before the cross-over occurred. We are currently awaiting confirmation from the author regarding this information.

Data from each period of cross-over were not available. We are therefore unable to perform paired analysis and compare the results with the other parallel-group trials, until further information from the trial authors.

One of the authors, who is a co-principal investigator and contributed to conception of the study, study design, grant writing, data collection, clinical follow-up, data analysis, data interpretation, and writing of the report, reports grants from Dutch Epilepsy Foundation during the conduct of the study and grants and non-financial support from Novartis outside the submitted work. The authors' institution received honoraria from Novartis for educational lectures presented by the author. Concerns were acknowledged regarding conflict of interests of an author in this study, but due to limited number of studies we were unable to assess (through sensitivity analyses or subgroup analyses) if this has affected trial results.

Overwater 2019
Study characteristics

Methods	Randomised, double-blind, placebo-controlled, single centre (the Netherlands).
Participants	32 participants (16 males and 16 females, age 4 - 17 years old) with TSC as per the Northrup 2013 new criteria were randomised to the study, 28 participants received intended treatment, AEs resulted in discontinuation of treatment in 4 participants.
Interventions	2 treatment arms 1. Everolimus (15 participants): 2.5 mg once daily 2. Placebo (17 participants) Duration of treatment was 12 months.
Outcomes	1. Frequency of seizure 2. Cognitive ability measured by IQ using WPPSI-III-NL17 or WISC-III-NL 3. Autistic features measured by ADOS 4. Social and communication skills (SRS 20 and Dutch Children's Communication Checklist) 5. Working memory and attention 6. Visual motor integration measured by the Beery-Buktenica Developmental Test of Visual-Motor Integration 7. Child behaviour with CANTAB and the parental rating scale BRIEF 8. Executive functioning with CANTAB and the parental rating scale BRIEF 9. Sleeping problems (SDSC), 10. Child health 11. Sensory related difficulties (Short Sensory Profile)
Notes	The trial was funded by the Dutch Brain Association (Hersenstichting) and the Sophia Children's Hospital Fund (Stichting Sophia Kinderziekenhuis Fonds, Rotterdam, the Netherlands). Novartis AG provided everolimus and placebo tablets and provided additional financial support.

Risk of bias

Overwater 2019 (Continued)

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	All participants were randomly assigned (1:1) to receive everolimus or placebo using a permuted-block (block size 4) computer-generated randomisation list.
Allocation concealment (selection bias)	Low risk	Allocation performed centrally.
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Blinding of participants and personnel were clearly described.
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Blinding of outcome assessors were clearly mentioned for all outcomes.
Incomplete outcome data (attrition bias) All outcomes	Low risk	ITT analysis was done, for all except 1 secondary outcome (pharmacokinetics).
Selective reporting (reporting bias)	Low risk	All outcomes that were mentioned in 'Methods' were reported in 'Results' sections of the study report.
Other bias	Unclear risk	One of the study's funders is Novartis, which is also the manufacturer of the investigational product. One of the authors, who contributed to trial design, trial implementation, data collection, statistical analysis, interpretation of the data, and development of the manuscript, reported grants from Sophia Foundation (SSWO), non-financial support from Novartis International AG, and grants from Novartis International AG during the conduct of the study; personal fees from Hoffmann-La Roche; and other from Novartis International AG. Concerns were acknowledged regarding conflict of interests of an author in this study, but due to limited number of studies we were unable to assess (through sensitivity analyses or subgroup analyses) if this has affected trial results.

Wataya-Kaneda 2017
Study characteristics

Methods	Randomised, placebo-controlled, single centre (Osaka, Japan).
Participants	36 participants (13 males and 23 females) enrolled in this trial, aged 6 to 47 years and had at least 3 isolated angiofibromas that were 2 mm or larger in their longest diameters. Participants were diagnosed based on old criteria (Roach 1999).
Interventions	Participants were randomised into 4 arms, sirolimus or placebo gel applied to the participant's lesions twice per day: 1. 0.05% sirolimus – 8 participants 2. 0.1% sirolimus – 8 participants 3. 0.2% sirolimus – 8 participants 4. Placebo - 12 participants Duration of treatment was for 12 weeks + 4 weeks of treatment discontinuation.
Outcomes	1. Skin lesion response

Rapamycin and rapalogs for tuberous sclerosis complex (Review)

Wataya-Kaneda 2017 (Continued)

2. AEs
3. Ratio for the decrease in tumour volume,
4. Reduction of tumour colour,
5. Improvement factor 4 weeks after discontinuation of treatment
6. Mean general improvement at 12 weeks, mean participant satisfaction

Notes	The study was supported by an H24-intractable-general-008 grant from the Ministry of Health, Labor and Welfare of Japan, grant 25461690 from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and practical research project 15ek0109082h0001 for rare and intractable diseases from the Japan Agency for Medical Research and Development.
-------	---

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Participants randomly allocated using a web-response system in a 2:1 fashion to receive each sirolimus concentration or a placebo.
Allocation concealment (selection bias)	Low risk	Allocation was centralised as it uses the web-response system.
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Although double-blinding was mentioned, details on who were blinded were not mentioned. We are still awaiting author's confirmation on the blinding status of the participants and personnel.
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	There was no description on blinding of the outcome assessors. We are still waiting for authors confirmation on the blinding status of the assessors.
Incomplete outcome data (attrition bias) All outcomes	Low risk	ITT analysis.
Selective reporting (reporting bias)	Low risk	All outcomes that were mentioned in 'Methods' were reported in 'Results' sections of the study report.
Other bias	Low risk	The authors reported no conflict of interest.

Wataya-Kaneda 2018
Study characteristics

Methods	Randomised, double-blind, placebo-controlled, multicentre (9 sites in Japan).
Participants	62 participants (28 males and 34 females) aged 6 - 53 years old with 3 or more reddish papules of facial angiofibroma. TSC diagnosis based on new criteria (Northrup 2013).
Interventions	Participants randomised into 2 arms. 1. 0.2% Sirolimus gel (30 participants) 2. Placebo gel (32 participants) Gels were applied topically twice daily for 12 weeks.

Wataya-Kaneda 2018 (Continued)

Each participant was instructed to evenly spread the investigational drug to skin lesions at the daily amounts of gel twice daily for 12 weeks; up to 400 mg for participants younger than 6 years, 600 mg for participants 6 to 11 years, and 800 mg for participants older than 11 years.

Outcomes	1. Skin lesion response 2. AEs, 3. Skin lesion change/improvement – composite improvement 4. QoL (DLQI and CDLQI)
Notes	The study was supported by Nobelpharma Co, Ltd, and in part by grants 27-Yaku, No. 369 orphan drug designation from the Japanese National Institutes of Biomedical Innovation, Health, and Nutrition, under the jurisdiction of the Japan Ministry of Health, Labor, and Welfare.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Participants randomly assigned (1:1) to receive sirolimus or placebo. An independent statistician generated the codes for permuted-block (size of 4) randomisation SAS Version 9.4 (SAS 2013).
Allocation concealment (selection bias)	Low risk	Central allocation was performed.
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Participants and study personnel were blinded until study completion.
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Blinding of outcome assessors were clearly described.
Incomplete outcome data (attrition bias) All outcomes	Low risk	All participants were included in the final analysis of the outcomes.
Selective reporting (reporting bias)	Low risk	All outcomes that were mentioned in 'Methods' were reported in 'Results' sections of the study report.
Other bias	Unclear risk	One of the funders of the study (Nobelpharma Co. Ltd.) is also manufacturer of the investigational product. The funders participated in the design and conduct of the study and preparation, review, or approval of the manuscript. Authors who received funding from or are consultants of or are employed by Nobelpharma Co. Ltd. were involved in study concept and design, acquisition, analysis, or interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, administrative, technical, or material support, and study supervision. Some of the authors also received funding or fees from other commercial entities: Novartis, Sanofi, Sumitomo-Dainippon, GlaxoSmithKline, AbbVie, Eli-Lilly, Boehringer-Ingelheim, Kyowa-Hakko-Kirin, Maruho, Terumo Foundation for Life Science and Arts, Nakatomi Foundation, and Mitsubishi-Tanabe Pharma, and Chugai. Concerns were acknowledged regarding conflict of interests among authors of these studies, but due to limited number of studies we were unable to assess (through sensitivity analyses or subgroup analyses) if this has affected trial results.

