Efficacy and safety of topical sirolimus for the treatment of angiofibromas in tuberous sclerosis: a systematic review and meta-analysis

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Abstract

Background: Facial angiofibromas (FAs) are common skin manifestations of tuberous sclerosis complex (TSC) that occur in up to 80% of patients. Rapamycin seems to be effective in decreasing FAs. Objective: The aim of our study was to investigate the efficacy and safety of topically applied rapamycin in TSC patients with FAs. Methods: The methods and the results were carried out according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement. PubMed/MEDLINE, SCOPUS, and Cochrane database were systematically searched until April 21, 2022, using the PICO tool (Patient, Interventions, Comparisons, Outcome). Studies regarding efficacy and/or safety of topical sirolimus for the treatment of FAs in TSC with a published full-text in English were included. Safety was assessed based on adverse effects and sirolimus' blood levels, and efficacy was documented by clinical improvement and reduction of Facial Angiofibroma Severity Index (FASI). For Meta-analysis, Review Manager (RevMan) 5.4.1 software was used, using random-effects model and standardized mean difference (SMD) with 95% confidence interval (CI). Results: Twenty-one final studies were included. Regarding safety, in the included studies the observed adverse effects were mainly local, while the blood levels of rapamycin were within safe limits, decreasing the likelihood of systemic immunosuppression. The meta-analysis revealed a statistically significant decrease in post-treatment FASI (SMD: -1.31, 95% CI: [-1.85,-0.77], p-value <0.00001). Subgroup and sensitivity analyses indicated similar findings. No publication bias was found to this association. Conclusion: The application of topical sirolimus to FAs can safely decrease their severity in patients with TSC.

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Abstract

Background: Facial angiofibromas (FAs) are common skin manifestations of tuberous sclerosis complex (TSC) that occur in up to 80% of patients. Rapamycin seems to be effective in decreasing FAs.

Objective: The aim of our study was to investigate the efficacy and safety of topically applied rapamycin in TSC patients with FAs.

Methods: The methods and the results were carried out according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement. PubMed/MEDLINE, SCOPUS, and Cochrane database were systematically searched until April 21, 2022, using the PICO tool (Patient, Interventions, Comparisons, Outcome). Studies regarding efficacy and/or safety of topical sirolimus for the treatment of FAs in TSC with a published full-text in English were included. Safety was assessed based on adverse effects and sirolimus' blood levels, and efficacy was documented by clinical improvement and reduction of Facial Angiofibroma Severity Index (FASI). For Meta-analysis, Review Manager (RevMan) 5.4.1 software was used, using random-effects model and standardized mean difference (SMD) with 95% confidence interval (CI).

Results: Twenty-one final studies were included. Regarding safety, in the included studies the observed adverse effects were mainly local, while the blood levels of rapamycin were within safe limits, decreasing the likelihood of systemic immunosuppression. The meta-analysis revealed a statistically significant decrease in post-treatment FASI (SMD: -1.31, 95% CI: [-1.85,-0.77], p-value <0.00001). Subgroup and sensitivity analyses indicated similar findings. No publication bias was found to this association.

Conclusion: The application of topical sirolimus to FAs can safely decrease their severity in patients with TSC.

Keywords: rapamycin; sirolimus; angiofibromas; tuberous sclerosis; FASI

Introduction

Sirolimus is a compound obtained from Streptomyces hygroscopicus, which was isolated in 1975 [1], after being collected in a soil sample in Easter Island (Rapa Nui) in 1965[2] and was first approved by the FDA for use in 1999 [3]. Since then, it has been used as an anticancer agent, such as in patients with advanced renal cell carcinomas, mantle cell lymphomas and endometrial cancer [4], as well as an immunosuppressive agent to prevent allograft rejection in pediatric kidney transplants [5]. More recently, because of its role in regulating cell growth and proliferation [6], it has been used in the treatment of various manifestations of Tuberous Sclerosis Complex (TSC) such as renal angiomyolipoma and subependymal giant cell astrocytoma [7]. Additionally, since it inhibits cellular responses to mitogenic stimuli, including critical signaling pathways

that regulate T cell activation [8], it has been involved in various treatments regarding dermatological conditions such as Port-Wine Stain and genodermatosis [9]. Where the last two applications of sirolimus overlap is in the treatment of TSC-related angiofibromas, which has been documented for over a decade [10]. Since angiofibromas manifest in about 80% of patients with TSC, their treatment is the focus of a large number of studies.

While systematic reviews on the use of sirolimus in dermatology and/or in the treatment of various manifestations of TSC have been conducted in 2015 [9], 2016 [7], 2019 [11] and 2021 [12,13], most of them neither included a meta-analysis nor they covered a broader spectrum of dermatological conditions that where treated with topical or even systemic application of sirolimus. We focused on reviewing studies that measured or described its potency as well as its safety as a topically applied agent against TSC-related angiofibromas, covering almost a decade's worth of studies, before performing our meta-analysis. As a result, we provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).

We reviewed both randomized and non-randomized clinical trials, as well as retrospective and cohort studies that assessed the effectiveness and safety of different concentrations of topically applied sirolimus compared either to placebo or previously received standard care (e.g. cryosurgery, ablative laser therapy), which was most often deemed unsatisfactory. The Facial Angiofibroma Severity Index (FASI) and Quality of Life (QOL) questionnaires to determine improvements for pediatric and adult populations that demonstrate TSC-related angiofibromas were utilized. Our objective is to provide an up-to-date overview of the efficacy and safety of topically applied sirolimus, as an alternative treatment for facial angiofibromas that result from TSC.

Materials and Methods

This systematic review was conducted based on the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) statement [14]. It is in line with the PRISMA checklist. The review protocol has been registered to PROSPERO (International Prospective Register of Systematic Reviews) with Identifier (ID) Number: CRD42022330123.

Inclusion and Exclusion Criteria

We searched for Randomized Controlled Trials (RCTs), Cohort and Case-Control studies examining the efficacy and safety of topical sirolimus treatment for facial angiofibromas in patients with tuberous sclerosis. We included only studies with a published full text in English.

