Année 2017/2018 N°

Thèse

Pour le

DOCTORAT EN MEDECINE

Diplôme d'État

par

Sophie LEDUCQ

Née le 04 Août 1989 à Rouen (76)

TITRE

Traitement des malformations cutanées lymphatiques microkystiques par sirolimus topique 0,1% : protocole pour un essai thérapeutique de phase II, randomisé, en double aveugle, en comparaison intra-individuelle versus véhicule

---

Présentée et soutenue publiquement le Jeudi 15 Novembre 2018 date devant un jury composé de :

Président du Jury : Professeur Patrice DIOT, Pneumologie, Faculté de Médecine – Tours

Membres du Jury :
Professeur Bruno GIRAUDEAU, Biostatistiques, Faculté de Médecine – Tours
Docteur Valérie GISSOT, Centre d'Investigation Clinique, PH, CHU –Tours
Docteur Anne LE TOUZE, Chirurgie Infantile, PH, CHU – Tours
Professeur Mahtab SAMIMI, Dermatologie-Vénéréologie, Faculté de Médecine – Tours
Directeur de thèse : Professeur Annabel MARUANI, Dermatologie-Vénéréologie, Faculté de Médecine – Tours

UNIVERSITE DE TOURS
FACULTE DE MEDECINE DE TOURS

DOYEN
Pr Patrice DIOT

VICE-DOYEN
Pr Henri MARRET

ASSESSEURS
Pr Denis ANGOULVANT, *Pédagogie*
Pr Mathias BUCHLER, *Relations internationales*
Pr Hubert LARDY, *Moyens – relations avec l'Université*
Pr Anne-Marie LEHR-DRYLEWICZ, *Médecine générale*
Pr François MAILLOT, *Formation Médicale Continue*
Pr Patrick VOURC'H, *Recherche*

RESPONSABLE ADMINISTRATIVE
Mme Fanny BOBLETER

****

DOYENS HONORAIRES
Pr Emile ARON (†) – *1962-1966*
Directeur de l'Ecole de Médecine - 1947-1962
Pr Georges DESBUQUOIS (†) - *1966-1972*
Pr André GOUAZE - *1972-1994*
Pr Jean-Claude ROLLAND – *1994-2004*
Pr Dominique PERROTIN – *2004-2014*

PROFESSEURS EMERITES
Pr Daniel ALISON
Pr Philippe ARBEILLE
Pr Catherine BARTHELEMY
Pr Christian BONNARD
Pr Philippe BOUGNOUX
Pr Alain CHANTEPIE
Pr Pierre COSNAY
Pr Etienne DANQUECHIN-DORVAL
Pr Loïc DE LA LANDE DE CALAN
Pr Alain GOUDEAU
Pr Noël HUTEN
Pr Olivier LE FLOCH
Pr Yvon LEBRANCHU
Pr Elisabeth LECA
Pr Anne-Marie LEHR-DRYLEWICZ
Pr Gérard LORETTE
Pr Roland QUENTIN
Pr Alain ROBIER
Pr Elie SALIBA

PROFESSEURS HONORAIRES
P. ANTHONIOZ – A. AUDURIER – A. AUTRET – P. BAGROS – P.BARDOS – J.L. BAULIEU – C. BERGER – JC. BESNARD – P. BEUTTER – P. BONNET – M. BROCHIER – P. BURDIN – L. CASTELLANI – B. CHARBONNIER – P. CHOUTET – T. CONSTANS – C. COUET - J.P. FAUCHIER – F. FETISSOF – J. FUSCIARDI – P. GAILLARD – G. GINIES – A. GOUAZE – J.L. GUILMOT – M. JAN – J.P. LAMAGNERE – F. LAMISSE – Y. LANSON – J. LAUGIER – P. LECOMTE – E. LEMARIE – G. LEROY – Y. LHUINTRE – M. MARCHAND – C. MAURAGE – C. MERCIER – J. MOLINE – C. MORAINE – J.P. MUH – J. MURAT – H. NIVET – L. POURCELOT – P. RAYNAUD – D. RICHARD-LENOBLE – J.C. ROLLAND – D. ROYERE - A. SAINDELLE – J.J. SANTINI – D. SAUVAGE – D. SIRINELLI – B. TOUMIEUX – J. WEILL

PROFESSEURS DES UNIVERSITES - PRATICIENS HOSPITALIERS

ANDRES Christian.....Biochimie et biologie moléculaire
ANGOULVANT Denis .....Cardiologie
AUPART Michel.....Chirurgie thoracique et cardiovasculaire
BABUTY Dominique .....Cardiologie
BALLON Nicolas.....Psychiatrie ; addictologie
BARILLOT Isabelle.....Cancérologie ; radiothérapie
BARON Christophe .....Immunologie
BEJAN-ANGOULVANT Théodora .....Pharmacologie clinique
BERNARD Anne .....Cardiologie
BERNARD Louis .....Maladies infectieuses et maladies tropicales
BLANCHARD-LAUMONNIER Emmanuelle ....Biologie cellulaire
BLASCO Hélène.....Biochimie et biologie moléculaire
BODY Gilles .....Gynécologie et obstétrique
BONNET-BRILHAULT Frédérique .....Physiologie
BRILHAULT Jean.....Chirurgie orthopédique et traumatologique
BRUNEREAU Laurent .....Radiologie et imagerie médicale
BRUYERE Franck.....Urologie
BUCHLER Matthias.....Néphrologie
CALAIS Gilles.....Cancérologie, radiothérapie
CAMUS Vincent .....Psychiatrie d'adultes
CHANDENIER Jacques.....Parasitologie, mycologie
COLOMBAT Philippe.....Hématologie, transfusion
CORCIA Philippe.....Neurologie
COTTIER Jean-Philippe .....Radiologie et imagerie médicale
DE TOFFOL Bertrand .....Neurologie
DEQUIN Pierre-François.....Thérapeutique
DESOUBEAUX Guillaume.....Parasitologie et mycologie
DESTRIEUX Christophe .....Anatomie
DIOT Patrice.....Pneumologie
DU BOUEXIC de PINIEUX Gonzague .....Anatomie & cytologie pathologiques
DUCLUZEAU Pierre-Henri.....Endocrinologie, diabétologie, et nutrition
DUMONT Pascal .....Chirurgie thoracique et cardiovasculaire
EL HAGE Wissam.....Psychiatrie adultes
EHRMANN Stephan .....Réanimation
FAUCHIER Laurent .....Cardiologie
FAVARD Luc.....Chirurgie orthopédique et traumatologique
FOUGERE Bertrand .....Gériatrie
FOUQUET Bernard.....Médecine physique et de réadaptation
FRANCOIS Patrick.....Neurochirurgie
FROMONT-HANKARD Gaëlle .....Anatomie & cytologie pathologiques
GAUDY-GRAFFIN Catherine.....Bactériologie-virologie, hygiène hospitalière
GOGA Dominique.....Chirurgie maxillo-faciale et stomatologie
GOUPILLE Philippe .....Rhumatologie
GRUEL Yves.....Hématologie, transfusion
GUERIF Fabrice.....Biologie et médecine du développement et de la reproduction
GUYETANT Serge .....Anatomie et cytologie pathologiques
GYAN Emmanuel.....Hématologie, transfusion
HAILLOT Olivier.....Urologie
HALIMI Jean-Michel.....Thérapeutique
HANKARD Régis.....Pédiatrie
HERAULT Olivier .....Hématologie, transfusion
HERBRETEAU Denis .....Radiologie et imagerie médicale
HOURIOUX Christophe.....Biologie cellulaire
LABARTHE François .....Pédiatrie
LAFFON Marc .....Anesthésiologie et réanimation chirurgicale, médecine d'urgence
LARDY Hubert.....Chirurgie infantile
LARIBI Saïd.....Médecine d'urgence
LARTIGUE Marie-Frédérique.....Bactériologie-virologie
LAURE Boris.....Chirurgie maxillo-faciale et stomatologie
LECOMTE Thierry.....Gastroentérologie, hépatologie
LESCANNE Emmanuel.....Oto-rhino-laryngologie
LINASSIER Claude .....Cancérologie, radiothérapie
MACHET Laurent .....Dermato-vénéréologie
MAILLOT François .....Médecine interne
MARCHAND-ADAM Sylvain .....Pneumologie

MARRET Henri .....Gynécologie-obstétrique
MARUANI Annabel .....Dermatologie-vénéréologie
MEREGHETTI Laurent .....Bactériologie-virologie ; hygiène hospitalière
MORINIERE Sylvain.....Oto-rhino-laryngologie
MOUSSATA Driffa .....Gastro-entérologie
MULLEMAN Denis .....Rhumatologie
ODENT Thierry.....Chirurgie infantile
OUAISSI Mehdi .....Chirurgie digestive
OULDAMER Lobna .....Gynécologie-obstétrique
PAGES Jean-Christophe .....Biochimie et biologie moléculaire
PAINTAUD Gilles .....Pharmacologie fondamentale, pharmacologie clinique
PATAT Frédéric .....Biophysique et médecine nucléaire
PERROTIN Dominique .....Réanimation médicale, médecine d'urgence
PERROTIN Franck .....Gynécologie-obstétrique
PISELLA Pierre-Jean.....Ophtalmologie
PLANTIER Laurent .....Physiologie
REMERAND Francis .....Anesthésiologie et réanimation, médecine d'urgence
ROINGEARD Philippe.....Biologie cellulaire
ROSSET Philippe .....Chirurgie orthopédique et traumatologique
RUSCH Emmanuel.....Epidémiologie, économie de la santé et prévention
SAINT-MARTIN Pauline.....Médecine légale et droit de la santé
SALAME Ephrem.....Chirurgie digestive
SAMIMI Mahtab .....Dermatologie-vénéréologie
SANTIAGO-RIBEIRO Maria .....Biophysique et médecine nucléaire
THOMAS-CASTELNAU Pierre .....Pédiatrie
TOUTAIN Annick.....Génétique
VAILLANT Loïc.....Dermato-vénéréologie
VELUT Stéphane.....Anatomie
VOURC'H Patrick.....Biochimie et biologie moléculaire
WATIER Hervé .....Immunologie

PROFESSEUR DES UNIVERSITES DE MEDECINE GENERALE

LEBEAU Jean-Pierre

PROFESSEURS ASSOCIES

MALLET Donatien.....Soins palliatifs
POTIER Alain.....Médecine Générale
ROBERT Jean.....Médecine Générale

MAITRES DE CONFERENCES DES UNIVERSITES - PRATICIENS HOSPITALIERS

BAKHOS David.....Physiologie
BARBIER Louise.....Chirurgie digestive
BERHOUET Julien .....Chirurgie orthopédique et traumatologique
BERTRAND Philippe.....Biostat., informatique médical et technologies de communication
BRUNAULT Paul .....Psychiatrie d'adultes, addictologie
CAILLE Agnès .....Biostat., informatique médical et technologies de communication
CLEMENTY Nicolas.....Cardiologie
DOMELIER Anne-Sophie .....Bactériologie-virologie, hygiène hospitalière
DUFOUR Diane .....Biophysique et médecine nucléaire
FAVRAIS Géraldine .....Pédiatrie
FOUQUET-BERGEMER Anne-Marie.....Anatomie et cytologie pathologiques
GATAULT Philippe.....Néphrologie
GOUILLEUX Valérie.....Immunologie
GUILLON Antoine.....Réanimation
GUILLON-GRAMMATICO Leslie.....Epidémiologie, économie de la santé et prévention
HOARAU Cyrille .....Immunologie
IVANES Fabrice .....Physiologie
LE GUELLEC Chantal.....Pharmacologie fondamentale, pharmacologie clinique
MACHET Marie-Christine .....Anatomie et cytologie pathologiques
MOREL Baptiste.....Radiologie pédiatrique
PIVER Éric.....Biochimie et biologie moléculaire

REROLLE Camille.....Médecine légale
ROUMY Jérôme.....Biophysique et médecine nucléaire
SAUTENET Bénédicte.....Néphrologie
TERNANT David.....Pharmacologie fondamentale, pharmacologie clinique
ZEMMOURA Ilyess.....Neurochirurgie

MAITRES DE CONFERENCES DES UNIVERSITES

AGUILLON-HERNANDEZ Nadia.....Neurosciences
BOREL Stéphanie.....Orthophonie
DIBAO-DINA Clarisse.....Médecine Générale
MONJAUZE Cécile.....Sciences du langage - orthophonie
PATIENT Romuald.....Biologie cellulaire
RENOUX-JACQUET Cécile.....Médecine Générale

MAITRES DE CONFERENCES ASSOCIES

RUIZ Christophe.....Médecine Générale
SAMKO Boris.....Médecine Générale

CHERCHEURS INSERM - CNRS - INRA

BOUAKAZ Ayache.....Directeur de Recherche INSERM – UMR INSERM 1253
CHALON Sylvie.....Directeur de Recherche INSERM – UMR INSERM 1253
COURTY Yves.....Chargé de Recherche CNRS – UMR INSERM 1100
DE ROCQUIGNY Hugues.....Chargé de Recherche INSERM – UMR INSERM 1259
ESCOFFRE Jean-Michel.....Chargé de Recherche INSERM – UMR INSERM 1253
GILOT Philippe.....Chargé de Recherche INRA – UMR INRA 1282
GOUILLEUX Fabrice.....Directeur de Recherche CNRS – UMR CNRS 7001
GOMOT Marie.....Chargée de Recherche INSERM – UMR INSERM 1253
HEUZE-VOURCH Nathalie.....Chargée de Recherche INSERM – UMR INSERM 1100
KORKMAZ Brice.....Chargé de Recherche INSERM – UMR INSERM 1100
LAUMONNIER Frédéric.....Chargé de Recherche INSERM - UMR INSERM 1253
LE PAPE Alain.....Directeur de Recherche CNRS – UMR INSERM 1100
MAZURIER Frédéric.....Directeur de Recherche INSERM – UMR CNRS 7001
MEUNIER Jean-Christophe.....Chargé de Recherche INSERM – UMR INSERM 1259
PAGET Christophe.....Chargé de Recherche INSERM – UMR INSERM 1100
RAOUL William.....Chargé de Recherche INSERM – UMR CNRS 7001
SI TAHAR Mustapha.....Directeur de Recherche INSERM – UMR INSERM 1100
WARDAK Claire.....Chargée de Recherche INSERM – UMR INSERM 1253

CHARGES D'ENSEIGNEMENT

Pour l'Ecole d'Orthophonie
DELORE Claire.....Orthophoniste
GOUIN Jean-Marie.....Praticien Hospitalier
PERRIER Danièle.....Orthophoniste

Pour l'Ecole d'Orthoptie
LALA Emmanuelle.....Praticien Hospitalier
MAJZOUB Samuel.....Praticien Hospitalier

Pour l'Ethique Médicale
BIRMELE Béatrice.....Praticien Hospitalier

SERMENT D'HIPPOCRATE

En présence des Maîtres de cette Faculté,
de mes chers condisciples
et selon la tradition d'Hippocrate, je promets et je jure d'être fidèle aux lois de l'honneur et de la probité dans l'exercice de la Médecine.