ADOS: Autism Diagnostic Observation Schedule; **AE:** adverse event; **AGS:** Angiofibroma Grading Scale; **BASC2:** Behavior Assessment System for Children, 2nd Edition; **BRIEF:** Behavior Rating Inventory of Executive Function; **CANTAB:** Cambridge; Neuropsychological Test Automated Battery; **CDLQI:** Child's Dermatology Life Quality Index; **CT:** computed tomography; **DLQI:** Dermatology Life Quality Index; **EEG:** electroencephalogram; **EVT2:** Expressive Vocabulary Test 2nd Edition; **FDLQI:** Family Dermatology Life Quality Index; **IQ:** intelligent quotient; **ITT:** intention to treat; **MRI:** magnetic resonance imaging; **PPTV4:** Peabody Picture Vocabulary Test 4th Edition; **QoL:** quality of life; **SDQ:** Strengths and Difficulties Questionnaire; **SDSC:** Sleep Disturbance Scale for Children; **SEGA:** subependymal giant cell astrocytomas; **SRS:** Social Responsiveness Scale; **TSC:** tuberous sclerosis complex; **VABS2:** Vineland Adaptive Behaviour Scale 2; **WISC-III-NL:** Wechsler Intelligence Scale for Children; **WPPSI-III-NL:** Wechsler Preschool and Primary Scale of Intelligence; **WRAML2:** Wide Range Assessment of Memory and Learning Second Edition; **WRAT4:** Wide Range Achievement Test 4

Characteristics of excluded studies [ordered by study ID]

Study	Reason for exclusion
Birca 2010	Case report.
Bissler 2019	Non-randomised, non-controlled study
Cabrera 2011	Non-randomised, non-controlled study.
ChiCTR-OPC-14005488	Non-randomised, non-controlled study.
ChiCTR-OPN-16008236	Non-randomised, non-controlled study.
Cuevas 2012	Non-randomised, non-controlled study.
Dabora 2011	Non-randomised, non-controlled study.
Davies 2008	Non-randomised, non-controlled study.
Davies 2011	Non-randomised, non-controlled study.
DRKS00005584	Non-randomised, non-controlled study.
EUCTR2007-005978-30-ES	Non-randomised, non-controlled study.
EUCTR2010-022583-13-DE	Non-randomised, non-controlled study
Foster 2012	Case series.
Franz 2006	Case series.
Franz 2018	Non-randomised, non-controlled study.
Gupta 2019	Only 9% of the subjects were diagnosed as TSC. There was no clear separation between TSC and non-TSC subjects in the analysis.
Herry 2007	Case report.
Hofbauer 2008	Case report.
JPRN-UMIN000002844	Non-randomised, non-controlled study.
JPRN-UMIN000006108	Non-randomised, non-controlled study.
JPRN-UMIN000015114	Non-randomised, non-controlled study.

Study	Reason for exclusion
JPRN-UMIN000011559	Non-randomised, non-controlled study.
JPRN-UMIN000024270	Non-randomised, non-controlled study.
Kenerson 2002	Non-randomised, non-controlled study.
Koenig 2008	Case report.
Krueger 2010	Non-randomised, non-controlled study.
Krueger 2013a	Single arm, interventional study.
Krueger 2013b	Non-randomised, non-controlled study.
Lam 2010	Case series.
NCT00001465	Observational case-control.
NCT00001532	Observational case-control
NCT00001975	Observational Cohort.
NCT00457808	Non-randomised, non-controlled study.
NCT00457964	Single arm interventional study
NCT00490789	Non-randomised, non-controlled study
NCT00552955	Non-randomised, non-controlled study.
NCT00792766	Single arm interventional study
NCT01092208	Non-randomised, non-controlled study
NCT01217125	Single arm interventional study
NCT01266291	Non-randomised, non-controlled study.
NCT01767779	Non-randomised, non-controlled study.
NCT01780441	Non-randomised, non-controlled study.
NCT01929642	Single arm interventional study
NCT02104011	single arm, interventional study
NCT02201212	Single arm interventional study
NCT02325505	Non-randomised, non-controlled study.
NCT02451696	Non-randomised, non-controlled study
NCT02461459	Non-randomised, non-controlled study.
NCT02634931	Single arm interventional study

Study	Reason for exclusion
NCT02654340	Non-randomised, non-controlled study.
NCT03047213	Non-randomised, non-controlled study.
NCT03213678	Non-randomised, non-controlled study.
NCT03525834	Non-randomised, non-controlled study
NCT03649919	Non-randomised, non-controlled study.
Pressey 2010	Case report.
Salido 2012	Case series.
Sallas 2015	Pharmacokinetic-Pharmacodynamic modelling article
Sparagana 2010	Case report.
Staehler 2012	Case series.
Tanaka 2013	Non-randomised; left-right cheek comparison of topical rapamycin vs vehicle on facial angiofibroma of the same participants.
Wataya-Kaneda 2011	Case series.
Wataya-Kaneda 2012	Case report.
Wataya-Kaneda 2015	Non-randomised, non-controlled study.
Wheless 2013	Case report.
Wienecke 2006	Case report.
Yalon 2011	Case report.
Young 2013	Patients are a mix-up of LAM only and LAM-TSC. No clear separation.
Zhang 2018	Non-randomised, non-controlled study.

LAM: lymphangioleiomyomatosis; **TSC:** tuberous sclerosis complex

Characteristics of studies awaiting classification *[ordered by study ID]*

[NCT03140449](#)

Methods	Randomised, double-blind, placebo-controlled, unclear about the number of centres (Taiwan)
Participants	52 participants with unclear diagnostic criteria were randomised. Trial authors will be contacted regarding the diagnostic criteria.
Interventions	Unclear about number of arms. Trial authors will be contacted regarding the trial arms. Topical rapamycin (0.1%) or calcitriol (3 mcg/g) single-agent therapy versus their combination / twice a day

NCT03140449 (Continued)

Duration of treatment is 12 weeks

Outcomes	1. Skin lesion response 2. Reduction for the grade of erythema, papule size, elevation and extension of the lesions using FASI 3. Reduction of VAS evaluated by the participants themselves
Notes	

Randell 2016

Methods	rRandomised, double-blind, placebo-controlled, single centre (UK)
Participants	48 participants between 16 and 60 years, with unclear diagnostic criteria, were randomised. Trial authors will be contacted regarding the diagnostic criteria.
Interventions	Participants randomised into 2 arms: 32 participants in everolimus arm, dose of 2x 2.5 mg daily 16 participants in placebo arm Duration of treatment is 24 weeks or 6 months
Outcomes	1. AE 2. List Learning test (from the BIRT Memory and Information Processing Battery) 3. Complex Figure test (from the BIRT Memory and Information Processing Battery) 4. CANTAB - SOC 5. CANTAB - SWM 6. Telephone search dual task (from the Test of Everyday Attention) 7. CANTAB - RVIP Battery 8. CANTAB - SSP 9. CANTAB - Attentional Set-shifting (IDED) 10. Verbal Fluency from Controlled Oral Word Association Test 11. Cancellation task from the Controlled Oral Word Association Test 12. Symptom Checklist 90-Revised 13. Quality of Life in Epilepsy 14. Liverpool Seizure Severity Scale 15. Vineland Adaptive Behavior Scales-II (survey form) 16. Social Responsiveness Scale – Adult version 17. Social communication questionnaire 18. National Adult Reading Test 19. Wechsler Abbreviated Scale of Intelligence (4 subtests) 20. Edinburgh Handedness Test
Notes	

AE: adverse effect; **BIRT:** Brain Injury Rehabilitation Trust; **CANTAB:** Cambridge Neuropsychological Test Automated Battery; **FASI:** facial angiofibroma severity index; **IQ:** intelligence quotient; **RVIP:** Rapid Visual Information Processing; **SOC:** Stockings of Cambridge; **SSP:** Spatial Span; **SWM:** Spatial Working; Memory; **TSC:** tuberous sclerosis complex; **VAS:** visual analysis score

Characteristics of ongoing studies [ordered by study ID]