Search Strategy and Sources

The research strategy was designed based on the Peer Review of Electronic Search Strategies (PRESS) checklist [15] using free text and Medical Subject Heading (MeSH) terms and their synonyms. Search terms were "sirolimus", "topical", "angiofibromas" and "tuberous sclerosis" with synonyms and alternatives. Apart from language, no filters, geographical, publication status, and year restrictions were applied.

The following databases were searched by two reviewers (EP, PT) independently: Pubmed/ Medline, Scopus, and Cochrane Library. PROSPERO (International Prospective Register of Systematic Reviews) database was likewise searched for ongoing SRMAs. The last searches were conducted on the 21st of April 2022.

Study Selection and Data Extraction

Two reviewers (EP, PT) conducted study selection and data extraction separately. Any discrepancies were resolved by a third reviewer (GNK) through discussion and consensus. Mendeley© (v.1.19.8) was used as a reference manager, and duplicates were removed. Predefined collection forms proposed by Cochrane collaboration for Intervention Reviews [16] were used for data extraction. In case of questions about study eligibility or data provided by the studies, the paper authors were contacted.

Risk of Bias Assessment

We used the ROBINS-I Cochrane Tool for assessing the risk of bias in non-randomized studies [17]. Studies with low/moderate risk of bias were included in the quantitative synthesis. A sensitivity analysis was conducted for studies with serious/critical risk of bias. Graphics visualizing the risk of bias were created using the Robvis tool [18]. Two reviewers (EP and PT) independently conducted the risk of bias assessment, and the third reviewer (GNK) settled any discrepancies.

Synthesis

The treatment effect of all outcomes was measured using mean/median, SD/IQR with 95% Confidence Interval (CI), as all of them were quantitative data. First, a robust qualitative synthesis was conducted. Second, we conducted a quantitative synthesis with RevMan (v.5.4.1). Different forest plots were created. Statistical heterogeneity was evaluated using the Higgins I^2 test and Chi-Squared Cochran Q-test (α=0.1). When I^2 was over 75%, was regarded as high statistical heterogeneity. Inverse Variance statistical method with standardized mean difference (SMD) as effect measure was conducted. The random-effects model was applied due to the heterogeneity of the studies. We conducted sensitivity analysis (excluding studies of serious/critical risk of bias). Subgroup analyses were performed based on treatment dosage (0.1% vs >0.1%), continent (Europe vs Asia) and treatment type (ointment vs cream). In case of missing data, we tried to contact the authors by e-mail.

Publication bias was assessed for FASI (Facial Angiofibroma Severity Index). RevMan 5.4.1 was used to create funnel plots.

Results

Search Results

The flow diagram of our search strategy results is shown in Figure 1. After the removal of 106 duplicates, 219 studies were screened per Title and Abstract. A total of 22 studies qualified for assessment of eligibility. Finally, 1 study [19] was excluded according to the exclusion criteria, while 21 studies [20–40] were found eligible for qualitative and quantitative analysis including 683 patients having received rapamycin treatment.

Study Characteristics

Five studies were randomized clinical trials [22,23,26,28,31], while 15 where non-randomized studies [20,21,24,25,27,29,30,32,34–40] (Table 1). Five studies [21,25,35,37,39] were conducted in Europe, 11 studies [22,24,26,28–32,36,38,40] were conducted in Asia, 2 studies [20,27] were conducted in Americas, one study [34] was conducted in Oceania, and one study [23] was conducted in Americas and Oceania. Of the 20 included studies, 5 studies [25,30,34,38,40] were conducted only in paediatric population. Regarding the dosage of rapamycin, in 8 studies [20,27,28,34,35,37,38,40] rapamycin [?] 0.1% was used, while in 12 studies [21–26,29–32,36,39] rapamycin [?] 0.1% was used.

Risk of Bias Assessment

The Risk of Bias Assessment regarding the efficacy outcomes revealed one study [28] with low risk of bias, 17 studies [20–24,26,27,29–34,36–38,41] with moderate risk of bias or some concerns and 3 studies [25,39,40] with high risk of bias (Figure 2).

Qualitative Analysis

Efficacy

The measures for the efficacy outcomes of each study are shown in Table 1. Seven out of the 21 studies used the Facial Angiofibroma Severity Index (FASI) [21,24,36–40], 2 used a modified version (mFASI) [25,28] and one study used the Angiofibroma Grading Scale (AGS) [23] as an indicator for the drug's efficacy. Four studies used Quality of Life questionnaires such as the Dermatology Quality of Life Index (DLQI) [22,23,31] which was modified for children (CDLQI) and families (FDLQI) or the 36 Item Short Form Health Survey (SF-36) [29,37], 4 studies estimated the improvement levels of the patients [20,30,34,41], 3 studies used photographs for appearance evaluation [22,23,27], 2 studies used improvement scales [32,33] and one study

used improvement factor determination [26]. Due to the significant number of different formats, as well as the fact that "the DLQI questionnaire may not be established as an appropriate QOL instrument for TSC-related facial angiofibroma" and was thought to not be "sensitive enough to capture the effect of the disease at baseline" and as a result "not an appropriate tool for the assessment of any change from baseline in Quality of Life" [23], the Quality of Life questionnaires were not included in Table 2, where the rest of the outcome measures are shown. All studies concluded that the treatment is effective. Twelve of the 21 studies [22,24–26,30–32,34,37,38,40,41] support the fact that paediatric patients have quicker results and an overall greater improvement regarding redness and flattening compared to adults, especially during the first 12 weeks [22,31].