Je donnerai mes soins **gratuits à l'indigent, et n'exigerai jamais un salaire au**-dessus de mon travail.

Admis dans l'intérieur des maisons, mes yeux ne **verront pas ce qui s'y passe, ma langue taira**
les secrets qui me seront confiés et mon état ne servira pas à corrompre l**es mœ**urs ni à favoriser le crime.

Respectueux et reconnaissant envers mes Maîtres, je rendrai à leurs enfants
l'instruction que j'ai reçue de leurs pères.

Que les hommes m'accordent leur estime si je suis fidèle à mes promesses. **Que je sois couvert d'opprob**re et méprisé de mes confrères **si j'y manque.**

Vu, le Directeur de Thèse

Vu, le Doyen
De la Faculté de Médecine de Tours
Tours, le

REMERCIEMENTS

Au président du jury,

Monsieur le Professeur Patrice DIOT,

Doyen de la Faculté de Médecine de Tours, Professeur des Universités, Praticien Hospitalier

Service de Pneumologie

Monsieur le Professeur, Monsieur le Doyen,

Vous me faites l'honneur de présider cette thèse et de juger mon travail.

Je vous en remercie infiniment.

Je vous prie de trouver ici l'expression de mon respect profond et sincère et de ma plus vive admiration.

A ma directrice de thèse,

Madame le Professeur Annabel MARUANI,

Professeur des Universités, Praticien Hospitalier

Service de Dermatologie

Je te suis profondément reconnaissante de me soutenir et de me guider tant dans mon parcours universitaire que dans mes travaux de recherches.

Je te remercie pour ta grande disponibilité ainsi que pour tes connaissances et l'expérience que tu nous fais partager dans le domaine des malformations vasculaires.

Je souhaite poursuivre mon apprentissage à tes côtés.

Puisse ce travail exprimer toute mon admiration et témoigner de ma profonde reconnaissance.

Aux membres du jury

Monsieur le Professeur Bruno GIRAUDEAU,

Professeur des Universités

Centre d'Investigation Clinique INSERM 1415

Je te remercie de me faire l'honneur de juger cette thèse.

Tu m'as accueillie avec bienveillance dans l'unité Biométrie du Centre d'Investigation Clinique où j'ai beaucoup appris et à présent au sein de l'Unité de recherche INSERM SPHERE.

Trouve ici l'expression de ma profonde reconnaissance et de ma vive estime.

Aux membres du jury

Madame le Professeur Mahtab SAMIMI,

Professeur des Universités, Praticien Hospitalier

Service de Dermatologie

Je te remercie de me faire l'honneur de faire partie de mon jury et d'évaluer mon travail.

Je tiens à te remercier pour toutes les compétences acquises à tes côtés, pour ta disponibilité, pour ton dynamisme, ainsi que pour tout le temps que tu consacres aux internes.

Puisse ce travail exprimer toute mon admiration et traduire ma profonde estime.

Aux membres du jury

Madame le Docteur Valérie GISSOT

Praticien Hospitalier

Centre d'Investigation Clinique INSERM 1415

Je vous remercie d'avoir accepté de participer à mon jury de thèse et de m'avoir prodigué vos conseils en apportant votre expertise.

Trouvez ici l'assurance de ma reconnaissance et de mon profond respect.

Aux membres du jury

Madame le Docteur Anne LE TOUZE,

Praticien Hospitalier

Service de Chirurgie Pédiatrique Viscérale et Plastique

Vous me faites l'honneur de participer à mon jury de thèse.

Je vous remercie pour les connaissances que vous avez su nous faire partager au cours des consultations pluridisciplinaires. Veuillez trouver ici l'expression de mes sincères remerciements et de mon profond respect.

Au Pr Laurent MACHET

Je vous remercie pour votre disponibilité, pour l'intérêt que vous portez dans la formation des internes et pour le partage de vos connaissances, notamment lors du staff hebdomadaire. Veuillez trouver ici l'expression de mes sincères remerciements.

Au Pr Gérard LORETTE

Merci pour votre savoir transmis lors des consultations de dermato-pédiatrie. Apprendre à vos côtés fut un plaisir et un honneur. Veuillez trouver dans ce travail, l'expression de mon plus profond respect.

Au Pr Denis HERBRETEAU

Je vous remercie pour votre savoir que vous avez su partager lors des consultations pluridisciplinaires. Recevez ici l'expression de ma profonde gratitude et de mon plus grand respect.

Aux Dr Gabriela GEORGESCOU, Dr Valérie TAUVERON et Pr Loïc VAILLANT : je vous remercie pour votre confiance et votre enseignement au cours de ces années d'internat.

Au Dr Emmanuelle LE BIDRE : merci pour ta gentillesse et ta patience, qui sont très appréciables, et merci pour ta pédagogie lors des visites.

Aux Dr Éric ESTEVE, Dr Guido BENS, Dr Raphaëlle BINOIS, Dr Aude ROUSSEL, et à toute l'équipe paramédicale : pour votre encadrement et votre accueil dans le service de Dermatologie au CHR d'Orléans.

Aux infirmières et secrétaires de la consultation de Dermato-pédiatrie : merci pour votre accueil si chaleureux et votre gentillesse que j'ai énormément apprécié.

A toutes les infirmières, aides-soignantes d'hospitalisation et de consultation, aux secrétaires du service de Dermatologie, qui nous facilitent le travail au quotidien : merci pour votre professionnalisme et vos petites attentions envers les internes.

Au Dr Agnès CAILLE et à toute l'équipe du Centre d'Investigation Clinique : Amélie, Anne, Carine, Elie, Elsa, Elody, Frédérique, Julie, Samia et Véronique, pour m'avoir fait partager votre savoir sur la méthodologie et les biostatistiques. Merci pour votre chaleureux accueil au sein de votre unité, pour avoir répondu à toutes mes questions et m'avoir aidé à me dépatouiller avec R et SAS !

Aux équipes médicales et paramédicales des services de Médecine Interne, Rhumatologie et Maladies infectieuses et tropicales du CHR d'Orléans : pour avoir su transmettre votre savoir et pour avoir partagé votre passion pour vos spécialités respectives.

Aux Dr Agnès D'ALTEROCHE, Dr Jean-Paul CLAUDEL et Anne : merci pour votre accueil dans votre cabinet. C'est avec grand plaisir que je m'y rends le samedi matin !

Au Dr Myriam BRUNAULT : je vous remercie de m'avoir accueilli aux consultations « cheveux » et pour votre savoir que vous m'avez fait partager.

A mes chefs et amies,

Dr Hélène CORNILLIER, merci pour tes précieux conseils que tu m'as prodigué au cours de mon internat. Merci également pour ton implication dans le service. Travailler avec toi est un réel plaisir, mais tu le sais déjà !

Dr Margaux GARNIER, ma chef modeuse, notre Cristina Cordula de la dermatologie, je te remercie pour ta disponibilité, pour ton coaching en petite chirurgie et pour ton amitié !

Dr Julia ZARAGOZA, je suis très contente d'avoir fait ta connaissance et d'avoir passé un semestre sous ta responsabilité. Ta bonne humeur et ton humour rendaient les visites en C mémorables !

Dr Marion NADAL, je garde un très bon souvenir des consultations du vendredi après-midi à tes cotés à Clocheville.

Dr Laura CHAPUT, merci pour l'aide que tu apportes aux internes et pour ta bonne humeur.

Au Dr Romain GABEFF, merci pour ta formation lors de mon semestre à Clocheville.

A mes co-internes de Dermatologie, Sarah et Chakib (avec qui j'ai beaucoup apprécié partager ces semestres), Frédéric (pour tous les séminaires passés ensemble !), Victoire, Alice, Camille, Juliette, Antoine, Diane et Laura, merci pour ces bons moments passés ensemble au travail et en dehors.

A mes amis et famille,

A mes amis rencontrés au cours de l'internat, Irène (pour toutes tes histoires que tu nous fais partager), Théo (merci pour ton incroyable twerk), Antoine (notre voisin à jamais), Ophélie (pour ta motivation qui sait rendre chaque soirée unique), Tristan (pour nous avoir fait connaitre Makassy), Anne-Sophie et Jonathan (pour votre coaching dans nos travaux), Mathieu et Aurélie (que nous aimerions voir sur Rennes bientôt), Quitterie et Gaëlle (nos copines chir). Du jeu de l'oie, à Madère, aux Antilles, à la Croute, au « Galette, Saucisse, Aurélia, Je t'aime », au ski et aux innombrables chutes de Najou, que de bons souvenirs !

Aux copains de Rouen, Agathe et Clément, Chloé et Edouard, Florian et Camille, Lucie, Gabriel et Avélig, Mathilde, Matthieu et Laurène, et les plus jeunes, Jules, Léopold, Noah, Alice et Clémence, pour tous les bons moments passés ensembles et ceux à venir.

A mes amies de longue date, Marie-Antonia, Anne-Laure et Géraldine.

A Marie, mon amie de toujours, que j'espère voir très prochainement à Londres.

A ma belle-famille, Isabelle, Pauline, Geneviève, Eric et Patricia, la famille Guillem et la famille Auger, merci pour votre présence ce jour et pour m'avoir si bien accueillie.

A mes grands-mères, Marcelle et Thérèse, **à mes grands-pères,** Daniel ┼et Etienne ┼, pour votre présence tout au long de ces années, je pense fort à vous en ce jour important.

A Vincent, mon oncle, un grand merci pour avoir fait le déplacement aujourd'hui.

A mes parents, pour votre soutien indéfectible, pour vos précieux conseils et pour votre grande disponibilité. Merci de nous avoir transmis le goût du travail. J'espère vous rendre fiers autant que je le suis de vous avoir.

A mon frère et ma sœur, Guillaume et Pauline, pour tous les moments magiques passés ensemble et les futurs à venir, et pour le goût de la photographie que nous partageons. Guillaume, merci d'être présent à chaque moment important, et pour information, j'ai déjà booké pour l'Oktoberfest en 2019 ! Pauline, je te souhaite de réussir cette année importante. Nous nous retrouverons donc dans 4 ans pour ta thèse !

A Thomas, toi qui as su me supporter, me conseiller et me soutenir. De la P2, au concours de l'ECN et maintenant l'internat, nous avons passés toutes ces étapes à 2. J'espère que beaucoup d'autres sont à venir. Merci pour tes sourires, ta bonne humeur, tes qualités de cuisinier et de me coacher pour chacune de mes présentations. Merci pour tout.

Table des matières

I- INTRODUCTION GÉNÉRALE

Les malformations lymphatiques kystiques sont des malformations vasculaires rares touchant l'enfant et l'adulte (prévalence estimée < 0.1%). Ce sont des malformations à bas débit, résultant d'une anomalie du développement embryologique des vaisseaux lymphatiques. Elles peuvent être macrokystiques, microkystiques ou mixtes et peuvent toucher les viscères ou la peau. Les malformations lymphatiques microkystiques cutanées (MLMC), également appelée lymphangiectasies, se manifestent par des amas de vésicules à contenu clair ou hématique, localisées habituellement sur les zones segmentaires, en particulier la tête et le cou, les membres inférieurs et les zones génitales. Elles peuvent se compliquer de saignements et parfois d'anémie, d'inflammation, de suintements, de douleurs, de cicatrices, de surinfections, et sont responsables d'une altération majeure de la qualité de vie. L'histoire naturelle des MLMC est l'aggravation des signes, avec augmentation du nombre de lésions et survenue de complications. Actuellement, il n'existe pas de recommandations pour la prise en charge des MLMC. Les options thérapeutiques incluent la sclérothérapie, les traitements physiques (laser ablatif, laser à colorant pulsé, radiofréquence, …) et la chirurgie, mais ces traitements sont douloureux, entrainent des cicatrices et poussées inflammatoires et leur efficacité est habituellement incomplète et transitoire. L'abstention thérapeutique est donc une attitude fréquemment adoptée.

Mammalian target of rapamycin (mTOR) est une sérine/thréonine kinase qui intervient dans la prolifération cellulaire, le métabolisme cellulaire, l'apoptose et l'angio/lymphangiogenèse. Les inhibiteurs de mTOR, en bloquant la voie mTOR, inhibent la prolifération cellulaire et l'angiogenèse, et en particulier la lymphangiogenèse. Parmi les inhibiteurs de mTOR, le sirolimus (ou rapamycine) est le plus ancien et le plus fréquemment utilisé. Du fait de ses propriétés immunosuppressives, il est utilisé par voie systémique depuis plus de 15 ans, principalement pour la prévention de rejet d'organe (transplantation rénale).