EUCTR2011-006308-12-ES

Study name	Phase II clinical trial, to study the efficacy and safety of topical rapamycin in reducing facial angiofibromas, single centre (Spain)
Methods	RandomiSed, placebo-controlled, with unclear blinding and diagnosis criteria
Participants	50 participants aged above 13 years with FA, allocation is unclear
Interventions	2 arms Topical sirolimus/Rapamune with unclear dosage and frequency of administration 3 months duration of treatment
Outcomes	1. Tumor size 2. Reduction of at least 50% in size and number of tumours 3. Decrease in the punctuation of the FASI of at least 40% at the end of the treatment period (3 months) 4. Proportion of patients achieving a total response at the end of the treatment period (3 months) 5. Proportion of participants achieving a total response at the first month of the study 6. Proportion of participants achieving a complete or a partial response after 1 week of treatment 7. Proportion of participants achieving a total response at the 1st week of the study 8. Proportion of participants achieving a complete or a partial response after 1 month of treatment 9. Time of relapse 10. Proportion of participants who suffer any AE 11. Proportion of local reactions 12. Proportion of participants whose serum rapamycin level is detectable 13. Proportion of participants whose laboratory tests became abnormal when at screening were not 14. Proportion of participants whose blood pressure or whose respiratory/ heart rate became abnormal
Starting date	29/03/2012
Contact information	Fundación Investigación Hospital Ramón y Cajal, Fundación Hospital Ramón y Cajal, ipablo.hrc@salud.madrid.org
Notes	

NCT02860494

Study name	Topical Everolimus in Patients With Tuberous Sclerosis Complex (EVEROST)
Methods	Randomised, placebo-controlled, unclear blinding, unclear diagnosis criteria, multicentre (France)
Participants	146 participants aged more than 2 years, with a diagnosis of TSC, with 3 or more facial angiofibroma
Interventions	Topical everolimus with unclear dose and frequency of administration, intended to be treated for 6 months
Outcomes	1. AE 2. Mean reduction of the validated and published score for assessment of angiofibromas 3. FASI after 6 months of treatment 4. Clinical assessment of FA: mean reduction of a composite clinical score (0 - 12) for FA

NCT02860494 (Continued)

5. Blinded assessment of the redness and extension using Reactiv'IP system
6. FA size (in mm) of the 3 largest targeted FA papules
7. Dermatologist's global assessment of efficacy
8. Participant or parents self-assessment
9. DLQI for adults and CDLQI for children to assess dermatological QoL
10. Correlation of the results of FASI blindly assessed and the clinical assessment of FA,
11. Participant self-assessment: composite scores of 5 items (stinging, burning, itching, dryness, and scaling), each item consists of 3 states: 0 = absent, 1 = mild, 2 = moderate and 3 = severe
12. The physicians will assess dryness and scaling scores
13. Quantification of blood levels of topical everolimus
14. Laboratory assessments (creatinine, serum electrolytes including sodium, potassium, chlorine, carbon dioxide, calcium, total protein and urea, liver enzymes, total cholesterol, triglycerides, glucose, complete blood count)

Starting date	Registered on August 9 2016, estimated starting date December 2020
Contact information	Alice PHAN, MD, 4 27 85 61 26 ext +33, alice.phan01@chu-lyon.fr
Notes	

NCT03363763

Study name	Topical Sirolimus Ointment for Cutaneous Angiofibromas in Subjects With Tuberous Sclerosis Complex
Methods	Randomised, unclear diagnostic criteria, double-blind, placebo-controlled, multicentre (USA).
Participants	Males or non-pregnant females aged 2 to 18 years, with visible FAs of at least grade 2, unclear number of expected participants
Interventions	3 arms 1. Sirolimus 0.2% 2. Sirolimus 0.4% 3. Placebo Applied topically once daily at night before going to bed, duration of treatment intended is 12 weeks
Outcomes	1. Skin lesion response, 2. The proportion of participants with a clinical response of treatment success
Starting date	06/12/2017
Contact information	Cynthia S Sandy, RN, BSN, MS, PMP, 770-545-4030, cindy.sandy@peachtreebrs.com
Notes	

NCT03826628

Study name	Dose-Ranging Efficacy and Safety Study of Topical Rapamycin Cream for Facial Angiofibroma Associated With Tuberous Sclerosis Complex
------------	--

NCT03826628 (Continued)

Methods	Randomised, double-blind, new diagnostic criteria Northrup 2013 , placebo-controlled, multicentre (USA, Australia, New Zealand, Spain)
Participants	120 participants aged between 6 and 65 years will be randomised
Interventions	3 arms, topically applied once a day for 26 weeks 1. 0.5% Rapamycin cream 2. 1.0% Rapamycin cream 3. Placebo
Outcomes	1. Improvement of FA 2. Success on the Investigator Global Assessment scale, 3. The change in grading on the FASI from baseline
Starting date	Study start date July 28 2019
Contact information	Jennifer Zhang, + 64 9 488 0232 ext 710, jenniferz@aftpharm.com
Notes	

AE: adverse event; **LQI:** Children's Dermatology Life Quality Index; **DLQI:** Dermatology Life Quality Index; **FA:** facial angiofibroma; **FASI:** Facial Angiofibroma Severity Index ; **IQ:** intelligence quotient; **QoL:** quality of life; **TSC:** tuberous sclerosis complex

DATA AND ANALYSES

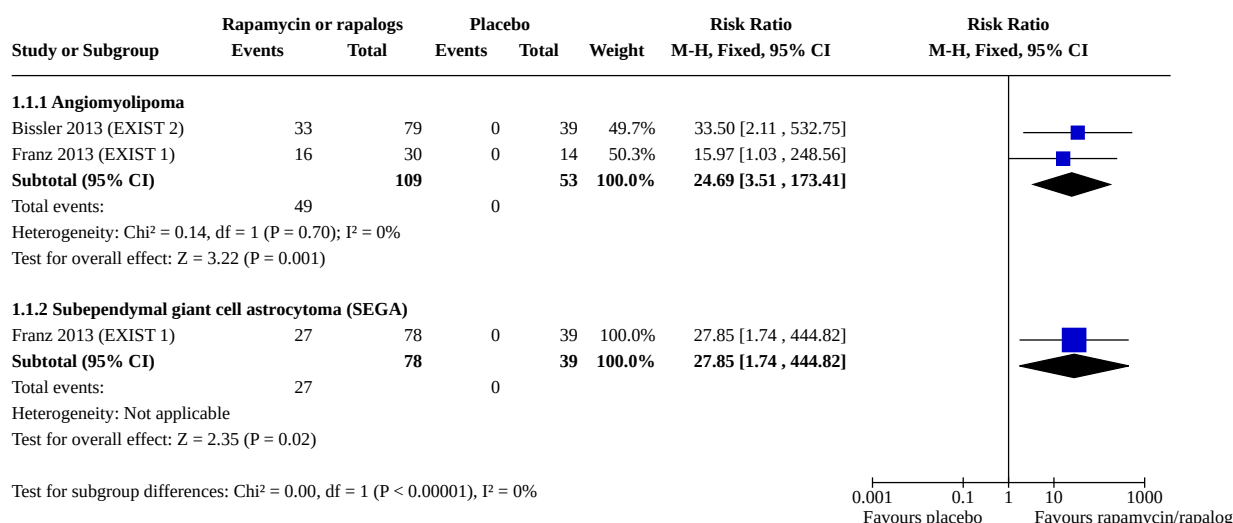
Comparison 1. Systemic administration of rapamycin or rapalogs versus placebo

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1.1 Response to tumour size (50% reduction from baseline)	2		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
1.1.1 Angiomyolipoma	2	162	Risk Ratio (M-H, Fixed, 95% CI)	24.69 [3.51, 173.41]
1.1.2 Subependymal giant cell astrocytoma (SEGA)	1	117	Risk Ratio (M-H, Fixed, 95% CI)	27.85 [1.74, 444.82]
1.2 Response to skin lesions	2		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
1.2.1 At 6 months	2	224	Risk Ratio (M-H, Fixed, 95% CI)	5.78 [2.30, 14.52]
1.3 Increased creatinine levels	1		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
1.3.1 At 6 months	1	118	Risk Ratio (M-H, Fixed, 95% CI)	0.16 [0.02, 1.53]
1.4 Reduction in seizure frequency	1		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
1.4.1 Seizure-free at 18 weeks	1	366	Risk Ratio (M-H, Fixed, 95% CI)	5.30 [0.69, 40.57]
1.4.2 50% reduction in seizure frequency	1	366	Risk Ratio (M-H, Fixed, 95% CI)	2.28 [1.44, 3.60]