Safety

Out of the 21 total studies, 14 mentioned any type of adverse events as a result of the treatment (Table 1), 6 of which mentioned that the adverse events were mild or moderate (Table 3). Ten out of the 21 studies [22–26,29,31,38,39,41] mentioned irritation, such as at the site of application or of the patients' skin. Eight studies [22,23,25,28–31,39] mentioned acne as an adverse event, as an aggravation of already present inflammatory acne, as drug-induced or at the site of application. Six studies [22–24,28,31,37] reported on the appearance of erythema, 5 studies [22,23,28,29,31] mentioned pruritus as an adverse event of the treatment and 4 studies [22,26,29,31] mentioned dry skin. Two studies [22,31] mentioned dermatitis acneiform, dermatitis contact and eye irritation. One study [34] mentioned perioral dermatitis, one study [24] mentioned increased sebum, one study [28] mentioned burning/stinging, one study [23] mentioned pain at the application site and one study [23] mentioned cutaneous eruption. Two studies [22,23] mentioned multisystemic adverse events such as nasopharyngitis and stomatitis. However, only the adverse events that were dermatologic in nature were found to be significantly higher in the group that was treated than in the placebo group and as such, only they were considered related to the treatment. No study mentions systemic absorption of rapamycin as the cause of any side effects, as blood levels were either undetectable or well below the lower limit that is required to induce immunosuppression (5-15 ng/mL or 8-20 μg/L or 2.6 mmol/L) [42]. It should be noted that any adverse events that occurred in vehicle only or placebo groups were not included in Table 3.

Quantitative Analysis

We meta-analyzed the results of seven studies [21,24,25,28,36–38] that assessed the Facial Angiofibroma Severity Index (FASI) at baseline and post-treatment period. The meta-analysis showed a statistically significant reduction of the FASI post-treatment [SMD: -1.31, 95% CI (-1.85,-0.77); $p < 0.00001$; I^2: 74%] (Figure 3). No publication bias was detected (Figure 4). We conducted a sensitivity analysis by excluding the study with high risk of bias, which showed no alteration in our results [SMD: -1.51, 95% CI (-2.13,-0.89); $p < 0.00001$; I^2: 76%] (Figures 5 and 6). The subgroup analysis by dosage showed statistically significant reduction in both rapamycin [?]0.1% and [?]0.1%, with a slight superiority of the [?]0.1% dosage (Figure 7). The subgroup analysis by moisturizer showed statistically significant reduction in both ointment and cream, with a superiority of ointment (Figure 8). The subgroup analysis by region showed statistically significant reduction in both European and Asian patients, with a superiority in the Asia ones, but the high statistical heterogeneity (82%) of the result (Figure 9) makes the quantitative synthesis prohibitive.

Discussion

Tuberous sclerosis complex is a genodermatosis marked by extensive hamartomas in several organs such as the brain, heart, skin, eyes, kidney, lung, and liver[43]. TSC is caused by mutations in the tuberous sclerosis 1 and 2 genes, which cause overactivation of the mTOR signaling pathway that regulates cell growth, proliferation, and survival. TSC1 encrypting hamartin and TSC2 encrypting tuberin are responsible for TSC [6]. One of the most frequently affected organs is the skin. Skin manifestations of the syndrome are angiofibromas, facial angiofibromas, subungual, periungual and scalp fibromas, connective tissue nevi on the back and white, oval, leaf-shaped macules. Among these, angiofibromas are the most common and cosmetically concerning [44]. Invasive treatments such as cryosurgery, dermabrasion, photodynamic therapy and ablative or pulsed dye laser therapies have been tried in patients with facial angiofibromas, but provided

limited therapeutic benefits in the long run [45–47].

Rapamycin is a lipophilic macrocyclic lactone produced by the Easter Island soil bacterium Streptomyces hygroscopicus. It was revealed to have anti-fungal effects and later anti-T-cell activity, making it beneficial as an immunosuppressant in the prevention of graft rejection. Currently, rapamycin belongs to the class of anti-cancer medications known as mTOR inhibitors due to its effectiveness as a tumor growth suppressor [48]. Patients with TSC exhibit abnormal mTOR activation in fibroblast-like cells of the dermis which results in the synthesis of epiregulin, a growth factor that stimulates the proliferation of epidermal cells [49]. Rapamycin inhibits the mTOR pathway, which may diminish the production of vascular endothelial growth factor (VEGF) since it inhibits the expression of hypoxia-inducible factor and represses endothelial-cell proliferation [50]. Sirolimus has a molecular weight of 914.2 g/mol, allowing for its absorption through the superficial layers of the epidermis to the deep dermis [20].

In the present study, we investigated the efficacy and safety of topical sirolimus treatment for facial angiofibromas in 682 patients with TSC. The topical treatment was in the form of a gel, cream, powder or ointment with a standard concentration of 0.1% to 0.2%. In order to determine the safety of the drug, its blood levels were monitored and the side effects of its application were recorded. All the adverse effects observed were mainly local while blood levels were within safe limits, thus decreasing the likelihood of systemic immunosuppression. Koenig et al.[20] described a case of pneumonia in a subject of their study, who required prolonged hospitalization. This incident was attributed to aspiration during a seizure and was not considered a systemic adverse effect of topical sirolimus as it was not detected in the blood. Furthermore, Wataya-Kaneda et al. [22] reported the development of acute pancreatitis and gastric hemorrhage in a 20-year-old man who was receiving topical sirolimus. The patient recovered soon after his hospitalization and there was no need for treatment discontinuation. In order to determine the responsiveness to the drug, parameters such as the papules' size, redness, flatness and the Facial Angiofibroma Severity Index (FASI) scores were evaluated. All of the tested indicators improved, particularly the FASI, for which data were meta-analyzed. We also evaluated the difference in its efficacy depending on dosage, treatment formulation and the continent tested. More specifically, the subgroup analysis revealed that sirolimus' concentration [?]0.1% is more effective than that of [?]0.1%, as well as ointment is preferable to cream as regards the selection of moisturizer. In accordance with our results, Wataya-Kaneda et al. mention that the optimal concentration of topical sirolimus is 0.2% [22,26]. Tanaka et al. investigated the *in vitro* percutaneous absorption of rapamycin and deduced that it was significantly greater with the gel compared with the ointment. However, there was no evidence of this in our review. Moreover, according to seven studies, young children appear to respond better to treatment than adolescents and adults [24,25,32,36,37,41] and most authors suggest early initiation of the medication so as to achieve the best results. Nevertheless, our review found no proof of this, as we did not perform an age-related subgroup analysis. Last but not least, regarding the follow-up period after discontinuation of treatment, Chen et al.[28] and Okanishi et al.[30] underlined that a recurrence of the lesion was noticed, although the severity was still significantly milder than at baseline. On the contrary, Wataya-Kaneda et al.[22], Cinar et al. [38] and Tanaka et al. [32] observed a relapse shortly after treatment, emphasizing its transient effectiveness. Malissen et al.[39] reported that 6 patients relapsed within 7 months and 1 was still responding at 1 year of treatment, which raises interest about the causes of patients' different post-treatment clinical severity.