Son utilisation par voie orale est bien codifiée. D'autres indications se sont développées secondairement : angiomyolipomes dans la sclérose tubéreuse de Bourneville (STB), astrocytomes à cellules géantes dans la STB, malformations vasculaires, etc. Les autres inhibiteurs de mTOR (évérolimus, deforolimus, temsirolimus), appelés *rapalogs*, s'administrent par voie injectable et sont principalement indiqués en cancérologie.

L'utilisation topique du sirolimus s'est développée depuis 2005, d'abord pour le psoriasis (Ormerod et al., 2005) et secondairement pour les angiofibromes liés à la STB (Haemel et al., 2011). Depuis, de nouvelles indications ont été ponctuellement rapportées. Les modes d'utilisation des inhibiteurs de mTOR par voie topique trouvés dans la littérature sont très hétérogènes, que ce soient par l'excipient utilisé, la concentration en produit actif, le nombre d'applications ou la durée. Ceci est probablement lié à l'absence de produit commercialisé à ce jour, les traitements consistant en préparations magistrales à partir de sirolimus en suspension buvable ou en comprimés.

Récemment, 2 publications ont rapporté une efficacité du sirolimus topique pour les MLMC. Dans le 1er cas, il s'agissait d'un patient d'une vingtaine d'années avec une MLMC primaire de la région glutéale, traitée par sirolimus topique appliqué une fois par jour pendant 3 mois permettant une régression de la lymphorrhée et une diminution des vésicules lymphatiques (Ivars et al., 2017). La 2ème publication rapportait 2 cas : une jeune fille de 13 ans avec une MCLM de la fesse droite et un jeune garçon de 5 ans avec une MLMC de fesse gauche, traitées efficacement par sirolimus topique appliqué pendant 4 et 6 moins respectivement (Garcia-Montero et al., 2017). Pour les 2 publications, aucun effet secondaire n'a été rapporté. Enfin, nous avons publié le cas d'un jeune homme de 18 ans avec une MLMC glutéale traitée efficacement par sirolimus topique, qui a permis une diminution des suintements et des lésions et chez qui le traitement est toujours en cours (Leducq et al., 2018).

Ainsi, notre travail s'est déroulé en 2 temps. Dans un premier temps, nous avons réalisé une revue systématique de la littérature avec méta-analyse. L'objectif a été de faire un état des lieux de toutes les pathologies dermatologiques pour lesquelles l'utilisation d'un inhibiteur de mTOR par voie topique a été publiée, et d'évaluer son efficacité et sa tolérance dans les différentes indications. Cela nous a permis d'appréhender le meilleur mode d'utilisation des inhibiteurs de mTOR par voie topique et de pouvoir anticiper les effets secondaires potentiellement rencontrés avec ce traitement. Ce travail a été fait en collaboration avec l'équipe SPHERE (MethodS in Patients-centered outcomes and HEalth ResEarch). Les résultats de ce travail ont été présentés aux Journées Dermatologiques de Paris en décembre 2017 (communication orale) et ont été soumis pour la Journée de Recherche biomédicale Angers-Tours en Novembre 2018. L'article a été accepté pour publication au *Journal of the American Academy of Dermatology*. Dans un deuxième temps, nous avons rédigé un protocole de recherche qui a pour objectif d'évaluer l'efficacité du sirolimus topique 0,1% dans les MLCM au moyen d'une étude randomisée, contrôlée, en comparaison intra-individuelle (*split body),* en double aveugle. Ce projet de recherche a été déposé à l'appel d'offre de la Direction Générale de l'Offre de Soins (DGOS) et a été retenu comme Programme Hospitalier de Recherche Clinique National (PHRCN) en 2017. Les inclusions de patients doivent débuter au 1er semestre de l'année 2019.

Ces travaux ont été réalisés grâce à une collaboration entre le Centre d'Investigation Clinique - INSERM 1415 de Tours, l'équipe SPHERE – INSERM UMR 1246 et l'équipe de dermatologie de Tours, labellisée en 2017 comme site constitutif du centre de référence MAGEC (MAladies GEnétiques rares d'Expression Cutanée à début pédiatrique). Ce centre de référence multi-site inclut les équipes de dermatologie d'Angers, de Cochin, de Dijon, de Necker, de Robert-Debré et de Tours, ce dernier site ayant la valence « malformations vasculaires et mosaïques ».

II- REVUE SYSTÉMATIQUE DE LA LITTÉRATURE ET MÉTA-ANALYSE

1. Résumé en français

Contexte : Les inhibiteurs de mTOR (*mammalian target of rapamycin*) sont actuellement utilisés par voie systémique dans de nombreuses indications. Leur utilisation par voie topique est récente et peu codifiée, probablement du fait de l'absence de formulation commercialisée.

Objectif : L'objectif de ce travail a été d'évaluer l'efficacité et la tolérance des inhibiteurs de mTOR par voie topique dans toutes les utilisations dermatologiques recensées, en réalisant une revue systématique de la littérature avec méta-analyse si possible.

Méthodes : La recherche documentaire a été faite sur MEDLINE via PubMed, CENTRAL, LILACS, et EMBASE en Janvier 2017. Elle a porté sur tout article original, quelle que soit la langue, rapportant l'utilisation topique d'inhibiteurs de mTOR chez l'humain, dans toutes les pathologies dermatologiques. Etaient exclus les articles rapportant une utilisation par voie systémique ou une utilisation sur les muqueuses. La sélection et l'extraction des données ont été faites par des doublons indépendants. L'évaluation des risques de biais a été réalisée selon les recommandations de la Cochrane (*the Cochrane Collaboration risk of bias tool*). L'efficacité du traitement a été exprimée en risque relatif (RR) avec des intervalles de confiance (IC) à 95%.

Résultats : La recherche initiale a identifié 1042 articles ; pour 48 d'entre eux, le texte intégral a été analysé. Nous avons finalement inclus 40 études dans notre revue systématique (262 patients) : 7 essais randomisés contrôlés incluant 134 patients et 33 cas cliniques/séries de cas totalisant 128 patients. L'âge moyen était de 23,9 ans, dont 124 enfants et 93 adultes (45 données manquantes). Onze pathologies dermatologiques différentes étaient étudiées, la plus fréquente étant les angiofibromes faciaux liés à la sclérose tubéreuse de Bourneville (STB), impliquant 157 patients. Le sirolimus topique était le produit utilisé dans toutes les publications sauf une (évérolimus). Il était significativement plus efficace que le placebo pour

les angiofibromes faciaux (RR, 2,52, 95% IC [1,27; 5,00]; $I^2 = 0\%$) sur le critère de jugement de l'efficacité auto-rapportée, seul critère analysable en méta-analyse. La concentration médiane de sirolimus dans la formulation était de 0,1%, avec une application médiane de 1 fois par jour. La durée médiane du traitement était de 12 semaines. Les inhibiteurs de mTOR topiques étaient globalement bien tolérés, avec uniquement des effets secondaires locaux légers ou modérés, principalement de type irritatif (picotement, sécheresse, prurit localisé). Le dosage de sirolimus sanguin était négatif chez 153/170 patients (90%). Lorsque la concentration de sirolimus sanguine était détectée, elle était toujours inférieure à celle requise pour obtenir une immunosuppression (4 ng/ml).

Conclusions : Cette revue systématique de la littérature avec méta-analyse montre l'efficacité du sirolimus topique dans les angiofibromes faciaux liées à la STB, chez les adultes et les enfants, avec uniquement des effets secondaires locaux rapportés. Les autres indications nécessitent des études supplémentaires.

2. Article scientifique en anglais

Topical use of mammalian target of rapamycin inhibitors in dermatology: a systematic review with meta-analysis

Sophie Leducq[1,2], MD; Bruno Giraudeau[1,3], PhD; Elsa Tavernier[1,3], PhD; Annabel Maruani[1,2], MD, PhD

[1]Universities of Tours and Nantes, INSERM 1246 – SPHERE, 37000 Tours, France

[2]Department of Dermatology and Reference Center for Rare Diseases and Vascular Malformations (MAGEC), CHRU Tours, 37044 Tours Cedex 9, France

[3]Clinical Investigation Center, INSERM 1415, CHRU Tours, 37000 Tours, France

References: 59

One table, 3 figures

Conflict of interest: the authors have no conflicts of interest to declare

Funding: None

Key words: mTOR inhibitor, rapamycin, sirolimus, topical, systematic review, dermatology

Abstract

Background: Systemic mammalian target of rapamycin (mTOR) inhibitors are currently used in many dermatological indications. Their topical use is recent and poorly codified, probably because of no marketed formulation.

Objective: A systematic review to provide an overview of the topical use of all mTOR inhibitors in dermatologic conditions and a meta-analysis to evaluate efficacy and safety.

Data Sources: A literature search of MEDLINE via PubMed, CENTRAL, LILACS, and EMBASE was performed in January 2017 for articles of topical mTOR inhibitor use whatever the language.

Study Selection: Reports of all studies investigating the use of topical mTOR inhibitors in humans in any dermatology diseases were included. Exclusion criteria were systemic use and mucosal administration.

Data Extraction and Synthesis: Two authors independently reviewed publications to determine eligibility. Risk of bias was assessed by using the Cochrane Collaboration Risk of Bias tool.

Main Outcomes and Measures: Therapy effects (patient self-assessment) were expressed in terms of risk ratio (RRs) with 95% confidence intervals (CIs).

Results: The initial search yielded 1 042 records; for 48 of these, the full text was screened. We included 40 studies in the systematic review (262 patients); 7 randomized controlled trials included 134 patients and 33 case reports and case studies included 128 patients. Mean age was 23.9 years, 124 were children and 93 were adults (45 missing data). Eleven dermatologic conditions were found, the most frequent being facial angiofibromas linked to tuberous sclerosis complex (TSC) (157 patients). Topical mTOR inhibitors were significantly more efficient than placebo for angiofibromas (RR, 2.52, 95% CI [1.27; 5.00]; $I^2 = 0\%$). Overall, 39

reports described the use of sirolimus and 1 everolimus. The median concentration of sirolimus was 0.1%, with a median application of 1 per day. Median treatment duration was 12 weeks. Topical mTOR inhibitors were well tolerated, with only mild or moderate local side effects reported, mostly irritative (stinging, dryness, itching). Sirolimus blood level was not detected in 153/170 (90%) patients.

Conclusions and Relevance: This systematic review and meta-analysis supports the efficacy of topical sirolimus in adults and children for facial angiofibromas linked to TSC, with only local side effects reported. Other indications require further research.

Introduction

Mammalian target of rapamycin (mTOR) inhibitors are systemic drugs used in various conditions. mTOR is a serine/threonine protein kinase that belongs to the phosphoinositide-3 kinase (PI3K)-related kinase family.[1] It is a catalytic subunit of two biochemically distinct complexes called mTORC1 and mTORC2. mTORC1 controls cell autonomous growth in response to nutrient availability and growth factors, whereas mTORC2 mediates cell proliferation and cell survival.[2,3] mTOR inhibitors include sirolimus (also called rapamycin), which was first developed in the 1990s, and rapalogs such as everolimus, temsirolimus and deforolimus. Sirolimus is currently approved by the US Food and Drug Administration for preventing allograft rejection in renal transplantation[4]. It is also used to treat renal and brain tumors linked to tuberous sclerosis complex (TSC) [5]; in addition, sirolimus-eluting coronary stents have been marketed.[6] Rapalogs are mainly used in therapies for various cancers.[7–10]

Oral sirolimus is increasingly used in dermatologic conditions, especially vascular tumors and complicated vascular malformations, because of its antiproliferative and anti-angiogenic and -lymphangiogenetic properties. [11–15] For very superficial cutaneous anomalies, topical sirolimus administration has been tested since 2010, especially for facial angiofibromas linked to TSC.[16]

The molecular weight of sirolimus is 914.17 Dalton, which is a high weight and does not easily allow percutaneous absorption.[17] Currently, topical sirolimus is increasingly being tested in varied cutaneous conditions, such as cutaneous vascular malformations or inflammatory diseases.[18,19] Topical applications of the drug seem safe, but topical sirolimus is not yet marketed, and modalities of its use are heterogeneous, with widespread uncontrolled use.

The aim of this systematic review was to provide an overview of the dermatologic indications of topical mTOR inhibitors, with a meta-analysis aggregating data from randomized trials to estimate their efficacy and safety.

Methods

PRISMA guidelines were followed for reporting.

Search strategy

We searched electronic databases including MEDLINE via PubMed, CENTRAL, LILACS, and EMBASE from inception to January 2017 by using the terms “mTOR inhibitor”, “sirolimus”, “rapamycin”, “everolimus”, “TOR serine-threonine kinases/antagonists”, combined with “topical”, “local”, “ointment”, “cream”, “topical administration”, “skin cream” and “gels”.

Inclusion and exclusion criteria

We included all original reports (study, case series, case reports and correspondence) whatever the language, describing use of any topical mTOR inhibitor, alone or in association with other treatments, in any cutaneous condition, in humans. Reports of systemic use and mucosal administration (oral or conjunctival) were excluded, as were reports with non-extractable data (on drug or condition).

Study selection strategy

According to the pre-defined criteria, 2 authors (SL, AM) independently selected studies based on their title and abstract. Any disagreements were resolved by consensus. The 2 authors then examined the full texts of articles. In case of duplicates, the most complete report was chosen.

Data extraction

Two authors (SL, AM) extracted data by using a standardized extraction file. The following data were extracted from each report: first author, publication year, journal, country/site, study design, demographic characteristics of patients, condition, type of mTOR inhibitor, regime, concentration and duration of treatment, drug efficacy and side effects, blood level of mTOR inhibitor, co-interventions and follow-up. Any disagreements in extraction were resolved by consensus.