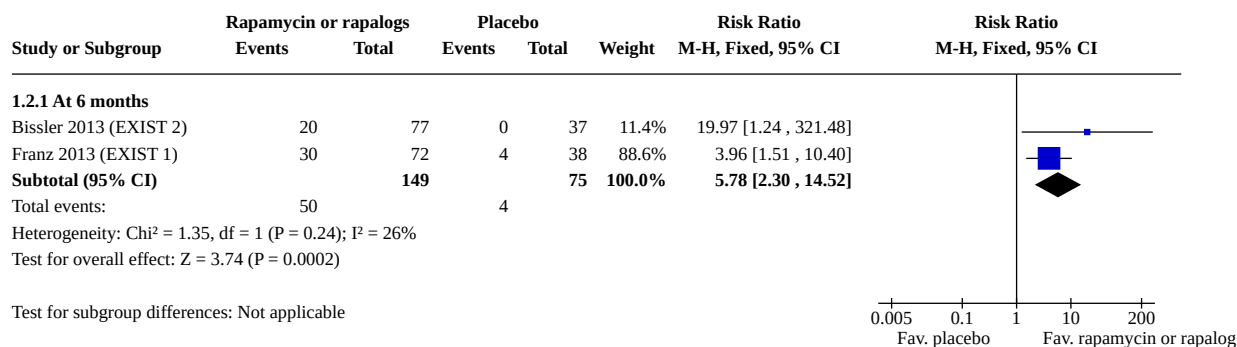
Rapamycin and rapalogs for tuberous sclerosis complex (Review)

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1.4.3 25% reduction in seizure frequency	1	366	Risk Ratio (M-H, Fixed, 95% CI)	1.63 [1.27, 2.09]
1.5 Change in attention and executive function - Stockings of Cambridge (CANTAB)	1		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
1.5.1 At 6 months	1	42	Mean Difference (IV, Fixed, 95% CI)	-1.26 [-2.03, -0.49]
1.6 Socialization and behaviour (Social Cognition - SRS)	1		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
1.6.1 At 6 months	1	42	Mean Difference (IV, Fixed, 95% CI)	-4.98 [-17.34, 7.38]
1.7 Adverse events	5		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
1.7.1 Any adverse event	5	680	Risk Ratio (M-H, Random, 95% CI)	1.09 [0.97, 1.22]
1.7.2 Adverse events leading to dose reduction, interruption or withdrawal	4	633	Risk Ratio (M-H, Random, 95% CI)	2.61 [1.58, 4.33]
1.7.3 Severe adverse events	2	413	Risk Ratio (M-H, Random, 95% CI)	2.35 [0.99, 5.58]

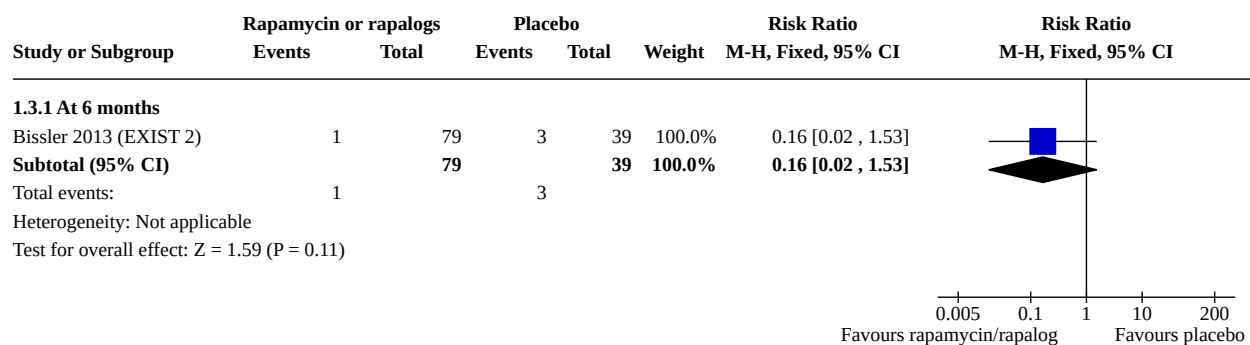
Analysis 1.1. Comparison 1: Systemic administration of rapamycin or rapalogs versus placebo, Outcome 1: Response to tumour size (50% reduction from baseline)

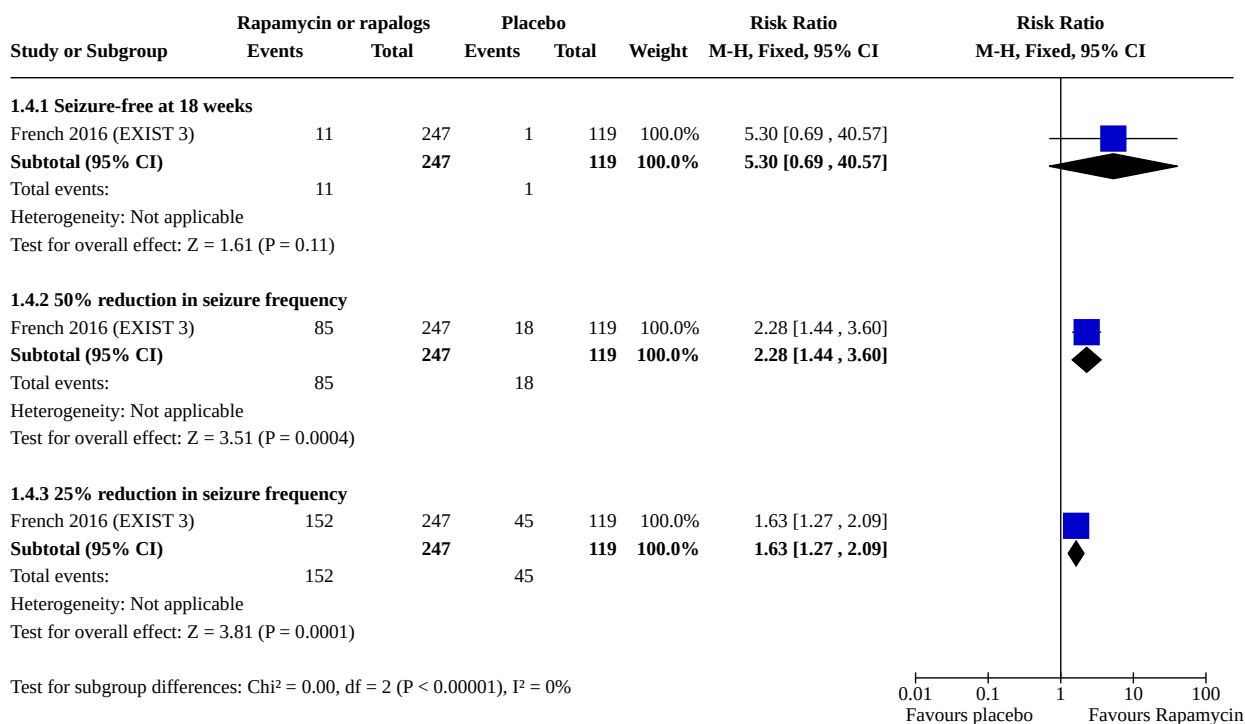
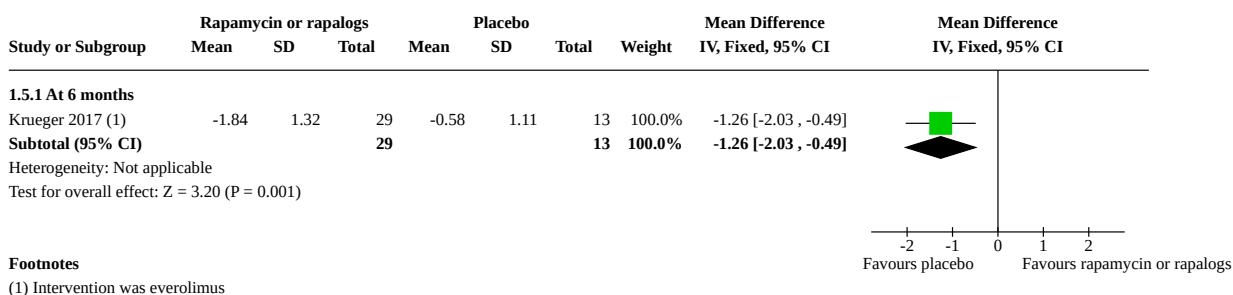
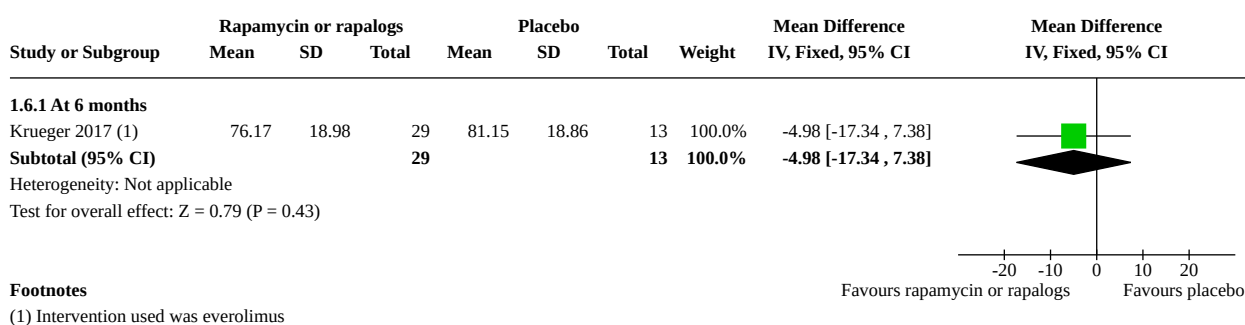