As far as we are aware, our meta-analysis is more focused on the management of angiofibromas related to TSC and thus more detailed in comparison with previous studies. Namely, our meta-analysis included 669 cases, a sample much larger than the population examined by Leducq et al.[11] and Balestri et al.[48], who included a total of 157 and 84 participants respectively. In addition, apart from the quantitative analysis of pre- and post-treatment FASI, we performed three different subgroup analyzes to reinforce our conclusions.

Limitations : Finally, it is essential to clarify the limitations of the present study. Firstly, owing to the lack of randomized clinical trials in literature, we have also included in our review and meta-analysis cohort and prospective and retrospective studies with a full-text available in English. As a result, articles with content relevant to our study but written in another language may have been overlooked. Secondly,

both the baseline characteristics of the research population (sex, age, comorbidities) and the methodology varied among the included studies (concentration, dosage, type and duration of treatment, co-interventions). Thirdly, it should be noted that the assessment of the clinical picture of patients before and after treatment was based on certain criteria such as redness, flatness or FASI, which depend on the subjective judgment of the examiner. Such variations may have influenced the clinical improvement and were confusing factors that could not be weighed out. Last but not least, the majority of the studies were performed in Asia and Europe. Nevertheless, according to the I^2 test the heterogeneity was low.

Conclusions

In conclusion, in accordance with the systematic review and meta-analysis performed, topical sirolimus is a safe and effective medication for facial angiofibromas linked to TSC.

Declarations:

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

Conflicts of interest: The authors have no conflicts of interest to disclose.

Authors' Individual Contributions : Eleni Paschalidou and Georgios N. Katsaras: conceived the study, participated in its design, data collection and interpretation, and also performed the data extraction, as well as the statistical analyses, and helped to draft the manuscript. Philippos Tassioudis: participated in conceptualization and the design of the study, collection and interpretation of the data; and also performed the data extraction, as well as the statistical analyses, and drafting the manuscript. Thomas Papoulakis and Dorothea Kapoukranidou: contributed to the analysis of the results and in drafting the manuscript. Theodora Papamitsou: supervised the methodology and statistical analyses and edited the final draft of the manuscript.

All authors reviewed the work critically for significant intellectual content and approved the final version for publication.

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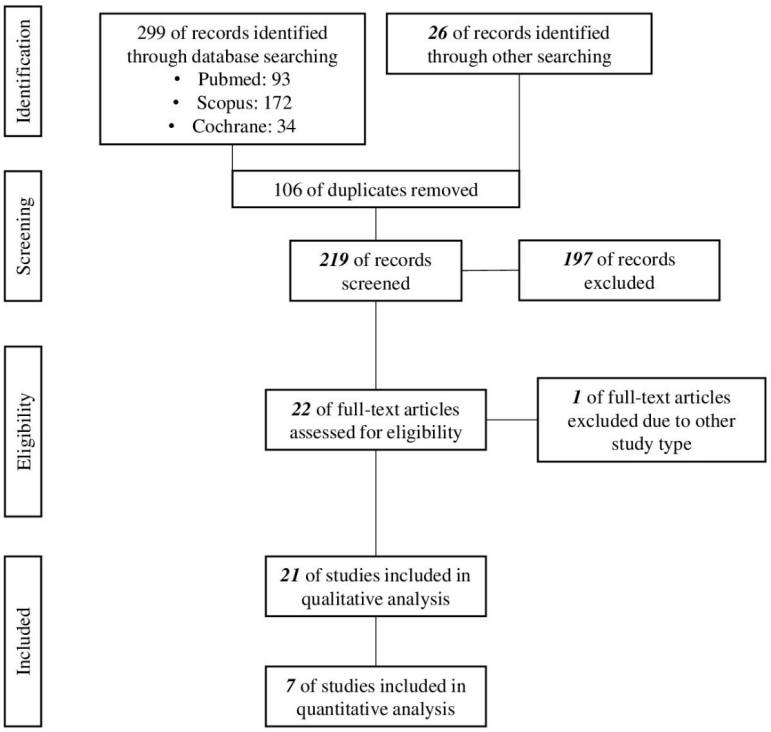

Fig. 1 Flow diagram of PRISMA results

Table 1. Study Characteristics

Author (Year)	Country	Study Design	N of Patients (M:F)	Age (Years)	Dosage	Type of Treatment	Treatment Duration (months)	Control	Efficacy Outcomes
Wataya-Kaneda (2011)	Japan	Pilot	9 (3:6)	9-46*	0.2% rapamycin twice daily	Ointment	3	Yes	Improvemen scale (range 0-4) (size, redness, and flatness)
Foster (2012)	Australia	Cohort	4 (2:2)	5-17*	0.1% rapamycin	Petrolatum and oral solution	6	No	Improvemen level evaluation (percentage)
Koenig (2012)	USA	RCT	28 (15:13)	23ˆ	0.003% or 0.015% sirolimus	Cream	6	Yes	Improvemen level evaluation
Salido (2012)	Spain	Case series	10 (5:5)	6-43* (13)ˆ	0.4% sirolimus	Ointment	9	No	FASI
Tanaka (2013)	Japan	Comparative	11 (7:4)	2-36*	0.2% rapamycin	Ointment and gel	3	Yes	Improvemen scale (range -2 – 4) (size, redness, flatness)
Tu (2014)	Australia	Case series	19 (13:6)	4.5 -18.5* (10.5)ˆ	0.1–1% rapamycin	Crushed tablet and powder	8-30	No	Improvemen level evaluation (percentage)
Viswanath (2016)	India	Cohort	5 (0:5)	6-44*	0.1–1% rapamycin	Ointment	1-6	No	FASI
Amin (2017)	U.K.	Cohort	14 (7:7)	9-40* (16)ˆ	0.1% sirolimus	Ointment	6	No	FASI, PedsQL, SF-36
Cinar (2017)	Turkey	Prospective	12 (7:5)	7-14*	0.1% sirolimus	Cream	3	Yes	FASI