Quality and risk of bias assessment

Two authors (SL, AM) independently assessed the risk of bias of each randomized controlled trial (RCT) by using the Cochrane Collaboration Risk of Bias tool.[20] The following sources of bias were considered: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, and selective reporting. In case of disagreement, a methodologist (ET) gave the final decision.

Statistical analyses

Descriptive data are expressed with median and quartiles (Q1; Q3) for quantitative data and number (%) for categorical data. A meta-analysis was planned if we could pool data from randomized controlled trials (RCTs). For missing data, the first author was contacted. Relative risk of self-reported improvement was the primary measure of treatment effect. The meta-analysis was performed by computing relative risks (RRs) using random-effects model. RR and 95% confidence intervals (CIs) were calculated. Heterogeneity of results across RCTs was assessed by the Q and I^2 statistics, and any heterogeneity was predefined as $p < 0.05$ for the Q statistic or I^2 value $\geq 50\%$. Statistical analyses involved use of R v3.3.1 and SAS 9.4 (SAS Inst. Inc., Cary, NC).

Results

Characteristics of included reports (Table 1)

Our systematic search of the literature identified 1 042 reports describing use of topical mTOR inhibitors in dermatologic conditions. We included 40 reports of studies involving 262 patients (Figure 1) that were published between 2005 and 2017: 33 were observational studies (n=128 patients) and 7 were RCTs (n=134 patients). Nineteen reports were from Europe, 10 from the United States, 9 from Asia and 2 from Oceania. The median [Q1; Q3] age of participants was 25.5 years [12; 33]; 124/246 (50.4%) were male.

Eleven dermatologic conditions were identified, including tumors (angiofibromas, subungual fibromas and hypomelanotic macules linked to TSC, fibrofolliculomas, trichoepitheliomas, familial discoid fibromas, Kaposi's sarcoma), inflammatory diseases (psoriasis plaques, lichen planus) and vascular malformations (port-wine stain [PWS] and cutaneous microcystic lymphatic malformations). Among the 40 reports, 39 reported the use of sirolimus and 1 everolimus. Sirolimus formulations were described as ointments for 90 patients (37.8%), creams for 58 (24.4%), gels for 38 (16.0%) and solutions for 52 (21.9%). Formulations were not detailed for 24 patients.

Assessment of risk of bias

Figure 2 summarizes the assessment of risk of bias of the 7 RCTs, including the 2 on angiofibromas which were meta-analyzed.

Results for efficacy

Detailed data on each study are provided in Supplemental File 1.

Angiofibromas in TSC

Overall, 26 reports (157 patients) described the use of a topical mTOR inhibitor for angiofibromas in TSC (3 RCTs and 23 observational studies).[16,17,21–44] Among 141 patients,

100 (70.9%) were children and 66 (46.8%) were male. Median age was 14.5 years [11; 38]. One case involved everolimus.[26] The median mTOR inhibitor concentration was 0.1% [0.1; 0.2], with a median number of 2 applications per day. The median treatment time was 16 weeks [12; 24]. Clinical criteria considering efficacy were heterogenous and were mainly the Facial Angiofibroma Severity Index (FASI) [45] and physician assessment. Topical mTOR inhibitors were reported as efficient in 115/121 (95.0%) patients. Follow-up data were available for 20 patients; 18 experienced recurrence between 2 and 12 weeks after withdrawal of the drug.[24,27,33-35]

We pooled data from 2 RCTs (n=39 patients treated/ n=59 patients included) comparing patient self-assessment of efficacy of topical sirolimus versus placebo in angiofibromas linked to TSC. In the third RCT on angiofibromas, this criteria was not mentioned and there was no additional common outcome. In the meta-analysis, topical sirolimus use was associated with improved patient self-assessment (RR=2.52, 95% CI [1.27;5.00]). There was no evidence of heterogeneity (I^2=0%) (Figure 3).

Hypomelanotic macules and subungual fibromas linked to TSC

Two reports described the benefit of topical sirolimus 0.2% applied twice daily for 12 weeks in 7 of 8 patients with hypomelanotic macules linked to TSC.[46,47] One report described the complete regression of subungual fibromas after 6 months' daily application of topical sirolimus 0.1%, with no recurrence after 6-month follow-up.[48]

Benign cutaneous tumors

One double-blind, split-body, facial left-right controlled trial evaluated 0.1% sirolimus solution applied twice daily for 6 months for facial fibromas in 19 patients with Birt-Hogg-Dubé syndrome and showed no significant improvement with sirolimus in size or number of fibromas.[49] Sirolimus 1% was also tested in 2 children with multiple familial

trichoepithelioma syndrome and led to reduced growth of new lesions after 24 and 48 weeks.[50] Also, one case report described a 27-year-old man with familial multiple discoid fibromas treated with 0.1% sirolimus solution applied once daily for 16 weeks, with reduced size of lesions and redness.[51]

Kaposi's sarcoma

One case report described a 73-year-old man with Kaposi's sarcoma without HIV infection that was treated with 0.5% sirolimus ointment applied twice daily.[52] Clinically and histologically, lesions disappeared at 16 weeks, with no recurrence after 2-year follow-up.

Inflammatory skin diseases

One vehicle-controlled, split-body RCT included 24 patients with chronic plaque psoriasis; 2.2% topical sirolimus was applied once daily for 6 weeks and 8% topical sirolimus for 6 additional weeks.[19] Results showed a slight improvement with treatment versus vehicle at 12 weeks (mean composite score assessing erythema, thickness and scaling of 9.1/24 vs 11.2/24, p=0.032). One case report of a 79-year-old male with plantar erosive lichen planus treated with 0.1% sirolimus solution applied once daily during 20 weeks[53] showed a significant reduction in pain after 4 weeks and lesions healed after 20 weeks.

Vascular malformations

Four reports (2 RCTs and 2 observational studies) involving 47 patients described the efficacy of topical sirolimus for PWS.[54–57] Median age was 33 years [29; 33]; 16/47 (34.0%) were male. In all studies, sirolimus was used as adjunct therapy to pulsed dye laser (PDL) treatment. The median concentration of sirolimus in topical formulation was 1% [0.1; 1], with a median of 1 application per day. Efficacy outcomes were heterogenous (physician assessment, colorimetrical or digital photographic image scores). In the observational studies, sirolimus as add-on treatment to PDL seemed efficient in 2 out of 7 patients.[54,56] In the RCTs,

when the outcome was assessed with colorimetric tools,[55] percentage clearance did not differ between PDL and placebo versus PDL with topical sirolimus. When the outcome was a digital photographic image score, the scores were lower with PDL and topical sirolimus than PDL and placebo.[57] Data could not be aggregated because assessment outcomes differed in the 2 RCTs. In one case report, a man in his 20s who had cutaneous microcystic lymphatic malformation (lymphangiectasias) of the scrotum and penile root treated with 0.8% sirolimus petrolatum applied twice daily for 12 weeks showed nearly complete regression.[18]

Results for safety

Adverse effects

Safety data were reported in 37/40 reports, and 120 adverse effects (AEs) were reported. The number of patients who experienced AEs could not be calculated because usually only the number of AEs was reported. Among 212 patients with individual available data, 50 (23.6%) experienced at least one AE. In one study, a general AE was reported (respiratory sepsis) but was not considered linked to sirolimus because blood sirolimus was undetectable. [31] In other reports, only local AEs were described. The most frequent was irritation (n=82, 68.3%), pruritus (n=17, 14.2%), facial acne (n=8, 6.7%), topical allergy (n=5, 4.2%), transient numbness (n=4, 3.3%), sores (n=3, 2.5%) and herpes infections (n=1, 0.8%). The nomenclature for irritative AEs was heterogeneous (stinging, burning, irritation, erythema etc.). Among these 120 AEs, 57 were reported with use of sirolimus solution (47.5%). Regarding biological side effects, 1 of the 12 reports (n=134 patients) describing lab tests performed showed slight abnormalities, consisting of a slight increase in levels of transaminases (n=4), blood glucose (n=4), creatine kinase (n=4) and amylase (n=2) that were not considered related to topical sirolimus. [19]

Systemic passage of sirolimus

Detection of the blood level of sirolimus was described in 22 reports (n=170 patients). Overall, 17 patients were positive for blood sirolimus concentration, but in all cases, blood levels were below the level required for immunosuppression (5 ng/ml).[4,13] The highest blood sirolimus concentration was 3.39 ng/ml after 6-week treatment with 0.1% sirolimus applied once daily on facial PWS previously treated with PDL.[57]

Discussion

Main results

Our systematic review included 40 reports involving 262 patients who received topical application of mTOR inhibitors. Sirolimus was used in all patients except one who received everolimus. Eleven dermatologic conditions were found, the most frequent being facial angiofibromas linked to TSC, for which treatment seemed efficient. Children were more represented (57.1%) than were adults. Local AEs were frequent (120 AEs in 262 patients), mainly consisting of local irritation (n=82), and seemed related to the formulation rather than sirolimus concentration. Sirolimus was detected in blood in 10.0% of patients analyzed and in all cases were below the level required for immunosuppression.

Comments

Topical sirolimus is mainly used in facial angiofibromas in TSC, for which it seems efficient, and has also been tried in other anomalies linked to TSC. Indeed, TSC results from mutations in the TSC1 and TSC2 genes leading to overactivation of the mTOR signaling pathway, which controls cell functions including cell growth, proliferation, and survival.[1] Several reports suggested that in this indication, topical sirolimus was more effective in young than old patients, but this was not evidenced in our review.[17,21,28,29,35,36,41,43] The underlying hypothesis is that sirolimus would be efficient in proliferative tumors with less fibrosis.

The second most frequent indication is PWS, for which topical sirolimus is usually given as an adjunct to PDL to minimize post-laser revascularization, and seems more efficient than without PDL. Indeed, sirolimus has anti-angiogenic properties by downregulating hypoxia-inducible factor 1α, a transcriptional factor that regulates vascular endothelial growth factor expression.[58] However, efficacy was variable in studies, which could be explained by the different locations of PWS,[54] perhaps too-short treatment duration,[54] or local AEs that may affect compliance.[55]

Besides its use for these 2 main indications, topical sirolimus has been anecdotally reported in other dermatologic conditions and seems promising for subungual fibromas and lymphangiectasias. Clinical trials should be conducted to confirm the efficiency.

This review showed heterogeneity of the treatment regime in terms of concentration, number of applications per day and duration. This high heterogeneity is linked to lack of a marketed drug. The most frequent regimen was 0.1% sirolimus, with 1 or 2 applications per day for 12 weeks. Topical sirolimus is produced with the sirolimus used for oral administration, which is available in solution (1 mg/ml) and tablets (1, 2 and 5 mg). Thus, sirolimus in tablet, solution and powder form can be combined with ointment, cream and gel to obtain a topical preparation. Different solvents were used in reports. Bouguéon et al. developed a 0.1% sirolimus cream formulation with solubilized sirolimus that allowed for immediate bioavailability of the active molecule.[59] Sirolimus has been solubilized in a solvent (Transcutol®), which is an excellent permeation agent that enhances drug diffusion through the skin.[59]

Topical sirolimus was generally well tolerated; most symptoms reported by patients were mild to moderate irritation limited to the site of application. Local side effects were not linked to concentration of sirolimus but were more frequent with sirolimus solution. We found no

systemic AEs related to the treatment. Indeed, in this systematic review, we found a positive blood sirolimus concentration in only 10.0% of patients with the dosage, both in children and adults, with different concentrations, frequencies of application per day and formulations. In all cases, blood levels were below the level required for immunosuppression. Sirolimus has a high molecular weight, 914.17 Dalton[17], which allows for diffusion limited to the skin. In contrast, betamethasone has a molecular weight of 392.467 Dalton. However, absorption could be enhanced by additional physical treatment, PDL for instance, or with wide surface application. The surface area of application was never indicated in any studies.

Sirolimus was the mTOR inhibitor used in all patients, except one, who used everolimus.[26] This high use is probably due to sirolimus being the oldest mTOR inhibitor, with a well-known safety profile and better penetration of the skin. However, Dill et al. chose everolimus because of presumed advantages (better water solubility because of its additional hydroxy group and shorter half-life). An RCT is ongoing in France to access everolimus for facial angiofibromas in TSC versus placebo (ClinicalTrials.gov NCT02860494).

Finally, long-term maintenance therapy might be necessary, but in our reports, data on recurrence after treatment were too few to assess the frequency and time to recurrence.

Study limitations

The first limitation is that most RCT data could not be pooled for meta-analysis. Criteria for assessing efficacy were heterogenous: only a few studies used objective scoring systems (eg FASI); most used subjective criteria (cosmetic improvement). Second, the systematic review showed 95.0% efficacy of a topical mTOR inhibitor for facial angiofibromas in TSC, which might be overestimated. Indeed, reports showing treatments as effective are usually published as compared with negative reports (publication bias). Third, formulations of topical sirolimus, concentrations, duration and monitoring were heterogeneous, so comparisons were difficult.

Conclusions

This systematic review and meta-analysis supports the efficacy of topical sirolimus in adults and children for facial angiofibromas linked to TSC. The treatment appears to be safe and non-invasive but locally often irritative, especially when using solutions. Other indications require further research.

References

1. Laplante M, Sabatini DM. mTOR signaling at a glance. *J Cell Sci*. 2009;122(20):3589–94.

2. Saxton RA, Sabatini DM. mTOR signaling in growth, metabolism, and disease. *Cell*. 2017;168(6):960–76.

3. Kaur A, Sharma S. Mammalian target of rapamycin (mTOR) as a potential therapeutic target in various diseases. *Inflammopharmacology*. 2017;25(3):293–312.

4. Kasap B. Sirolimus in pediatric renal transplantation. *Pediatr Transplant*. 2011;15(7):673–85.

5. Sasongko TH, Ismail NFD, Zabidi-Hussin Z. Rapamycin and rapalogs for tuberous sclerosis complex. *Cochrane Database Syst Rev*. 2016;7:CD011272.