Analysis 1.2. Comparison 1: Systemic administration of rapamycin or rapalogs versus placebo, Outcome 2: Response to skin lesions

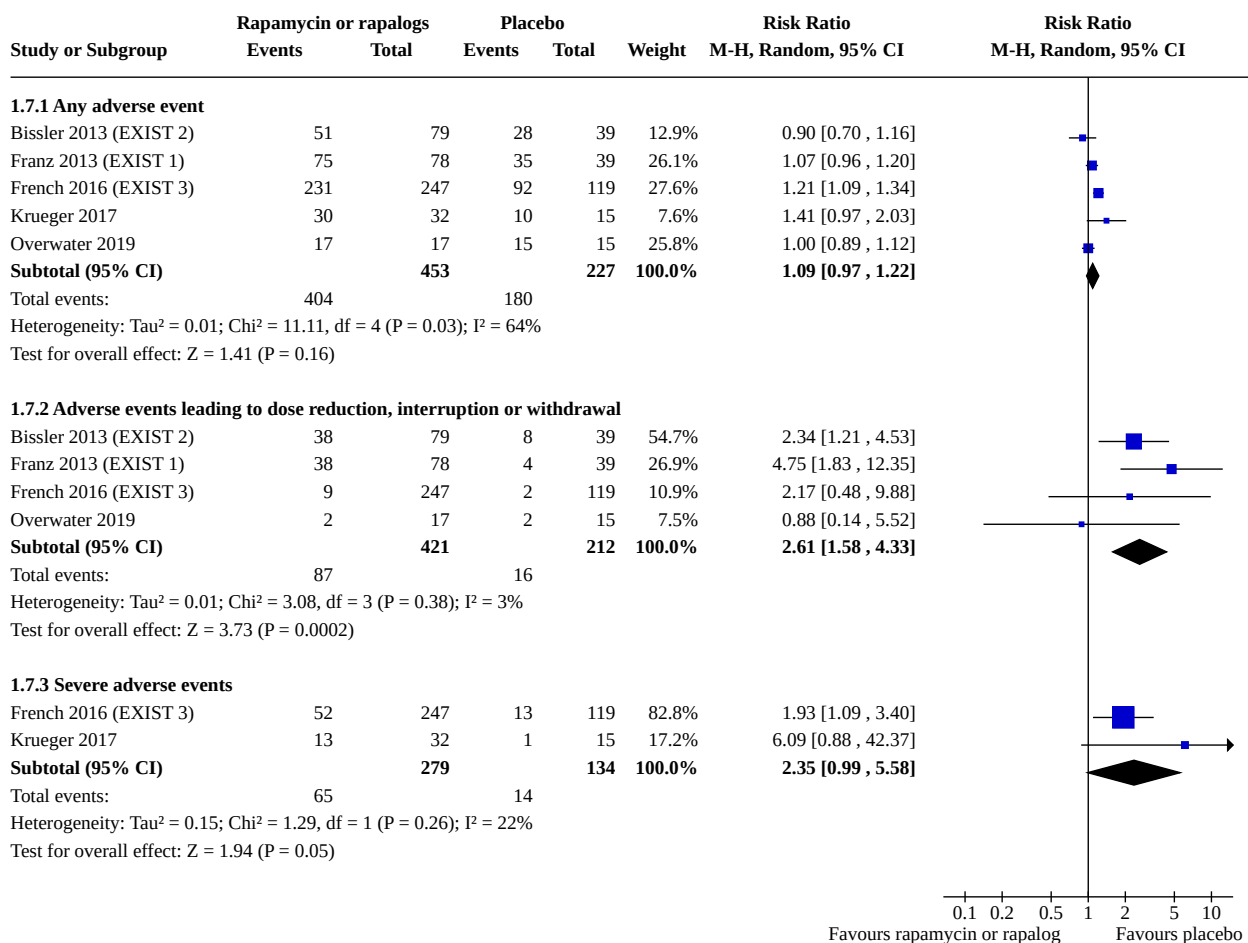


Analysis 1.3. Comparison 1: Systemic administration of rapamycin or rapalogs versus placebo, Outcome 3: Increased creatinine levels



Analysis 1.4. Comparison 1: Systemic administration of rapamycin or rapalogs versus placebo, Outcome 4: Reduction in seizure frequency**Analysis 1.5. Comparison 1: Systemic administration of rapamycin or rapalogs versus placebo, Outcome 5: Change in attention and executive function - Stockings of Cambridge (CANTAB)****Analysis 1.6. Comparison 1: Systemic administration of rapamycin or rapalogs versus placebo, Outcome 6: Socialization and behaviour (Social Cognition - SRS)**

Analysis 1.7. Comparison 1: Systemic administration of rapamycin or rapalogs versus placebo, Outcome 7: Adverse events