Malissen (2017)	France	Prospective	25 (9:16)	4-47* (14)**	1% sirolimus	Cream	18	No	FASI
Wang (2017)	China	Prospective	29 (19:10)	2-14* (7.9 ± 2.9)ˆ	0.1% sirolimus	Ointment	9	No	FASI
Wataya-Kaneda (2017)	Japan	RCT	36 (13:23)	6-47* (40)**	0.05%, 0.1% and 0.2% sirolimus	Gel	3	Yes	Improvemen Factor (tumor volume reduc-tion and lessen-ing of redness)
Koenig (2018)	USA, Australia	RCT	179 (80:99)	3-61* (20.5)ˆ	1% and 0.1% sirolimus	Cream	6	Yes	DLQI/CDL AGS, EOT photo rating
Lee (2018)	South Korea	Retrospective	36 (16:20)	2-48*	0.2% sirolimus	Ointment	5 (FASI)	No	FASI
Norrenberg (2018)	Switzerland	Retrospective	14 (7:7)	6-18* (11.4 ± 4.7)ˆ	0.1% sirolimus	Cream and powder[a]	6-13 (10.4 ± 4.5) and 7-17 (10±3.4)[a]	No	FASI (modified)

Wataya-Kaneda (2018)	Japan	RCT	62 (28:34)	22.5ˆ	0.2% sirolimus	Gel	3	Yes	Response rates (photo-graph re-view), DLQI/CDL
Wheless (2019)	USA	Prospective	11 (N/A)	9-27*	0.1% rapamycin	Cream	1, 5, 6.5, 7.5 or >12	No	Appearance, redness improvemen
Chen (2020)	Taiwan	RCT	52 (20:32)	7-67* (23)ˆ	0.1% sirolimus or 0.0003% cal-citriol or combination	Ointment	2 3-month periods	No	FASI (modified)
Hatano (2020)	Japan	Prospective	33 (17:16)	14-55* (25)**	0.2% sirolimus	Gel	3	No	SF-36
Okanishi (2020)	Japan	Prospective	9 (5:4)	3.5-11* (7.8)ˆ	0.2% sirolimus	Gel	6	No	Improvemen level evalua-tion (percentage)

Wataya-Kaneda (2020)	Japan	Clinical Trial (open)	94 (44:50)	3-53* (21)ˆ	0.2% sirolimus	Gel	< 13, 13-25.75, >= 26 (maximum 36)	No	Response rates, DLQI/CDL patient satisfaction questionnair

Abbreviations: AF(s), Angiofibroma(s); AGS, Angiofibroma Grading Scale; (C- or F-)DLQI, (Children's or Family) Dermatology Life Quality Index; EOT, End-of-Trial; F, Females; FASI, Facial Angiofibroma Severity Index; M, Males; N/A, Not Available; PedsQL, Pediatric Quality of Life; RCT, Randomized Controlled Trial; SF-36, Short Form

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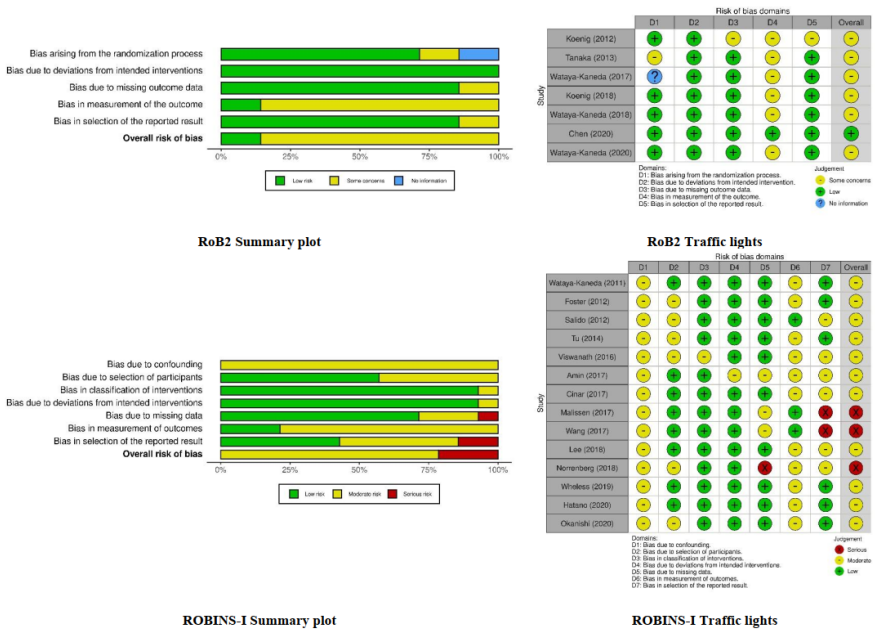

Fig 2. Risk of Bias Assessment

Table 2. Results of Efficacy outcomes.