6. Abizaid A. Sirolimus-eluting coronary stents: a review. *Vasc Health Risk Manag*. 2007;3(2):191–201.

7. Faivre S, Kroemer G, Raymond E. Current development of mTOR inhibitors as anticancer agents. *Nat Rev Drug Discov*. 2006;5(8):671-88.

8. Sathornsumetee S, Reardon DA, Desjardins A, Quinn JA, Vredenburgh JJ, Rich JN. Molecularly targeted therapy for malignant glioma. *Cancer*. 2007;110(1):13–24.

9. Lee L, Ito T, Jensen RT. Everolimus in the treatment of neuroendocrine tumors: efficacy, side-effects, resistance, and factors affecting its place in the treatment sequence. *Expert Opin Pharmacother*. 2018;19(8):909–28.

10. Mahalingam D, Sankhala K, Mita A, Giles FJ, Mita MM. Targeting the mTOR pathway using deforolimus in cancer therapy. *Future Oncol Lond Engl*. 2009;5(3):291–303.

11. Hammill AM, Wentzel M, Gupta A, Nelson S, Lucky A, Elluru R, et al. Sirolimus for the treatment of complicated vascular anomalies in children. *Pediatr Blood Cancer*. 2011;57(6):1018–24.

12. Adams MT, Saltzman B, Perkins JA. Head and neck lymphatic malformation treatment: a systematic review. *Otolaryngol Head Neck Surg*. 2012;147(4):627–39.

13. Nadal M, Giraudeau B, Tavernier E, Jonville-Bera A-P, Lorette G, Maruani A. Efficacy and safety of mammalian target of rapamycin inhibitors in vascular anomalies: a systematic review. *Acta Derm Venereol*. 2016;96(4):448–52.

14. González-Mateo GT, Aguirre AR, Loureiro J, Abensur H, Sandoval P, Sánchez-Tomero JA, et al. Rapamycin protects from type-I peritoneal membrane failure inhibiting the angiogenesis, lymphangiogenesis, and endo-MT. *BioMed Res Int*. 2015;2015:989560

15. Boccara O, Puzenat E, Proust S, Leblanc T, Lasne D, Hadj-Rabia S, et al. The effects of sirolimus on Kasabach-Merritt phenomenon coagulopathy. *Br J Dermatol*. 2018;178(2):e114–6.

16. Haemel AK, O'Brian AL, Teng JM. Topical rapamycin: a novel approach to facial angiofibromas in tuberous sclerosis. *Arch Dermatol*. 2010;146(7):715–8.

17. 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. *Br J Dermatol*. 2013;169(6):1314–8.

18. Ivars M, Redondo P. Efficacy of topical sirolimus (rapamycin) for the treatment of microcystic lymphatic malformations. *JAMA Dermatol*. 2017;153(1):103–5.

19. Ormerod AD, Shah S a. A, Copeland P, Omar G, Winfield A. Treatment of psoriasis with topical sirolimus: preclinical development and a randomized, double-blind trial. *Br J Dermatol.* 2005;152(4):758-64.

20. Higgins JPT, Altman DG, Gøtzsche PC, Jüni P, Moher D, Oxman AD, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ*. 2011;343:d5928.

21. Amin S, Lux A, O'Callaghan F. Novel treatment option of sirolimus ointment for facial angiofibromas in individuals with tuberous sclerosis complex (TSC). *Arch Dis Child*. 2016;101:A143.

22. Bae-Harboe Y-SC, Geronemus RG. Targeted topical and combination laser surgery for the treatment of angiofibromas. *Lasers Surg Med*. 2013;45(9):555–7.

23. Bloemen-boot MCT, Toonstra J, van Steensel MAM, Pasmans GMA. Topicale rapamycine voor de behandeling van faciale angio bromen bij tubereuzesclerosecomplex. *Ned Tijdschr Dermatol Venereol*. 2014;24(5):323-6.

24. Cinar SL, Kartal D, Bayram AK, Canpolat M, Borlu M, Ferahbas A, et al. Topical sirolimus for the treatment of angiofibromas in tuberous sclerosis. *Indian J Dermatol Venereol Leprol*. 2017 Feb;83(1):27–32.

25. DeKlotz CMC, Ogram AE, Singh S, Dronavalli S, MacGregor JL. Dramatic improvement of facial angiofibromas in tuberous sclerosis with topical rapamycin: optimizing a treatment protocol. *Arch Dermatol*. 2011;147(9):1117–8.

26. Dill PE, De Bernardis G, Weber P, Lösch U. Topical everolimus for facial angiofibromas in the tuberous sclerosis complex. A first case report. *Pediatr Neurol*. 2014;51(1):109–13.

27. Ebrahimi-Fakhari D, Müller CSL, Meyer S, Flotats-Bastardas M, Vogt T, Pföhler C. Topical rapamycin for facial angiofibromas in a child with Tuberous Sclerosis Complex (TSC): a case report and long-term follow-up. *Dermatol Ther*. 2017;7(1):175-179.

28. Foster RS, Bint LJ, Halbert AR. Topical 0.1% rapamycin for angiofibromas in paediatric patients with tuberous sclerosis: a pilot study of four patients. *Australas J Dermatol*. 2012;53(1):52–6.

29. Kaufman McNamara E, Curtis AR, Fleischer AB. Successful treatment of angiofibromata of tuberous sclerosis complex with rapamycin. *J Dermatol Treat*. 2012;23(1):46–8.

30. Knöpfel N, Martín-Santiago A, Bauza A, Hervás JA. Topical 0.2% rapamycin to treat facial angiofibromas and hypomelanotic macules in tuberous sclerosis. *Actas Dermosifiliogr*. 2014;105(8):802–3

31. 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. *Drugs R D*. 2012 12(3):121-6.

32. Mutizwa MM, Berk DR, Anadkat MJ. Treatment of facial angiofibromas with topical application of oral rapamycin solution (1mgmL(-1)) in two patients with tuberous sclerosis. *Br J Dermatol*. 2011;165(4):922–3.

33. Park J, Yun S-K, Cho Y-S, Song K-H, Kim H-U. Treatment of angiofibromas in tuberous sclerosis complex: the effect of topical rapamycin and concomitant laser therapy. *Dermatol Basel Switz*. 2014;228(1):37–41.

34. Pynn EV, Collins J, Hunasehally PRY, Hughes J. Successful topical rapamycin treatment for facial angiofibromata in two children. *Pediatr Dermatol.* 2015;32(3):e120-123.

35. Rodrigo-Nicolas B, Gimeno-Carpio E, Quecedo-Estébanez E, Millán-Parrilla F, Armengot-Carbó M, Molés-Poveda P, et al. Topical rapamycin in facial angiofibromas: assessment of clinical improvement and patient satisfaction in a series of 4 cases. *J Am Acad Dermatol.* 2013;68(4):AB182.

36. Salido R, Garnacho-Saucedo G, Cuevas-Asencio I, Ruano J, Galán-Gutierrez M, Vélez A, et al. Sustained clinical effectiveness and favorable safety profile of topical sirolimus for tuberous sclerosis – associated facial angiofibroma. *J Eur Acad Dermatol Venereol*. 2012;26(10):1315–8.

37. Truchuelo T, Díaz-Ley B, Ríos L, Alcántara J, Jaén P. Facial angiofibromas treated with topical rapamycin: an excellent choice with fast response. *Dermatol Online J*. 2012;18(1):15.

38. Tu J, Foster RS, Bint LJ, Halbert AR. Topical rapamycin for angiofibromas in paediatric patients with tuberous sclerosis: follow up of a pilot study and promising future directions. *Australas J Dermatol.* 2014;55(1):63-9

39. Valerón-Almazán P, Vitiello M, Abuchar A, Kerdel FA. Topical rapamycin solution to treat multiple facial angiofibromas in a patient with tuberous sclerosis. *Actas Dermosifiliogr*. 2012;103(2):165–6.

40. Vasani RJ. Facial angiofibromas of tuberous sclerosis treated with topical sirolimus in an indian patient. *Indian J Dermatol.* 2015;60(2):165–9.

41. Viswanath V, Thakur P, Pund P. Use of topical rapamycin in facial angiofibromas in Indian skin type. *Indian J Dermatol*. 2016;61(1):119.

42. 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. *Br J Dermatol.* 2011;165(4):912–6.

43. 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 Dermatol*. 2017;153(1):39–48.

44. Wheless JW, Almoazen H. A novel topical rapamycin cream for the treatment of facial angiofibromas in tuberous sclerosis complex. *J Child Neurol*. 2013;28(7):933–6.

45. Salido-Vallejo R, Ruano J, Garnacho-Saucedo G, Godoy-Gijón E, Llorca D, Gómez-Fernández C, et al. Facial Angiofibroma Severity Index (FASI): reliability assessment of a new tool developed to measure severity and responsiveness to therapy in tuberous sclerosis-associated facial angiofibroma. *Clin Exp Dermatol*. 2014;39(8):888–93.

46. 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 Dermatol.* 2015;151(7):722-30.

47. 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. *Arch Dermatol*. 2012;148(1):138–9.

48. Muzic JG, Kindle SA, Tollefson MM. Successful treatment of subungual fibromas of tuberous sclerosis with topical rapamycin. *JAMA Dermatol.* 2014;150(9):1024–5.

49. Gijezen LMC, Vernooij M, Martens H, Oduber CEU, Henquet CJM, Starink TM, et al. Topical rapamycin as a treatment for fibrofolliculomas in Birt-Hogg-Dubé syndrome: a double-blind placebo-controlled randomized split-face trial. *PLoS One.* 2014;9(6):e99071.

50. Tu JH, Teng JMC. Use of topical sirolimus in the management of multiple familial trichoepitheliomas. *Dermatol Ther.* 2017;30(2).

51. Wee JS, Chong H, Natkunarajah J, Mortimer PS, Moosa Y. Familial multiple discoid fibromas: unique histological features and therapeutic response to topical rapamycin. *Br J Dermatol*. 2013;169(1):177–80.

52. Díaz-Ley B, Grillo E, Ríos-Buceta L, Paoli J, Moreno C, Vano-Galván S, et al. Classic Kaposi's sarcoma treated with topical rapamycin. *Dermatol Ther*. 2015 Feb;28(1):40–3.

53. Munidasa D, Espinosa O, Burge SM. Topical sirolimus: a novel treatment for chronic erosive plantar lichen planus. *Br J Dermatol*. 2012;167(suppl 1):30.

54. Doh EJ, Ohn J, Kim MJ, Kim YG, Cho S. Prospective pilot study on combined use of pulsed dye laser and 1% topical rapamycin for treatment of nonfacial cutaneous capillary malformation. *J Dermatol Treat*. 2017;28(7):672-677.

55. Greveling K, Prens EP, van Doorn MB. Treatment of port wine stains using Pulsed Dye Laser, Erbium YAG Laser, and topical rapamycin (sirolimus)-A randomized controlled trial. *Lasers Surg Med*. 2017;49(1):104-9.

56. Griffin TD, Foshee JP, Finney R, Saedi N. Port wine stain treated with a combination of pulsed dye laser and topical rapamycin ointment. L*asers Surg Med*. 2016;48(2):193–6.

57. Marqués L, Núñez-Córdoba JM, Aguado L, Pretel M, Boixeda P, Nagore E, et al. Topical rapamycin combined with pulsed dye laser in the treatment of capillary vascular

malformations in Sturge-Weber syndrome: phase II, randomized, double-blind, intraindividual placebo-controlled clinical trial. *J Am Acad Dermatol.* 2015;72(1):151-8.e1.

58. Karar J, Maity A. PI3K/AKT/mTOR Pathway in Angiogenesis. *Front Mol Neurosci.* 2011;4:51.

59. Bouguéon G, Lagarce F, Martin L, Pailhories H, Bastiat G, Vrignaud S. Formulation and characterization of a 0.1% rapamycin cream for the treatment of Tuberous Sclerosis Complex-related angiofibromas. *Int J Pharm.* 2016;509(1–2):279–84.