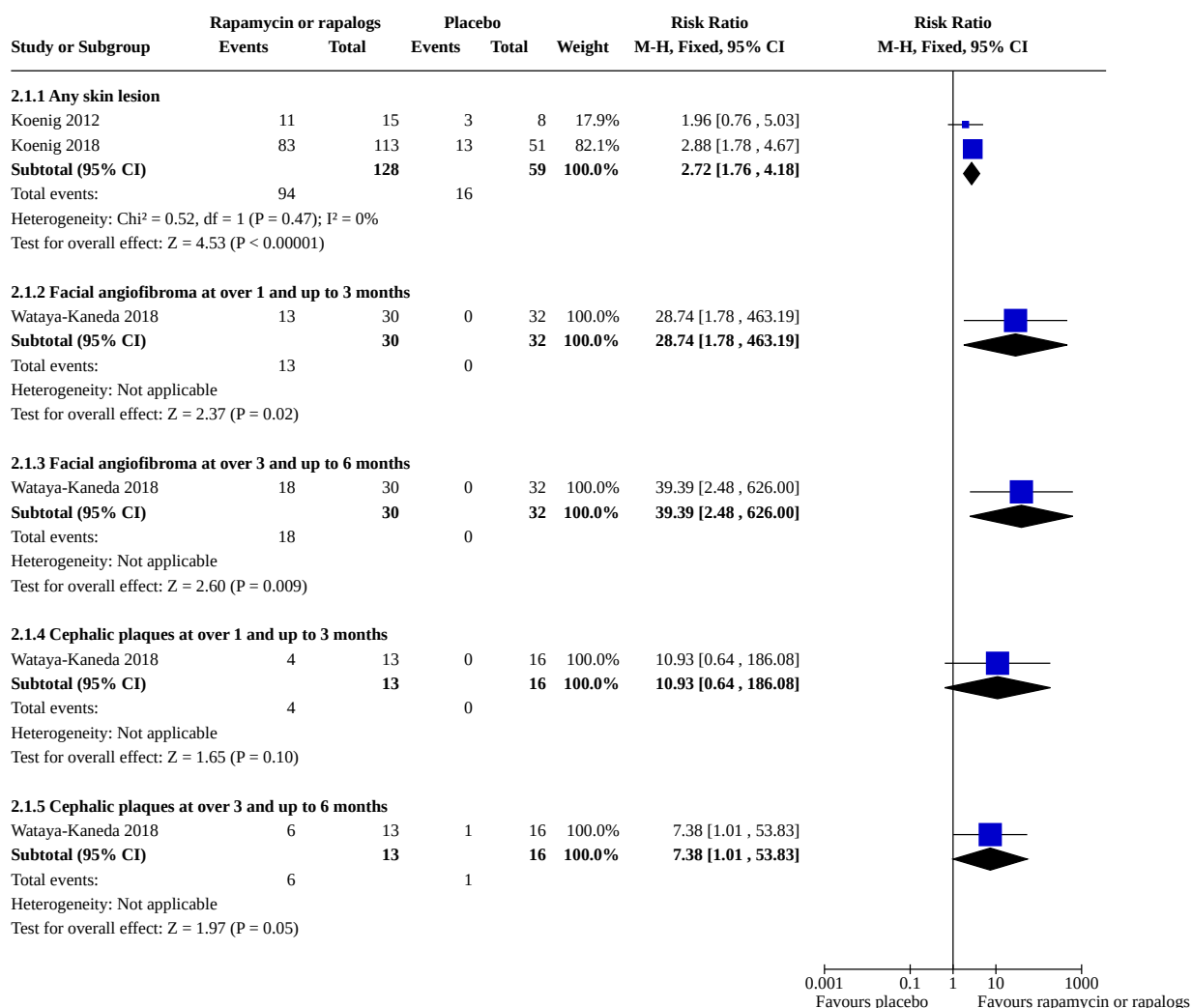


Comparison 2. Topical administration of rapamycin or rapalogs versus placebo

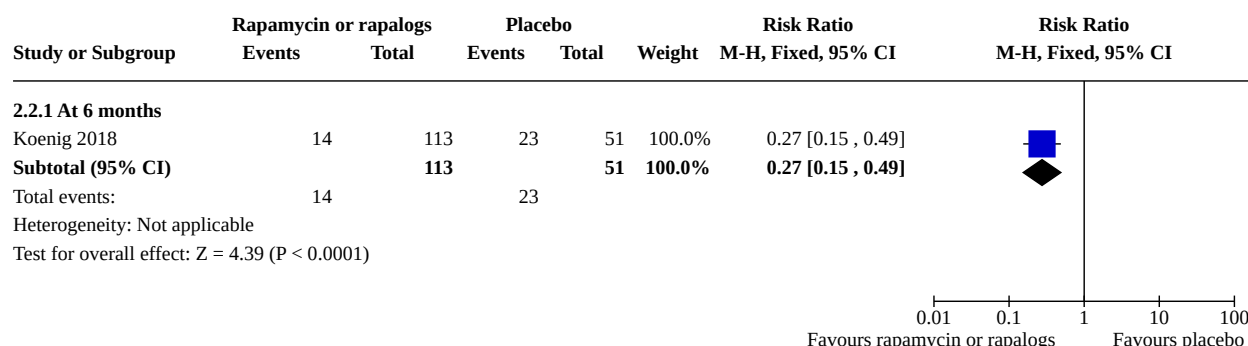
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
2.1 Improvement in skin lesions	3		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
2.1.1 Any skin lesion	2	187	Risk Ratio (M-H, Fixed, 95% CI)	2.72 [1.76, 4.18]
2.1.2 Facial angiofibroma at over 1 and up to 3 months	1	62	Risk Ratio (M-H, Fixed, 95% CI)	28.74 [1.78, 463.19]
2.1.3 Facial angiofibroma at over 3 and up to 6 months	1	62	Risk Ratio (M-H, Fixed, 95% CI)	39.39 [2.48, 626.00]
2.1.4 Cephalic plaques at over 1 and up to 3 months	1	29	Risk Ratio (M-H, Fixed, 95% CI)	10.93 [0.64, 186.08]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
2.1.5 Cephalic plaques at over 3 and up to 6 months	1	29	Risk Ratio (M-H, Fixed, 95% CI)	7.38 [1.01, 53.83]
2.2 Deterioration in skin lesions	1		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
2.2.1 At 6 months	1	164	Risk Ratio (M-H, Fixed, 95% CI)	0.27 [0.15, 0.49]
2.3 Mean general improvement in facial angiofibroma at 12 weeks	1	36	Mean Difference (IV, Fixed, 95% CI)	-1.01 [-1.68, -0.34]
2.3.1 Child Group	1	18	Mean Difference (IV, Fixed, 95% CI)	-1.50 [-2.64, -0.36]
2.3.2 Adult Group	1	18	Mean Difference (IV, Fixed, 95% CI)	-0.75 [-1.58, 0.08]
2.4 Change in quality of life (combined DLQI and CDLQI)	1		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
2.4.1 At 6 months	1	62	Mean Difference (IV, Fixed, 95% CI)	0.30 [-1.01, 1.61]
2.5 Mean participant satisfaction at 12 weeks	1	36	Mean Difference (IV, Fixed, 95% CI)	-0.92 [-1.79, -0.05]
2.5.1 Child Group	1	18	Mean Difference (IV, Fixed, 95% CI)	-1.50 [-2.69, -0.31]
2.5.2 Adult Group	1	18	Mean Difference (IV, Fixed, 95% CI)	-0.25 [-1.52, 1.02]
2.6 Adverse events	3		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
2.6.1 Any adverse event	3	277	Risk Ratio (M-H, Random, 95% CI)	1.72 [1.10, 2.67]
2.6.2 Severe Adverse Events	1	179	Risk Ratio (M-H, Random, 95% CI)	0.78 [0.19, 3.15]

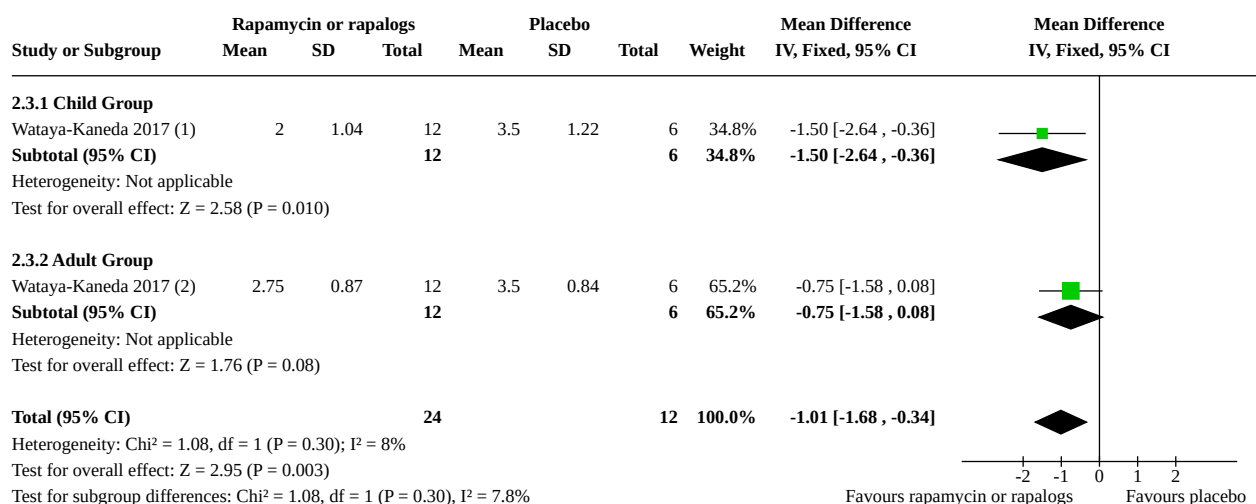
Analysis 2.1. Comparison 2: Topical administration of rapamycin or rapalogs versus placebo, Outcome 1: Improvement in skin lesions



Analysis 2.2. Comparison 2: Topical administration of rapamycin or rapalogs versus placebo, Outcome 2: Deterioration in skin lesions