Author (Year)	FASI_-pre mean (SD)	FASI_-post mean (SD)	AGS_-pre mean (SD)**	AGS_-post mean im-prove-ment	Improvement scale mean (SD)	Improvement factor mean (SD)	% of pts that achieved >50 % im-prove-ment (me-dian months of achieve-ment)	Respon rates o AF
Wataya-Kaneda (2011)	N/A	N/A	N/A	N/A	2.6 / 4 (0.874)	N/A	N/A	N/A
Foster (2012)	N/A	N/A	N/A	N/A	N/A	N/A	75% (1)	N/A
Koenig (2012)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Salido (2012)	6.325 (1.537)	2.725 (1.839)	N/A	N/A	N/A	N/A	N/A	N/A
Tanaka (2013)*	N/A	N/A	N/A	N/A	>2 / 4 (N/A)	N/A	N/A	N/A
Tu (2014)	N/A	N/A	N/A	N/A	N/A	N/A	89% (N/A)	N/A

Viswanath (2016)	6 (1.265)	3.33 (0.816)	N/A	N/A	N/A	N/A	N/A	N/A
Amin (2017)	8.07 (0.73)	6 (1.71)	N/A	N/A	N/A	N/A	N/A	N/A
Cinar (2017) [Left Side]	7.58 (0.9)	5.17 (1.34)	N/A	N/A	N/A	N/A	N/A	N/A
Cinar (2017) [Right Side]	7.42 (0.9)	7.17 (0.83)	N/A	N/A	N/A	N/A	N/A	N/A
Malissen (2017)*	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Wang (2017)*	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Wataya-Kaneda (2017)	N/A	N/A	N/A	N/A	N/A	0.2%: 1.94 (0.68) 0.1%: 1.06 (0.62) 0.05%:1.63(0.95)	N/A	N/A
Koenig (2018)	N/A	N/A	46.4 (31.0)	1%: 16.7 points 0.1%: 11 points Vehicle only: 2.1 points	N/A	N/A	N/A	N/A
Lee (2018)	7.2 (1.1)	4.4 (1.4)	N/A	N/A	N/A	N/A	N/A	N/A
Norrenberg (2018)	5.5 (1.3) (mFASI)	5.1 (1.8) (mFASI)	N/A	N/A	N/A	N/A	N/A	N/A
Norrenberg (2018) [more stable moisturizer]	5.1 (1) (mFASI)	3.6 (1.7) (mFASI)	N/A	N/A	N/A	N/A	N/A	N/A
Wataya-Kaneda (2018)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Childre 85% (size), 46% (color), Adults: 41% (size), 35% (color)
Wheless (2019)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Chen (2020)	-0.35 (0.79) (mFASI)	-1.07 (0.92) (mFASI)	N/A	N/A	N/A	N/A	N/A	N/A
Hatano (2020)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Okanishi (2020)	N/A	N/A	N/A	N/A	N/A	N/A	Papular AF: 100% (3), Miliary AF: 83% (5), redness of AF: 100% (4)	N/A
Wataya-Kaneda (2020)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Childre 74.5% (week 1 74.5% (week 5 Adults: 41.5% (week 1 82.1% (week 5

Abbreviations: AF, Angiofibroma(s); AGS, Angiofibroma Grading Scale; (m)FASI, (modified) Facial Angiofibroma Severity Index; N/A, Not Available; pt(s), patient(s); SD, Standard Deviation. *Tanaka (2013), Malissen (2017) and Wang (2017) do not provide accurate scores regarding pre-treatment and post-treatment, besides the figures within their respective publications.

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Abbrevi AF, Angiofi- broma(s AGS, Angiofi- broma Gradin Scale; (m)FAS (modi- fied) Facial Angiofi- broma Severity Index; N/A, Not Available; pt(s), patient(s) SD, Standard Deviation. *Tanak (2013), Malissen (2017) and Wang (2017) do not provide accurat scores regarding pre-treatme and post-treatme besides the figures within their re- spective publica

Table 3. Results of Safety Outcomes.

Author (Year)	Irritation	Acne (Aggravation, Drug-induced etc.)	Erythema	Pruritus	Dry Skin	Burning/Stinging	Worsening Acneiform	Increased Sebum	Pain (Application Site)	Blood level of mTOR inhibitor	Dermatitis Contact	Perioral Dermatitis	Eye Irritation
Wataya-Kaneda (2011)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	<0.3 ng/mL	N/A	N/A	N/A
Foster (2012)	2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0.8 mmol/L (1 pt)	N/A	N/A	N/A
Koenig (2012)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	<0.1 ng/mL	N/A	N/A	N/A
Salido (2012)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	<0.3 ng/mL	N/A	N/A	N/A
Tanaka (2013)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Undetectable	N/A	N/A	N/A
Tu (2014)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0.5 and 0.8 μg/L (2 pts)	N/A	1	N/A
Viswanath (2016)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Undetectable	N/A	N/A	N/A
Amin (2017)	N/A	N/A	1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Cinar (2017)	3	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Malissen (2017) *	8	3 (aggravation of inflammatory acne)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	<0.2 ng/mL	N/A	N/A	N/A
Wang (2017)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	<1 ng/mL	N/A	N/A	N/A
Wataya-Kaneda (2017) *	10	N/A	N/A	N/A	10	N/A	N/A	N/A	N/A	<0.25 ng/mL	N/A	N/A	N/A

Koenig (2018) *	3	6 (4 at site of application)	3	5	N/A	N/A	N/A	N/A	8	<0.5 ng/mL	N/A	N/A	N/A
Lee (2018)*	3	N/A	3	N/A	N/A	N/A	N/A	3	N/A	N/A	N/A	N/A	N/A
Norrenberg (2018)	3	2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Norrenberg (2018) [more stable moisturizer]	0	1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Wataya-Kaneda (2018)*	11(application site), 1(skin)	2	1	7	11	N/A	1	N/A	N/A	=<0.5 ng/mL	1	N/A	1
Wheless (2019)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Undetectable	N/A	N/A	N/A
Chen (2020)* ^	N/A	2 (Folliculitis/Acne)	3	5	N/A	2	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Hatano (2020)	3	4	N/A	2	3	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Okanishi (2020)	N/A	1 (Drug-induced)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Wataya-Kaneda (2020)	29	19	7	8	26	N/A	6	N/A	N/A	<1 ng/mL	5	N/A	8