Abbreviations used:

AEs: adverse effects

CIs: confidence intervals

FASI: facial angiofibroma severity index

mTOR: mammalian target of rapamycin

PDL: pulsed dye laser

PWS: port wine stain

RCT: randomized controlled trial

RRs: risk ratios

TSC: tuberous sclerosis complex

Tables

Table I: Characteristics of reports included in systematic review

Indications		Total number of publications (number of RCTs)	Total number of patients (number of patients included in RCTs)	Median of drug concentration in % [IQR]	Duration of treatment median of weeks [IQR]
Angiofibromas in TSC	Angiofibromas	26 (3)	157 (51)	0.1 [0.1;0.2]	16 [12;24]
Hypomelanotic macules and subungual fibromas linked to TSC	Hypomelanotic macules	2 (0)	8 (0)	0.2	12
	Subungual fibromas	1 (0)	1 (0)	0.1	24
Benign cutaneous tumors	Fibrofolliculomas	1 (1)	19 (19)	0.1	24
	Trichoepitheliomas	1 (0)	2 (0)	1	38 [24;48]
	FMDF	1 (0)	1 (0)	0.1	16
Kaposi's sarcoma	Kaposi's sarcoma	1 (0)	1 (0)	0.5	16
Inflammatory skin diseases	Chronic plaque psoriasis	1 (1)	24 (24)	2.2 then 8	12
	Lichen plan	1 (0)	1 (0)	0.1	20
Vascular malformations	Port wine stains	4 (2)	47 (40)	1 [0.1;1]	11 [10;12]
	CMLM	1 (0)	1 (0)	0.8	12

Abbreviations
effective.
CMLM: cutaneous microcystic lymphatic malformation
FMDF: familial multiples discoid fibromas
IQR: interquartile range
RCTs: randomized controlled trials
TSC: Tuberous Sclerosis Complex

Median and IQR were not mentioned in case of small

Figures

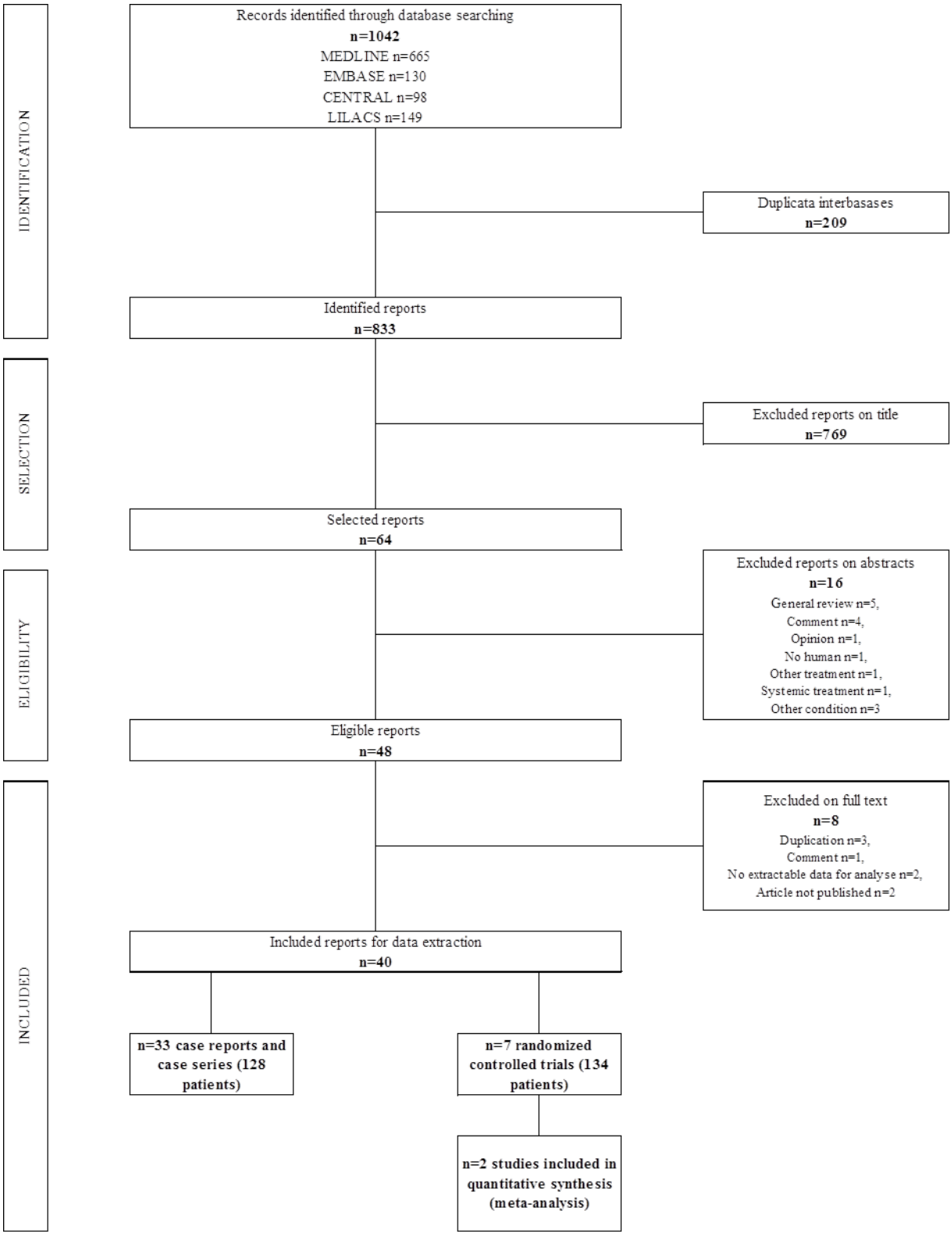


Figure 1. Flow chart of included reports

Figure 2. Risk of bias assessment of the included RCTs by the Cochrane Risk of Bias tool

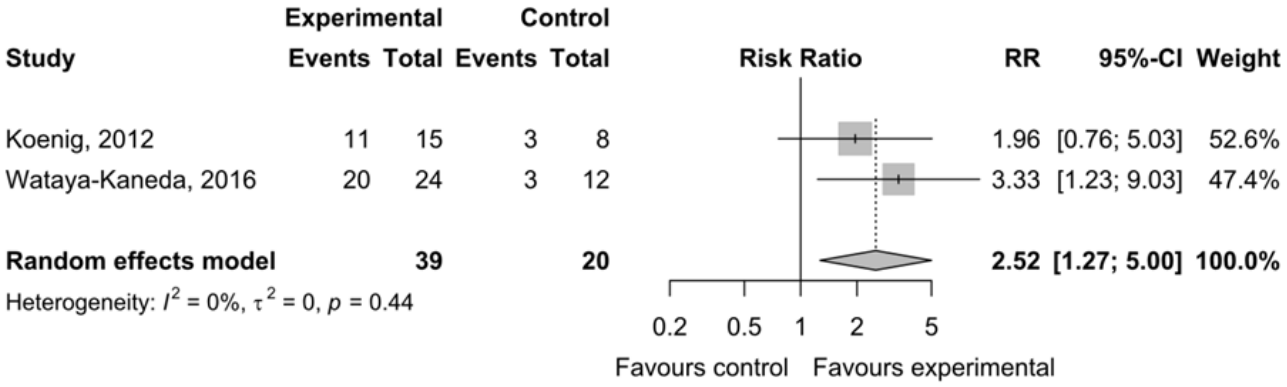


Figure 3. Forest plot showing the effect of topical sirolimus in facial angiofibromas linked to tuberous sclerosis complex

Supplemental files.

Supplemental file 1: Reports on topical mTOR inhibitors in dermatology

Conditions	Author (year)	Patients (demographic data)	Study design	Description of intervention (duration in weeks)	Outcome assessment (number of patients who experienced improvement)	Adverse effects (number of adverse effects)	Blood sirolimus level
Angiofibromas in tuberous sclerosis complex	Amin S. *et al*. (2017)	n=14 (9 children M: 7 Median age: 16 Y)	Prospective observational study	Sirolimus ointment 0.1% Once daily (24)	FASI (12/14)	Irritation (n=1)	NR
	Bae Harboe YS. *et al.* (2013)	n=1 (0 child M: 0 26 Y)	Case report	Sirolimus ointment 0.2% Twice daily In adjunction of physical treatments (12)	PA (1/1)	None	NR
	Bloemen-Boot MCT. *et al.* (2014)	n=10 (10 children M: 6 Median age: 10.5 Y)	Retrospective study	Sirolimus cream and solution 0.01. 0.02 or 0.1% Once daily (NR)	PA (10/10)	Irritation (n=5) and prurit (n=1)	NR
	Cinar SL. *et al.* (2017)	n=12 (12 children M: 7 7-14 Y)	Single-blinded, cross-over and split-body, non-randomized controlled trial (control: vehicle)	Sirolimus cream 0.1% Twice daily (12)	FASI (5.17 + /- 1.34 after 3 months vs 7.58 +/- 0.90 at the beginning (p=0.002))	Irritation (n=3)	-
	De Klotz CMC. *et al.* (2011)	n=1 (NR)	Case report	Sirolimus ointment 1% Twice daily (4)	PA (1/1)	NR	NR
	Dill PE. *et al.* (2014)	n=1 (1 child M: 0 10 Y)	Case report	Everolimus ointment 0.4% Once daily (24)	PA (1/1)	Irritation (n=1)	Undetectable
	Ebrahimi D. *et al.* (2017)	n=1 (1 child M: 0 12 Y)	Case report	Sirolimus ointment 0.1% Twice daily (48)	PA (1/1)	None	Undetectable
	Foster RS. et al. (2012)	n=4 (4 children M: 2 Median age : 6 Y)	Case series	Sirolimus (solution for 2 patients and ointment for 2 patients) 0.1% Twice daily (24)	PA and PRO (4/4)	Irritation (n=2)	Detectable (n=1/4) [0.8 mmol/l]
	Haemel AK. *et al.* (2010)	n=1 (1 child M: 0 16 Y)	Case report	Sirolimus ointment 1% Twice daily (12)	PA (1/1)	None	Undetectable

Kaufman Mc Namara E. *et al.* (2012)	n=1 (1 child M: 1 6 Y)	Case report	Sirolimus cream 0.1% Once daily (12)	PA (1/1)	None	NR
Knopfel N. *et al.* (2014)	n=1 (1 child M: 1 13 Y)	Case report	Sirolimus ointment 0.2% Once daily 5 days a week (48)	PA (1/1)	None	Undetectable
Koenig MK. *et al.* (2012)	n=28 (8 children M: 15 Mean age: 23 Y)	Double-blinded, parallel, RCT (control: vehicle)	Sirolimus cream 0.003% or 0.015% Once daily (24)	PRO (11/15)	None (One unrelated serious general adverse event)	Undetectable
Mutizwa MM. *et al.* (2011)	n=2 (1 child M: 0 15 and 26 Y)	Case report	Sirolimus solution 0.1% Twice daily (10 and 23)	PA (2/2)	Irritation (n=2)	Undetectable
Park J. *et al.* (2014)	n=4 (1 child M: 1 Median age : 29 Y)	Case series	Sirolimus ointment 0.1% Twice daily In adjunction of CO_2 laser (n=3) (12 to 16)	PA (4/4)	None	Undetectable
Pynn EV. *et al.* (2015)	n=2 (2 children M: 2 8 and 11 Y)	Case report	Sirolimus ointment 0.1% Once daily (20 and 36)	PA (2/2)	Irritation (n=1)	NR
Rodrigo-Nicolas B. *et al.* (2014)	n=4 (0 child M: 3 Median age: 44 Y)	Case series	Sirolimus ointment 1% Twice daily (12)	FASI (reduction of FASI at 3 months of 34. 11. 11 and 11.5% with an average of 16.9%) PRO (3/4)	Irritation (n=1)	-
Salido R. *et al.* (2012)	n=10 (7 children M: 5 Median age: 13 Y)	Prospective observational study	Sirolimus ointment 0.4% Once daily 3 days a week (36)	FASI (mean decrease value 60.2% [34.3-100])	None	Undetectable
Tanaka M. *et al.* (2013)	n=11 (7 children M: 7 Median age: 13 Y)	Open-labelled, split body, non-randomized controlled trial (control: vehicle)	Sirolimus ointment or gel 0.2% Twice daily (12)	3 outcome measures: redness, papule size and flatness scoring from -2 to 4 (the averages of patients for all outcome measures were > 2 and significantly higher on the rapamycin-treated cheek at the end of treatment)	None	Undetectable
Truchuelo T. *et al.* (2012)	n=1 (1 child M: 0 11 Y)	Case report	Sirolimus cream 1% Once daily (6)	PA (1/1)	None	Undetectable
Tu J. *et al.* (2014)	n=19 (18 children M: 13 Median age: 10.5 Y)	Retrospective study	Sirolimus ointment or solution 0.1%, 0.5% or 1% Twice daily (32 to 120)	PA (17/19 good or excellent improvement and 2/19 moderate improvement)	Irritation (n=2)	Detectable (n=2/10) [0.5 and 0.8 ng/ml]
Valerón-Almazan P. *et al.* (2012)	n=1 (0 child M: 0 27 Y)	Case report	Sirolimus solution 0.1% Twice daily (12)	PA (1/1)	None	NR

	Vasani RJ. *et al.* (2015)	n=1 (1 child M: 0 17 Y)	Case report	Sirolimus ointment 0.1% Twice daily (12) Then 1% sirolimus ointment twice daily (4)	PA (1/1)	None	-
	Viswanath V. *et al.* (2016)	n=5 (3 children M: 0 Median age: 11 Y)	Case series	Sirolimus ointment 0.1% or 1% Twice daily (4 to 24)	FASI (5/5)	None	NR
	Wataya-Kaneda M. *et al.* (2011)	n=9 (5 children M: 3 Median age: 17 Y)	Open-labelled split body non-randomized controlled trial (control: tacrolimus)	Sirolimus ointment 0.2% In adjunction of topical tacrolimus Twice daily (12)	3 outcome measures: redness, papule size and flatness scoring from 0 to 4 (all the scores significantly improved with sirolimus and tacrolimus compared with the side treated with tacrolimus alone)	None	Undetectable
	Wataya-Kaneda M. *et al.* (2017)	n=36 (18 children M: 17 Median age: 40 Y)	Double-blinded, parallel, RCT (control: placebo)	Sirolimus gel 0.05%, 0.1% or 0.2% Twice daily (12)	Improvement factor (primary outcome): statistically significant in all treatment subgroups except the 0.1% and 0.05% sirolimus adult subgroups	Intervention: irritation (n=20) Control: irritation (n=4)	Detectable (n= 10/24) [≤ 0.25 ng/mL]
	Wheless JW. *et al.* (2013)	n=2 (2 children M: 1 10 and 12 Y)	Case report	Sirolimus cream 0.1% Twice daily for 2 weeks and then once daily (48)	PA (2/2)	None	Undetectable
Hypomelanotic macules in tuberous sclerosis complex	Wataya-Kaneda M. *et al.* (2012)	n=2 (2 children M: 2 2 and 8 Y)	Case report	Sirolimus gel 0.2% Twice daily (12)	PA (2/2)	None	Undetectable
	Wataya-Kaneda M. *et al.* (2015)	n=6 (M: 4 Mean age: 11.7 Y)	Prospective observational study	Sirolimus gel 0.2% Twice daily (12)	Spectrophotometry assessment (5/6)	None	Undetectable
Subungual fibromas in tuberous sclerosis complex	Muzic JG. *et al.* (2014)	n=1 (1 child M: 0 Age NR)	Case report	Sirolimus solution 0.1% Twice daily (24)	PA (1/1)	None	NR
Fibrofolliculomas in Birt-Hogg-Dubé syndrome	Gijezen LMC. *et al.* (2014)	n=19 (0 child M: 13 Mean age: 52 Y)	Double-blinded, split-body, RCT (control: placebo)	Sirolimus solution 0.1% Twice daily (24)	PA (2/19)	Irritation (n=29), pruritus (n=3)	NR
Trichoepitheliomas	Tu JH. *et al.* (2017)	n=2 (2 children M: 1 8 and 6 Y)	Case report	Sirolimus cream 1% Twice daily In adjunction of laser CO_2 (n=1) (24 and 48 weeks)	PA (2/2)	None	NR
Familial multiple discoid fibromas	Wee JS. *et al.* (2013)	n=1 (0 child M: 1 27 Y)	Case report	Sirolimus solution 0.1% Once daily (16)	PA (1/1)	Irritation (n=1) with 2 applications per day	Undetectable