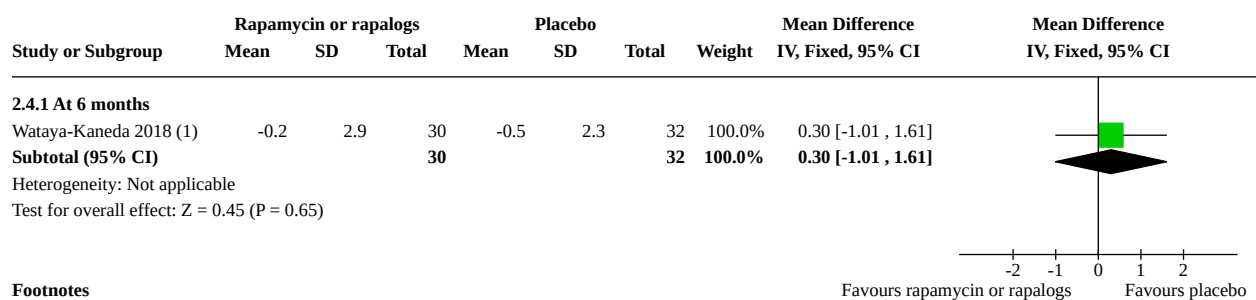
Analysis 2.3. Comparison 2: Topical administration of rapamycin or rapalogs versus placebo, Outcome 3: Mean general improvement in facial angiofibroma at 12 weeks



Footnotes

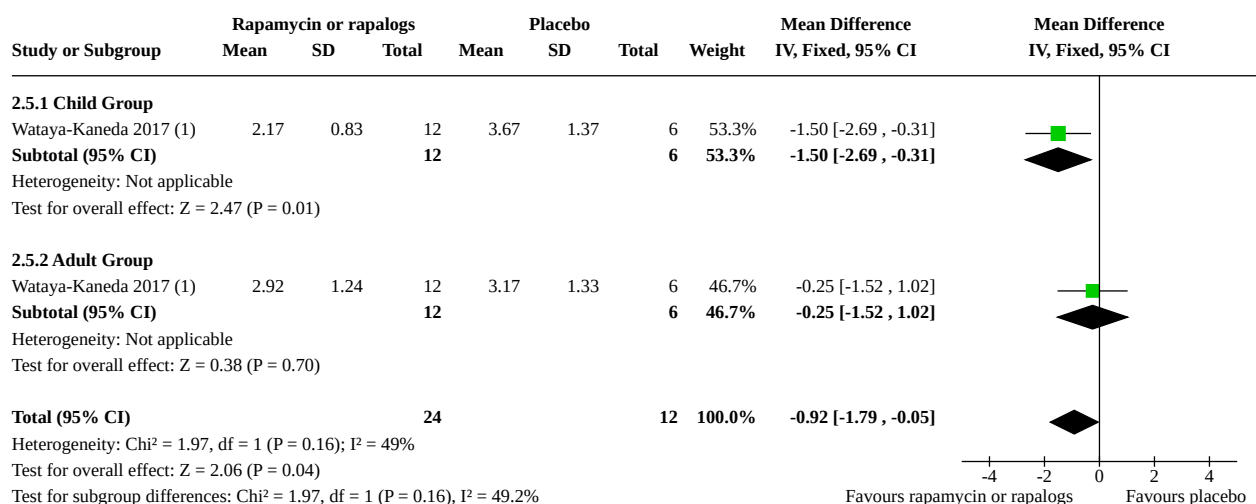
- (1) Intervention used was sirolimus. Scoring system such that 1 shows remarkable improvement up to 5 showing worse condition.
(2) Intervention used was sirolimus. Scoring system such that 1 shows remarkable improvement up to 5 showing worse condition

Analysis 2.4. Comparison 2: Topical administration of rapamycin or rapalogs versus placebo, Outcome 4: Change in quality of life (combined DLQI and CDLQI)

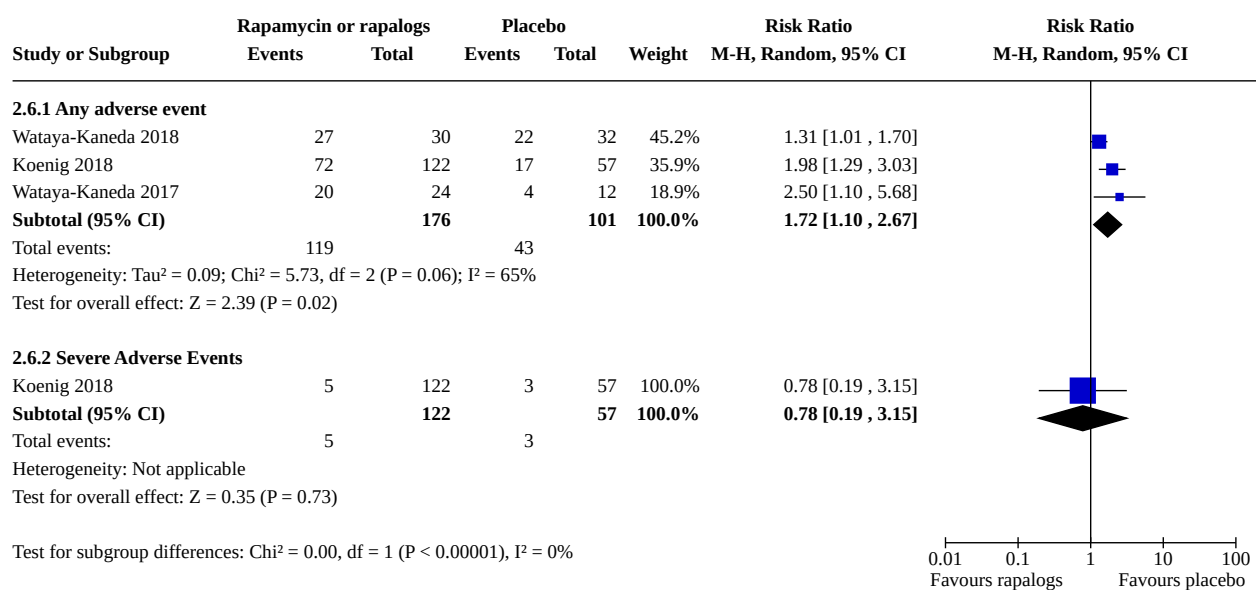


Footnotes

- (1) Intervention used was sirolimus. Combined Dermatology Life Quality Index (DLQI) and Child Dermatology Life Quality Index (CDLQI)

Analysis 2.5. Comparison 2: Topical administration of rapamycin or rapalogs versus placebo, Outcome 5: Mean participant satisfaction at 12 weeks**Footnotes**

(1) Intervention used was sirolimus.

Analysis 2.6. Comparison 2: Topical administration of rapamycin or rapalogs versus placebo, Outcome 6: Adverse events**ADDITIONAL TABLES****Table 1. List of adverse events in oral (systemic) administration of everolimus (rapalog) updated for 2020 review**

Adverse events	Everolimus (n = 404)	Placebo (n = 197)
Stomatitis	209 (51.7%)	22 (11.1%)

Table 1. List of adverse events in oral (systemic) administration of everolimus (rapalog) updated for 2020

review (Continued)		
Nasopharyngitis	70 (17.3%)	40 (20.3%)
Acne-like skin lesions/Acne	28 (6.9%)	5 (2.5%)
Rash	29 (7.2%)	5 (2.5%)
Convulsion	18 (4.5%)	10 (5.1%)
Pyrexia	58 (14.3%)	12 (6.1%)
Headache	31 (7.7%)	13 (6.6%)
Cough	52 (12.9%)	13 (6.6%)
Hypercholesterolaemia	31 (7.7%)	2 (1.0%)
Hypertriglyceridaemia	14 (3.5%)	2 (1.0%)
Aphthous stomatitis	15 (3.7%)	4 (2.0%)
Fatigue	25 (6.2%)	8 (4.1%)
Mouth ulceration	38 (9.4%)	4 (2.0%)
Nausea	13 (3.2%)	5 (2.5%)
Urinary tract infection	12 (3.0%)	6 (3.0%)
Bronchitis	8 (2.0%)	4 (2.0%)
Ear infection/ Otitis media	17 (4.2%)	3 (1.5%)
Pharyngitis	22 (5.4%)	2 (1.0%)
Vomiting	52 (12.9%)	18 (9.1%)
Anaemia	10 (2.5%)	1 (0.5%)
Arthralgia	10 (2.5%)	2 (1.0%)
Diarrhoea	68 (16.8%)	10 (5.1%)
Abdominal pain	9 (2.2%)	3 (1.5%)
Blood lactate dehydrogenase increased	9 (2.2%)	2 (1.0%)
Hypophosphataemia	9 (2.2%)	0 (0.0%)
Eczema	8 (2.0%)	3 (1.5%)
Leucopenia	8 (2.0%)	3 (1.5%)
Oropharyngeal pain	8 (2.0%)	4 (2.0%)
Upper respiratory tract infection	55 (13.6%)	24 (12.2%)

Table 1. List of adverse events in oral (systemic) administration of everolimus (rapalog) updated for 2020

review (Continued) Decreased appetite	19 (4.7%)	7 (3.6%)
Epistaxis	10 (2.5%)	1 (0.5%)
Influenzae	12 (3.0%)	4 (2.0%)
Rhinorrhoea	10 (2.5%)	1 (0.5%)

Data were analysed from 3 studies ([Bissler 2013 \(EXIST 2\)](#); [Franz 2013 \(EXIST 1\)](#); [French 2016 \(EXIST 3\)](#)), **excluding** other 2 studies [Krueger 2017](#), [Overwater 2019](#) due to general adverse events data.