Abbreviations:	Abbreviations:	Abbreviations:	Abbreviations:	Abbreviations:	Abbreviations:	Abbreviations:	Abbreviations:	Abbreviations:	Abbreviations:	Abbreviations:	Abbreviations:	Abbreviations:	Abbreviations:
mTOR, mammalian target of rapamycin; N/A, Not Available, pt(s), patient(s); * Mild or moderate side effects ** Referring to application site, unless stated otherwise ^ Safety outcomes regarding solely treatment with rapamycin (calcitriol, as well as ra-	mTOR, mammalian target of rapamycin; N/A, Not Available, pt(s), patient(s); * Mild or moderate side effects ** Referring to application site, unless stated otherwise ^ Safety outcomes regarding solely treatment with rapamycin (calcitriol, as well as ra-	mTOR, mammalian target of rapamycin; N/A, Not Available, pt(s), patient(s); * Mild or moderate side effects ** Referring to application site, unless stated otherwise ^ Safety outcomes regarding solely treatment with rapamycin (calcitriol, as well as ra-	mTOR, mammalian target of rapamycin; N/A, Not Available, pt(s), patient(s); * Mild or moderate side effects ** Referring to application site, unless stated otherwise ^ Safety outcomes regarding solely treatment with rapamycin (calcitriol, as well as ra-	mTOR, mammalian target of rapamycin; N/A, Not Available, pt(s), patient(s); * Mild or moderate side effects ** Referring to application site, unless stated otherwise ^ Safety outcomes regarding solely treatment with rapamycin (calcitriol, as well as ra-	mTOR, mammalian target of rapamycin; N/A, Not Available, pt(s), patient(s); * Mild or moderate side effects ** Referring to application site, unless stated otherwise ^ Safety outcomes regarding solely treatment with rapamycin (calcitriol, as well as ra-	mTOR, mammalian target of rapamycin; N/A, Not Available, pt(s), patient(s); * Mild or moderate side effects ** Referring to application site, unless stated otherwise ^ Safety outcomes regarding solely treatment with rapamycin (calcitriol, as well as ra-	mTOR, mammalian target of rapamycin; N/A, Not Available, pt(s), patient(s); * Mild or moderate side effects ** Referring to application site, unless stated otherwise ^ Safety outcomes regarding solely treatment with rapamycin (calcitriol, as well as ra-	mTOR, mammalian target of rapamycin; N/A, Not Available, pt(s), patient(s); * Mild or moderate side effects ** Referring to application site, unless stated otherwise ^ Safety outcomes regarding solely treatment with rapamycin (calcitriol, as well as ra-	mTOR, mammalian target of rapamycin; N/A, Not Available, pt(s), patient(s); * Mild or moderate side effects ** Referring to application site, unless stated otherwise ^ Safety outcomes regarding solely treatment with rapamycin (calcitriol, as well as ra-	mTOR, mammalian target of rapamycin; N/A, Not Available, pt(s), patient(s); * Mild or moderate side effects ** Referring to application site, unless stated otherwise ^ Safety outcomes regarding solely treatment with rapamycin (calcitriol, as well as ra-	mTOR, mammalian target of rapamycin; N/A, Not Available, pt(s), patient(s); * Mild or moderate side effects ** Referring to application site, unless stated otherwise ^ Safety outcomes regarding solely treatment with rapamycin (calcitriol, as well as ra-	mTOR, mammalian target of rapamycin; N/A, Not Available, pt(s), patient(s); * Mild or moderate side effects ** Referring to application site, unless stated otherwise ^ Safety outcomes regarding solely treatment with rapamycin (calcitriol, as well as ra-	mTOR, mammalian target of rapamycin; N/A, Not Available, pt(s), patient(s); * Mild or moderate side effects ** Referring to application site, unless stated otherwise ^ Safety outcomes regarding solely treatment with rapamycin (calcitriol, as well as ra-

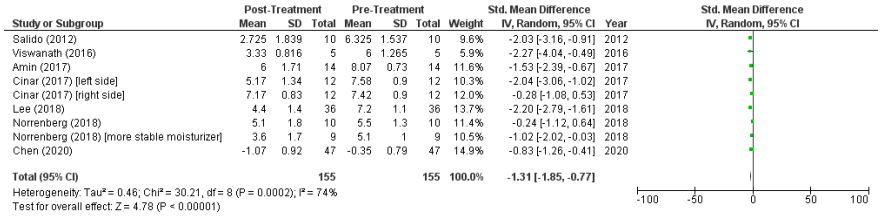

Fig. 3 Forest plot of the meta-analyzed effect sizes regarding FASI

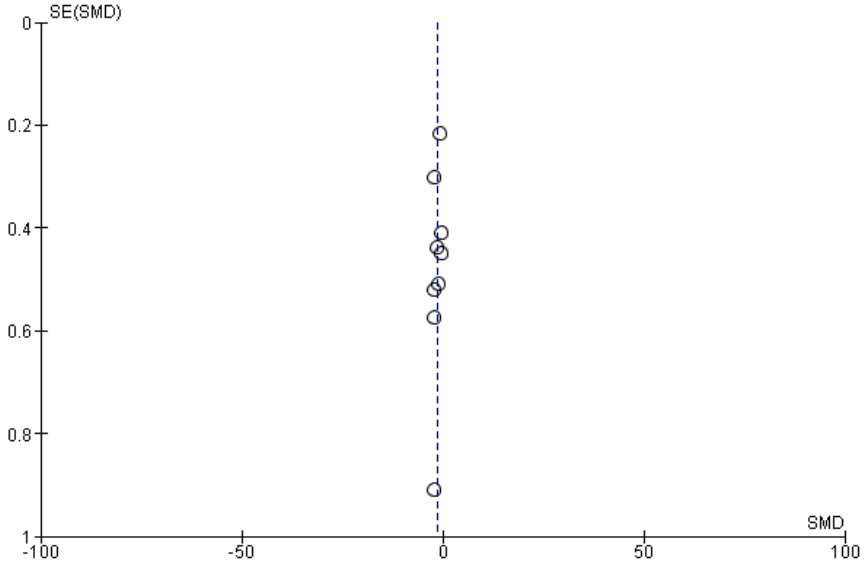

Fig 4. Funnel plot of the meta-analyzed effect sizes regarding FASI

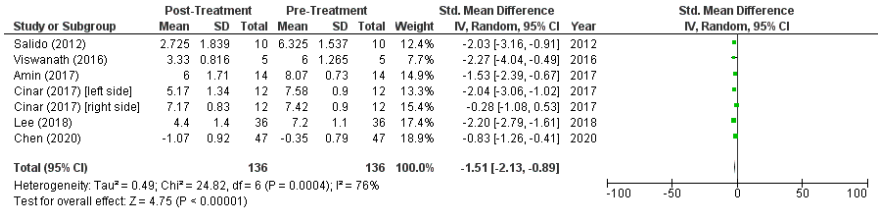

Fig. 5 Forest plot of the meta-analyzed effect sizes regarding FASI after sensitivity analysis