Kaposi's sarcoma	Díaz-Ley B. *et al.* (2014)	n=1 (0 child M: 1 73 Y)	Case report	Sirolimus ointment 0.5% Twice daily (16)	PA (1/1) Pathologic examinations (no residual tumors cells)	Pruritus (n=1)	Undetectable
Chronic plaque psoriasis	Ormerod AD. *et al.* (2004)	n=24 (0 child M: 18 Mean age: 47.5 Y)	Double-blinded, split body, RCT (control: vehicle)	Sirolimus ointment 2.2% (for 6 weeks) then 8% (for 6 weeks) Once daily (12)	Assessment by clinical score from 0 to 8 on erythema, thickening and scaling (mean score was 9.1 in group sirolimus and 11.2 in group control (p=0.032))	Topical allergy (n=2), increase of transaminases (n=4), blood glucose (n=4), creatine kinase (n=4) and amylase (n=2)	Undetectable
Plantar erosive lichen planus	Munidasa D. *et al.* (2012)	n=1 (0 child M: 1 79 Y)	Case report	Sirolimus solution 0.1% Once daily (20)	PA and PRO (1/1)	NR	NR
Port-wine stains	Doh EJ. *et al. (*2017)	n=6 (0 child M: 0 Median age: 26 Y)	Open labelled, split body, non-randomized controlled trial (control: PDL + vehicle)	PDL + sirolimus solution 1% Once daily (1 to 8)	Colorimeter assessment (change in erythema was -2.10 (SD=2.81) for PDL alone, -3.28 (SD=2.39) for PDL with rapamycin for 1 week and -3.12 (SD=2.66) for PDL with rapamycin for 8 weeks. The difference was not statistically siginificant (p=0.43)) PA (1/6)	None	NR
	Greveling K. *et al.* (2017)	n=17 (0 child M: 4 Median age: 29 Y)	Open-labelled split body RCT (control: PDL and PDL + laser Yag)	Sirolimus solution alone, or sirolimus + PDL or sirolimus + PDL + laser YAG 0.1% Once a week (10)	Colorimeter assessment (PDL (16%, SD=34) with PDL + rapamycin (8%, SD=29), p=0.21.	Pruritus (n=12) Topical allergy(n=1)	Detectable (n=2/9) 2.1 ng/ml and 2.2 ng/ml
	Griffin TD. *et al.* (2016)	n=1 (0 child M: 1 56 Y)	Case report	Sirolimus ointment with PDL 0.5% Twice daily (ND)	PA (1/1)	NR	NR
	Marques et L. *et al.* (2015)	n=23 (children NR M: 11 Median age: 33 Y)	Double blinded, split body, RCT (control: placebo and placebo + PDL)	Sirolimus cream or sirolimus + PDL 1% Once daily (12)	Digital photographic image score (-1.9 for PDL + placebo. -0.7 for placebo. -5.7 for PDL + rapamycin and -0.4 for rapamycin (p<0.001))	Irritation (n=14), facial acne (n=8), transient numbness (n=4), small canker sores (n=3), herpes infections (n=1) and topical allergy (n=1)	Number of patients NR Median blood level [0.69 ng/ml at 6 weeks], [1.07 ng/ml at 12 weeks] [max 3.89 ng/ml]
Cutaneous microcystic lymphatic malformation	Ivars M. *et al.* (2017)	n=1 (0 child M: 1 20 Y)	Case report	Sirolimus petrolatum 0.8% Once daily (12)	PA (1/1)	None	-

Abbreviations
PA: Physician assessment
RCT: Randomized controlled trial
PDL: Pulsed dye laser
NR: Not reported
PRO: Patient-reported outcomes
SD: Standard deviation
M: Male
Y: Year

Supplemental file 2: PRISMA checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	25
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	26-27
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	28
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	29
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	-
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	29
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	29
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	29
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	29
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	29-30
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	30
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	-
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	30
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	30

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	30
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	-
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	31
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	31
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	-
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	31-34
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	32
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	31
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	-
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	35-37
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	37
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for futue research.	38
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	25

III- PROTOCOLE DE RECHERCHE *TOPICAL*

1. Résumé en français

Rationnel : Les malformations lymphatiques microkystiques cutanées (MLMC) sont des maladies rares de l'enfant et de l'adulte. Elles se manifestent par des amas de vésicules à contenu clair ou hématique et peuvent se compliquer de suintements et de saignements réguliers. Actuellement, les traitements pour les MLMC sont décevants et leur prise en charge est difficile. Le sirolimus appartient aux inhibiteurs de mTOR (*mammalian Target Of Rapamycin*) ; mTOR est une sérine/thréonine kinase, impliquée notamment dans l'angio- et la lymphangiogenèse. Le sirolimus topique, qui est efficace et bien toléré pour le traitement des angiofibromes faciaux liés à la sclérose tubéreuse de Bourneville, a été récemment rapporté comme un traitement efficace dans quelques cas de MLMC.

Objectif : L'objectif de ce protocole est d'évaluer l'efficacité et la tolérance d'un traitement par sirolimus topique 0,1% pendant 12 semaines dans les MLMC chez les adultes et enfants, versus une crème placebo (véhicule).

Méthodes : Il s'agit d'une étude de phase 2 multicentrique, en split body, randomisée, contrôlée, en double aveugle visant à inclure 50 patients âgés de plus de 6 ans atteints de MLMC primaire. La MLMC sera divisée en 2 zones égales de sévérité équivalente sur lesquelles sera appliqué le sirolimus topique 0,1% ou le véhicule pour 12 semaines. A la fin des 12 semaines, l'ensemble de la MLMC sera traitée par le sirolimus topique pendant 8 semaines supplémentaires. Le critère de jugement principal consistera en l'évaluation de l'amélioration de la zone de la MLMC traitée par sirolimus topique 0,1% comparativement à la zone traitée par le véhicule, par un investigateur (en aveugle), en utilisant le score PGA (*Physician Global Assessment*), à 12 semaines. La qualité de vie, les suintements et saignements, les retentissements fonctionnel et esthétique seront évalués en critères

secondaires. Le passage systémique du sirolimus sera évalué par un dosage sanguin du sirolimus à 6, 12 et 20 semaines. Les événements indésirables et effets secondaires seront recueillis.

Discussion : Pour les patients atteints de MLMC, pathologie rare et sans traitement satisfaisant à ce jour, le sirolimus topique pourrait être une option thérapeutique non invasive et bien tolérée. Si l'étude démontre l'efficacité et la bonne tolérance du sirolimus topique 0,1% chez les patients ayant une MLMC, il deviendra vraisemblablement le traitement de première intention des MLMC, ce qui conduira ainsi à un réel changement dans la prise en charge de cette pathologie.

2. Article scientifique en anglais

0.1% topical sirolimus in the treatment of cutaneous microcystic lymphatic malformations in children and adults (TOPICAL): protocol for a multicenter phase 2, within-person, randomized, double-blind, vehicle-controlled clinical trial

Authors

Sophie Leducq[1,2,3], MD; Agnès Caille[1,3], MD, PhD; Sébastien Barbarot[4], MD, PhD; Nathalie Beneton[5], MD; Didier Bessis[6], MD, PhD; Olivia Boccara[7], MD ; Anne-Claire Bursztejn[8], MD; Christine Chiaverini[9], MD; Anne Dompmartin[10], MD; Catherine Droitcourt[11], MD; Valérie Gissot[3], MD; Dominique Goga[12], MD; Laurent Guibaud[13], MD; Denis Herbreteau[14], MD; Anne Le Touze[15], MD; Christine Léauté-Labrèze[16], MD, PhD; Gérard Lorette[2], MD; Stéphanie Mallet[17], MD; Ludovic Martin[18], MD, PhD; Juliette Mazereeuw-Hautier[19], MD, PhD; Alice Phan[20], MD; Patrice Plantin[21], MD; Pierre Vabres[22], MD, PhD; Bruno Giraudeau[1,3], PhD; Annabel Maruani[1,2,3], MD, PhD; Groupe de Recherche de la Société Française de Dermatologie Pédiatrique

Author informations

[1]Universities of Tours and Nantes, INSERM 1246 – SPHERE, 37000 Tours, France

[2]Department of Dermatology and Reference Center for Rare Diseases and Vascular Malformations (MAGEC), CHRU Tours, 37044 Tours Cedex 9, France

[3]Clinical Investigation Center, INSERM 1415, CHRU Tours, 37000 Tours, France

[4]Department of Dermatology, University Hospital Center of Nantes, 44000 Nantes, France

[5]Department of Dermatology Hospital Center of le Mans, 72037 le Mans, France

[6]Department of Dermatology, University Hospital Center of Montpellier, 34000 Montpellier, France

[7]Department of Dermatology and Reference center for genodermatoses and rare skin diseases (MAGEC), University Hospital Necker-Enfants Malades, 75015 Paris, France

[8]Department of Dermatology, University Hospital Center of Nancy, 54000 Nancy, France

[9]Department of Dermatology, University Hospital Center of Nice, 06000 Nice, France

[10]Department of Dermatology, University Hospital Center of Caen, 54000 Caen, France

[11]Department of Dermatology, University Hospital Center of Rennes, 35000 Rennes, France

[12]University of Tours, CHRU Tours, Department of Maxillo-Facial surgery, 37044 Tours Cedex 9, France

[13]University Hospital Center of Lyon, Consultation Multidisciplinaire Lyonnaise des Angiomes, 69229 Lyon Cedex 2, France

[14]University of Tours, CHRU Tours, Department of Neuroradiology, 37000 Tours, France

[15] Department of Pediatric Surgery, CHRU Tours, 37000 Tours, France

[16] Department of Dematology, Pellegrin Children's Hospital, 33076 Bordeaux, France

[17]Department of Dermatology, University Hospital Center of Marseille, 13885 Marseille Cedex 5, France

[18]Department of Dermatology, University Hospital Center of Angers, 49000 Angers, France

[19]Department of Dermatology, University Hospital Center of Toulouse, 31000 Toulouse, France

[20]Department of Dermatology, University Hospital Center of Lyon, 69229 Lyon Cedex 2, France

[21] Department of Dermatology, Hospital Center of Quimper, 29107 Quimper, France

[22]Department of Dermatology, University Hospital Center of Dijon, 21000 Dijon, France

Corresponding author

Dr Sophie Leducq, Department of Dermatology, CHRU Tours

Avenue de la République, 37044 Tours Cedex 9, France.

Tel: +33 2 47 47 90 80

Fax: +33 2 47 47 82 47

Email: soleducq@gmail.com

4232 Words, 31 references, 2 figures, 1 table, 2 additional files

Conflict of interest:

The authors declare no conflict of competing interests.

Trial registration

ClinicalTrials.gov Identifier: awaiting

EU Clinical Trials Register EudraCT Number: awaiting

Protocol version: version 1.2, October 19, 2018

Abstract

Background

Cutaneous microcystic lymphatic malformations (CMLMs) are rare conditions of children and adults resulting from abnormal embryologic development of lymphatic vessels. They present as clusters of vesicles full of lymph and blood of various extent. They ooze and bleed, inducing maceration, esthetic impairment, scars, pain, bacterial infections and impaired quality of life. Currently, treatments for CMLMs are disappointing, and their management is challenging. Sirolimus is an inhibitor of mammalian target of rapamycin (mTOR), a serine/threonine protein kinase involved in cell growth and proliferation, cellular metabolism, autophagy and angio-lymphangiogenesis. Topical sirolimus, known to be efficient and well tolerated in cutaneous angiofibromas linked to tuberous sclerosis, has recently been reported effective in few reports of patients with CMLMs.

Objective

The objective of this trial is to compare the efficacy and safety of a 12-week application of 0.1% topical sirolimus versus topical vehicle in CMLMs in children and adults.

Methods

This French blinded multicentre within person randomized controlled phase 2 trial aims to include 50 patients ≥ 6 years old who have a primary CMLM without an underlying malformation. The CMLM will be divided into 2 equal areas of the same severity that will be randomly allocated to 0.1% topical sirolimus or topical vehicle for 12 weeks. During the double-blind 12-week period, both topical products will be applied by a nurse to avoid inter-group contamination and for better compliance. At the end of the 12-week period, the patient/parent will treat the whole area of CMLM with 0.1% topical sirolimus on remaining

lesions, for 8 more weeks. Patients will also be seen at week 20 (treatment will be stopped) and at month 12 to evaluate long-term efficacy. The primary outcome will be improvement of the CMLM in the area treated with topical sirolimus compared to the area treated with topical vehicle by the investigator physician (blinded to the treatment) with the Physician Global Assessment score at week 12. Secondary outcomes will include assessment of efficacy by independent experts on the basis of standardized photographs, impact on quality of life; efficacy on oozing, bleeding, erythema and thickness evaluated by the investigators; and global efficacy as well as efficacy for functional and aesthetic impairment evaluated by the patient. Systemic passage of sirolimus will be measured at weeks 6, 12 and 20.