APPENDICES

Appendix 1. Electronic search strategies

Database	Search strategy	Date last searched
CENTRAL	A. MeSH-term-based search strategy: #1 MeSH descriptor Tuberous Sclerosis explode all trees #2 MeSH descriptor Sirolimus explode all trees #3 MeSH descriptor Everolimus explode all trees #4 #2 OR #3 #5 #1 AND #4 B. Free-text-based search strategy: (tuberous sclerosis OR Adenoma Sebaceum OR Bourneville* Disease OR Bourneville Phacomatosis OR Bourneville Phakomatosis OR Bourneville Pringle* Disease OR Bourneville Syndrome OR Bourneville Syndrome) AND ((Sirolimus OR Rapamycin OR Rapamune OR I-2190A OR I2190A OR I 2190A OR AY 22-989 OR AY 22989 OR AY 22 989) OR (Everolimus OR 001, RAD OR 40-O-(2-hydroxyethyl)-rapamycin OR Afinitor OR Certican OR RAD 001 OR RAD, SDZ OR RAD001 OR SDZ RAD OR SDZ-RAD) OR (temsirolimus OR torisel OR CCI-779 OR biolimus A9 OR zotarolimus OR ABT-578 OR deforolimus OR AP23573 OR MK-8669 OR ridaforolimus)):ti,ab,ky C. Filtering options for CENTRAL: We searched trial records only without any other filtering.	15 July 2022 (Issue 7, 2022)
Ovid MEDLINE	1 randomized controlled trial.pt. 2 controlled clinical trial.pt. 3 randomized.ab. 4 placebo.ab. 5 drug therapy.fs. 6 randomly.ab. 7 trial.ab.	15 July 2022

(Continued)

- 8 groups.ab.
- 9 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8
- 10 exp animals/ not humans.sh.
- 11 9 not 10
- 12 exp Tuberous Sclerosis Complex/
- 13 (tuberous sclerosis OR adenoma sebaceum OR bourneville* disease OR bourneville phacomatosis OR bourneville phakomatosis OR bourneville pringle* disease OR bourneville syndrome OR bourneville syndrome)
- 14 12 or 13
- 15 exp Sirolimus/
- 16 exp Everolimus/
- 17 (sirolimus OR rapamycin OR rapamune OR I-2190A OR I2190A OR I 2190A OR AY 22-989 OR AY 22989 OR AY 22 989)
- 18 (everolimus OR 001, RAD OR 40-O-(2-hydroxyethyl)-rapamycin OR Afinitor OR Certican OR RAD 001 OR RAD, SDZ OR RAD001 OR SDZ RAD OR SDZ-RAD)
- 19 (temsirolimus OR torisel OR CCI-779 OR biolimus A9 OR zotarolimus OR ABT-578 OR deforolimus OR AP23573 OR MK-8669 OR ridaforolimus)
- 20 15 or 16 or 17 or 18 or 19
- 21 11 and 14 and 20

Clinicaltrials.gov	ADVANCED SEARCH FORM	15 July 2022
	Condition or disease: tuberous sclerosis OR adenoma sebaceum OR bourneville* disease OR bourneville phacomatosis OR bourneville phakomatosis OR bourneville pringle* disease OR bourneville syndrome OR bourneville syndrome	
	Phase: Phase 2, Phase 3, Phase 4	
WHO ICTRP	ADVANCED SEARCH FORM	15 July 2022
	Condition: tuberous sclerosis OR adenoma sebaceum OR bourneville* disease OR bourneville phacomatosis OR bourneville phakomatosis OR bourneville pringle* disease OR bourneville syndrome OR bourneville syndrome	
	Phases: Phase 2, Phase 3, Phase 4	

WHAT'S NEW

Date	Event	Description
11 July 2023	New citation required and conclusions have changed	Conclusion currently covers more outcome areas namely seizure frequency and skin lesions. Conclusion on adverse events was changed.
11 July 2023	New search has been performed	New searches were carried out on 15 July 2022. We have included an additional seven studies (bringing the total number of

Date	Event	Description
		included studies to 10) and excluded an additional 48 studies (bringing the total number of excluded studies to 68).
		We now present the data by systemic administration (six studies) and topical administration (four studies).

HISTORY

Protocol first published: Issue 9, 2014

Review first published: Issue 7, 2016

CONTRIBUTIONS OF AUTHORS

Original review

NFDI searched for studies and applied the inclusion criteria for including studies into this review as well as extracted the data from the included studies and entered the data into RevMan. THS and NFDI carried out the analyses. THS and ZAMH interpreted the analyses. THS and ZAMH drafted the final review. THS and ZAMH secured funding for the review. All authors approved the final version of the review.

2022 update

SCSH searched for studies and applied the inclusion criteria for including studies into this review. SCSH and TXYJ extracted the data from the included studies and entered the data into RevMan. THS and KK verified the study screening and data extraction as well as carried out the analyses. THS, KK and ZAMH interpreted the analyses. THS, ZAMH, and KK drafted the final review. THS and ZAMH secured funding for the review. All authors approved the final version of the review.

DECLARATIONS OF INTEREST

Teguh Haryo Sasongko: none known.

ZAMH Zabidi-Hussin: attended as a consultant for Affinitor® at two meetings held by Novartis.

Kumaraswamy Kademane: none known.

Stanley Chai Soon Hou: none known.

Tan Xin Yi Jocelyn: none known.

SOURCES OF SUPPORT

Internal sources

- Grants, Malaysia

This work is supported by grants from the Universiti Sains Malaysia (1001/PPSP/812137 for Teguh Haryo Sasongko and 304/CNEURO/652205/K134 for Zabidi Azhar Mohd. Hussin).

External sources

- National Institute for Health Research, UK

This systematic review was supported by the National Institute for Health Research, via Cochrane infrastructure funding to the Cochrane Cystic Fibrosis and Genetic Disorders Group.

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

We included in this review additional secondary outcomes, skin lesion response and other outcomes relevant to individuals' well-being.

At the time of protocol writing, we did not consider other outcomes as previously listed in our protocol. However, we now realise the clinical relevance of 'skin lesion response' which is measured in the studies included in the original review (Bissler 2013 (EXIST 2); Franz 2013 (EXIST 1); Koenig 2012). In addition, because tuberous sclerosis complex is a systemic disorder affecting multiple organs and systems, it is reasonable to consider other outcomes that may affect individuals' well-being. As such, in the current updated version of the review we report quality of life, participant satisfaction, as well as neurocognitive, neuropsychiatry, behavioral, sensory and motor development outcomes.

We highlighted our changes as a post hoc change and added the new outcomes into the summary of findings tables.

Whenever applicable, we separated the analyses of efficacy and adverse effects into systemic administration and topical administration, including the summary of findings tables. For sensitivity analyses, we decided to remove the requirement "if 10 or more studies are included in the review" in view of the fact that this disorder is rare. For an assessment of heterogeneity, we decided to remove the following sentence "When there are more than five studies included, the authors will test for heterogeneity between studies using a standard χ^2 test and I^2 statistic". We added rapalogs to the search terms.

INDEX TERMS

Medical Subject Headings (MeSH)

*Angiofibroma; *Angiomyolipoma; *Astrocytoma [drug therapy]; Everolimus [adverse effects]; *Kidney Neoplasms [drug therapy]; MTOR Inhibitors; Sirolimus [adverse effects]; *Tuberous Sclerosis [complications] [drug therapy]

MeSH check words

Adult; Humans; Male