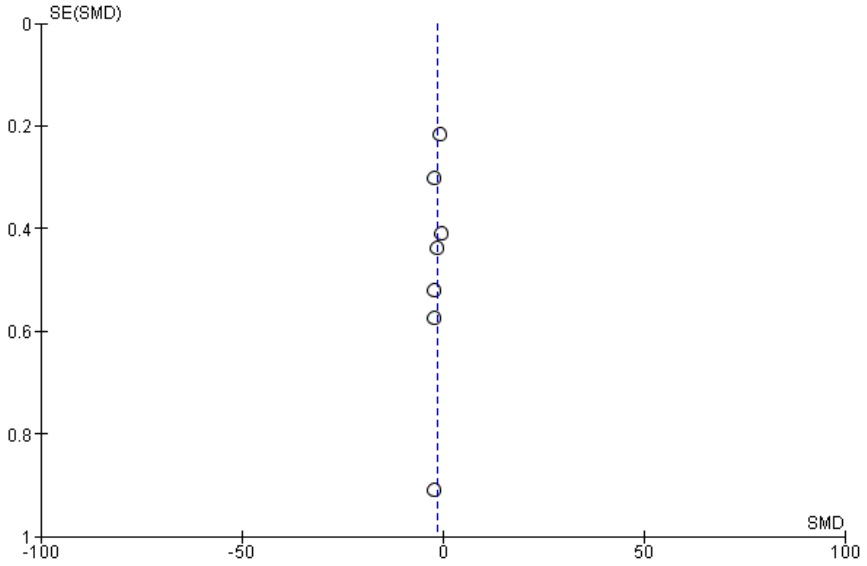

Fig. 6 Funnel plot of the meta-analyzed effect sizes regarding FASI after sensitivity analysis

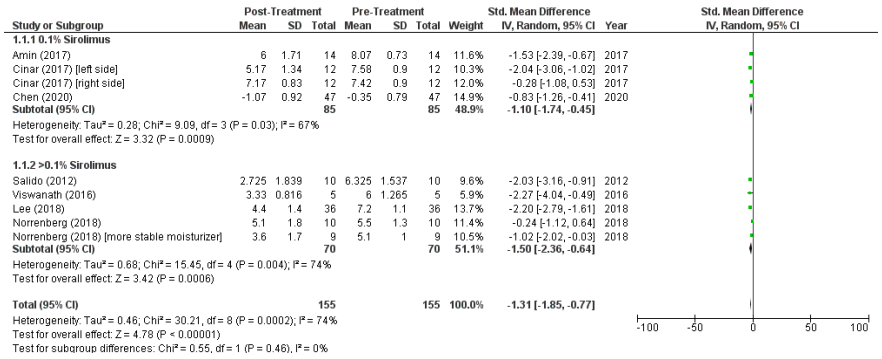

Fig. 7 Forest plot of the subgroup analysis by dosage regarding FASI

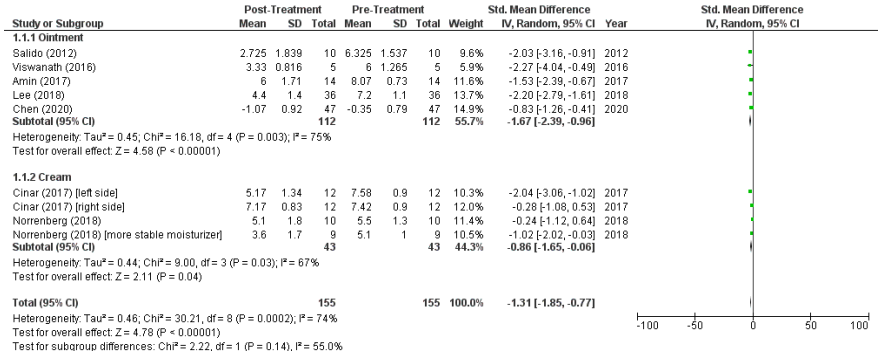

Fig. 8 Forest plot of the subgroup analysis by moisturizer regarding FASI

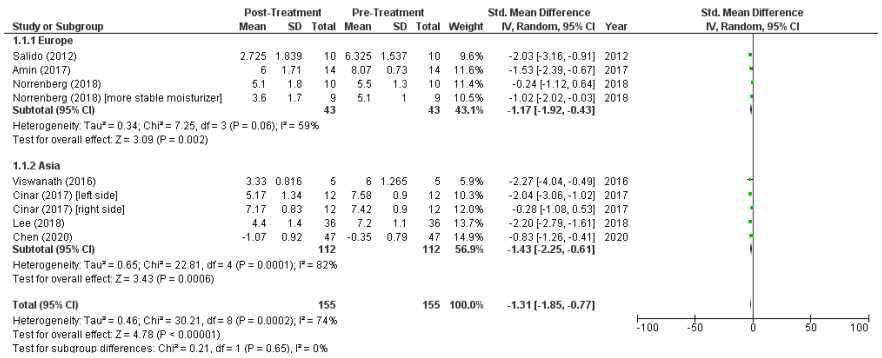

Fig. 9 Forest plot of the subgroup analysis by region regarding FASI