Discussion

For patients with CMLMs, topical sirolimus could be a non-invasive and well-tolerated therapeutic option. If the trial demonstrates efficacy and safety of this treatment in patients with CMLMs, this result will lead to a real change in the management of this condition, and 0.1% sirolimus cream would become the first-line treatment.

Keywords

Cutaneous microcystic lymphatic malformation, lymphangiectasia, sirolimus, rapamycin, topical rapamycin, mammalian target of rapamycin inhibitor, vascular malformation

Background

Background and rationale

Vascular malformations (VMs) are congenital anomalies that might involve four types of vessels (capillary, lymphatic, venous and arterial vessels) according to their classification as low- or high-flow VMs. The International Society for the Study of Vascular Anomalies updated their classification in 2014.[1] Among VMs, cystic lymphatic malformations are rare conditions in children and adults (estimated prevalence < 0.1%).[2,3] They consist of low-flow congenital VMs resulting from abnormal embryologic development of lymphatic vessels.[4] They might be macrocystic, microcystic or combined and can affect viscera, soft tissues and/or skin.

Cutaneous microcystic lymphatic malformations (CMLMs), also called lymphangiectasia, present as clusters of vesicles full of lymph and blood to various extents, usually located in a segmental area (head/neck, lower limbs, gluteal area).[5] They ooze and bleed, inducing maceration, esthetic impairment, scars, pain, bacterial infections, impaired quality of life and sometimes anemia. The natural history is progressive worsening during life (increase in lesions and complications). There are no guidelines for management of CMLMs, but therapeutic options are sclerotherapy (an efficient treatment for macrocystic lymphatic malformations but disappointing in CMLMs), physical treatments (pulsed-dye laser, CO_2 laser, radiofrequency, etc.)[6,7] and surgery[8,9], but these options are painful and induce inflammation and scars, and their efficacy is usually incomplete and transitory, the recurrence rate being very high.[10] Management requires pluridisciplinary care. The “wait and see” attitude is frequently chosen.

Sirolimus belongs to mammalian target of rapamycin (mTOR) inhibitors. mTOR is a serine/threonine kinase, regulated by phosphoinositide-3-kinase, that acts as a master switch

in cell proliferation, apoptosis, metabolism and angio/lymphangiogenesis. Sirolimus directly inhibits the mTOR pathway, which thereby inhibits cell proliferation, angiogenesis and lymphangiogenesis.[11,12] Oral sirolimus is commonly used to prevent rejection of kidney transplants (FDA-approved in this indication).[13] It was recently reported as an efficient drug to reduce the volume of and limit complications in VMs, especially with lymphatic components, in children of all ages.[14] Two randomized controlled trials (RCTs) of oral sirolimus in VMs are ongoing in France and in the United States (NCT02509468 and NCT02110069). For CMLMs without underlying painful involvement, many physicians consider oral sirolimus as a too-aggressive option, despite the high level of impairment linked to the condition.

Topical sirolimus is known to be efficient and well tolerated in cutaneous angiofibromas linked to tuberous sclerosis complex: several RCTs have been performed and the drug is currently used in general practice.[15,16] Recently, topical sirolimus was reported effective in CMLMs, producing decreased number of vesicles, bleeding, oozing and pain, in one retrospective study involving 11 patients and in 3 case reports.[17-20] In the reported cases of CMLM and angiofibromas, only local side effects were reported, mostly irritative. Systemic passage of sirolimus was not or almost not detectable, but the concentration of sirolimus in the preparations and the surface of the areas that receive topical sirolimus were heterogeneous.

Objectives

We aim to perform a clinical trial (TOPical sIrolimus in CutAneous Lymphatic malformation*;* TOPICAL) to assess the efficacy and safety of a 12-week application of 0.1% topical sirolimus versus topical vehicle for CMLMs in children and adults.

Trial design

TOPICAL is a within person randomized, vehicle-controlled, investigator- and patient-blinded, multicenter, superiority study, comparing 0.1% topical sirolimus and vehicle treatment. For each patient, the investigator will divide the CMLM area into two equal areas of homogeneous severity separated by a 2-cm–wide strip (Figure 1). Each area will be randomly allocated to receive 0.1% topical sirolimus or topical vehicle, applied by a nurse, once daily for 12 weeks.

At the end of the 12-week period, the patient/parent will treat the whole area of CMLM with 0.1% topical sirolimus on the remaining lesions, for 8 more weeks (Figure 2). The purpose of this extension phase is to strengthen patients' adherence to the protocol. Indeed, due to the design, each patient will be his/her own control. He/she may observe an improvement in one of the two areas during the 12 week period, and may therefore be attempted to apply the treatment allocated to this area to the whole CMLM, which would introduce group contamination. This is the reason why we planned to have nurses applying the treatments and also why patients will be offered to treat the whole CMLM area with the active drug at the end of the 12 week period. Doing so, we expect them to be compliant to the protocol and not to give up the study.

After week 20, treatments will be left to the discretion of the investigator.

The within person design allows for reducing the number of patients to be included; indeed, the population is too rare for a parallel-group trial. Furthermore, the location and severity of CMLMs are heterogeneous among patients, and a within person design has the advantage of reducing inter-observation variability since each patient is his/her own control. Finally, all patients will receive the experimental treatment, which would not be the case with a parallel-group trial.

Methods: participants, interventions, and outcomes

Study setting

The study will involve 16 French tertiary hospital centers, all involved in the management of vascular anomalies.

Eligibility criteria

Inclusion criteria

Eligible patients will be ≥ 6 years old (for a minimal size) and have a diagnosis of primary CMLM confirmed by histopathological or desmoscopic examination,[21,22] with or without an underlying malformation or a syndromic malformation (e.g., Proteus syndrome) responsible for impairment (oozing, bleeding and/or pain).

. We chose to include adults and children ≥ 6 years old because CMLMs can be seen in both children and adults; however, because the natural history of these malformations is a progressive worsening, impairments of CMLMs are usually not yet major in very young children. Moreover, we need a CMLM area large enough to apply both preparations, with a 2-cm central area in between, and consider that the required minimum area is 20 cm^2, which is usually not possible in children younger than 6 years. A 2-cm–wide space will delimit the two areas of the CMLM to avoid contamination between these 2 areas.

Exclusion criteria

Patients with a lymphatic malformation requiring continued background therapy (involving deep organs) will be excluded, as will patients with secondary lymphatic malformations (lymphangiectasia post-radiotherapy, etc.); immunosuppression; ongoing neoplasia; active chronic infectious disease such as chronic viral hepatitis, tuberculosis or HIV infection; and pregnant females and females of childbearing age not using birth control.

We will exclude patients who previously received systemic or topical mTOR inhibitors within 12 months before inclusion or oral or topical steroids within 10 days before inclusion. Finally, we will exclude patients with contraindications to topical sirolimus, such as local fungal infections, viral infections (e.g., herpes) or bacterial infection (impetigo, etc.) on the site of the CMLM and known allergy to one of the components of the topical sirolimus preparations or the vehicle.

Intervention

For each patient, the investigator will divide the CMLM area into two equal areas separated by an area 2 cm wide. Severity of CMLM is usually quite homogeneous; the two areas chosen by the investigator will have to be of similar severity. Each area will be randomly allocated to receive 0.1% topical sirolimus or topical vehicle, applied once daily for 12 weeks (Figure 1). The 0.1% topical sirolimus application will be prepared and packaged by the pharmacy of the Hospital University Center (CHU) Angers. The formulation of topical sirolimus was developed by the pharmacy team of CHU Angers. Its stability and conservation have been tested and published,[23] and regulatory formalities are under way with health authorities. Sirolimus cream will be prepared by first solubilizing rapamycin in Transcutol®. Then, the mixture will be progressively added to Excipial® hydrocrème. The formulation is 0.03 g rapamycin, 1.5 g Transcutol®, and QS 30 g Excipial® hydrocrème, corresponding to a 0.1% concentration. The cream will be packaged in 30-ml aluminum tubes. The stability of this formulation was studied by high-performance liquid chromatography: the rapamycin concentration remained above 95% of the initial concentration for at least 85 days. The odour, appearance and colour of the preparation remained unchanged during storage.

During the double-blind 12-week period, we planned that a nurse, trained to the protocol, will apply both topical treatments to avoid inter-group contamination and for better compliance. Also, treatments will be kept by the nurses.

A research nurse of CHRU Tours will coordinate and support the nurses at home (2 nurses will be trained for each patient). A nursing notebook with a guide (explanation for application) and material will be provided to the nurses.

Each of the two products will be applied with use of a different glove and massaged until complete penetration of thin thickness. The amount to be applied will be defined by using the fingertip unit (FTU), which is widely used in dermatology.[24] One FTU is equivalent to 20 to 25 mm cream squeezed onto the fingertip. One FTU is 0.5 g cream and is sufficient to treat an area of skin twice the size of an adult's hand (including palm and fingers). Thus, the amount of cream to be applied will be adjusted by using the FTU depending on the size of the area.

Regarding concomitant treatments, bandages in case of oozing and bleeding and topical or systemic antibiotics in case of local bacterial infection will be authorized. Topical steroids, topical immunosuppressive drugs, sclerotherapy, lasers and surgery on the area will be prohibited. Systemic steroids for a more than 3 days, systemic immunosuppressive drugs and oral mTOR inhibitors will be prohibited as well.

Outcomes

Primary outcome

The primary outcome will be the evaluation of the CMLM in the area treated with the intervention (0.1% topical sirolimus) compared with the area treated with topical vehicle

(inactive comparator) by the investigator physician (blinded to treatment) by using the 6-point Physician Global Assessment (PGA) score at week 12.

There are no specific scores for VMs. We chose the 6-point PGA score because it is relevant for this condition and is easy to understand. The PGA score ranges from 0 (clear) to 5 (severe) and is often used in trials investigating various dermatological conditions such as psoriasis.[25,26] We consider that a 1-point improvement in PGA score is clinically relevant for CMLM.

Secondary outcomes

Secondary outcomes are clinical assessments of the efficacy of sirolimus as well as safety. Clinical efficacy will be assessed by different endpoints (Table 1).

- Two blinded independent experts will qualitatively assess digital photographs. Experts will be provided photographs for each patient at baseline and at week 12; they will be asked to identify, at the end of the study, which area among the two areas received the active treatment.
- Comparison of improvement of CMLM in the area treated with topical sirolimus and that treated with topical vehicle will be evaluated by the PGA score at baseline, week 6, week 20 and month 20.
- Quality of life will be self-assessed by the patient at week 20 and month 12 in comparison to baseline by using the validated Dermatology Life Quality Index (DLQI) or Child-DLQI for children.
- Pain linked to the CMLM will be self-assessed by the patient (and parents for children < 16 years old) with a visual analog scale (VAS; 0–10) at baseline, week 20 and month 12.

- Improvement in terms of oozing, bleeding, erythema and thickness will be assessed by the investigator with blinding to treatment on a 0 to 10 VAS (0, no improvement; 10, recovery) at baseline (on both areas), week 12 (on both areas), week 20 (on the whole area), and month 12 (on the whole area).
- Global improvement in CMLM will be self-assessed by the patient on a 0 to 10 VAS (and parents for children < 16 years old) at week 12 (in both areas), week 20 (on the whole area) and month 12 (on the whole area).
- Functional and aesthetic impairment will be self-assessed by the patient (and parents for children < 16 years old) on a 0 to 10 VAS at baseline, week 20 and month 12.

Safety

Local adverse events (AEs) in both areas treated with topical sirolimus and vehicle before week 12 and the whole CMLM at week 20 will be recorded. The expected local AEs are mostly irritative (erythema, burning, dryness, itching, pruritus). Systemic passage of sirolimus will be assessed at weeks 6, 12 and 20 (dosage of serum level of sirolimus) and at week 16 with CMLM area ≥ 30 x 30 cm or ≥ 900 cm^2. Evaluation of biological safety will be assessed at weeks 12 and 20 in comparison to baseline (we will use biological measurements that are required for assessing safety of oral sirolimus: blood cell count, liver and renal functions, ionogram, lipids [cholesterol and triglycerides] and glycemia).

Classical AEs of oral sirolimus are clinical (mostly mucositis, gastrointestinal effects, fatigue, headaches, hypertension) and biological (thrombocytopenia, leucopenia, anemia, hyperlipidemia, hyperglycemia, hypokaliemia and increased liver enzyme levels). However, no general AE has ever been reported with topical sirolimus, but several local AEs were reported. Indeed, the risk of a systemic adverse reaction seems low with topical sirolimus, because systemic absorption is very low or hardly detectable. This risk will be assessed with blood samples in order to assess the safety of the treatment and systemic passage. If a

sirolimus level is detectable, the study treatment will not be stopped. With sirolimus level ≥ 15 ng/ml,[27] the treatment will be stopped.

Safety evaluation parameters will be performed at each visit by asking the patients/parents to report clinical AEs.

Participant timeline

Duration of participation will be 12 months for each patient. The time schedule of enrolment and visits is in the Table 1.

Sample size

The study is planned as a within person design, which means that data from the two areas of a patient will be matched. In addition, the PGA score is an ordered score ranging from 0 (clear) to 5 (severe). Therefore, we will use the Wilcoxon signed-rank test to compare the effect of the treatment at week 12. To the best of our knowledge, we do not have enough data to correctly formulate the required assumptions to estimate the sample size by using a Wilcoxon signed-rank test for paired data. Therefore, we used an approach based on continuous data and the Student *t* test to specify the sample size, assuming that the score difference (between the two zones) follows a normal distribution. Hypothesizing a 1-point difference and a 2.5-point standard deviation would lead to a 0.4 effect size. Assuming a power of 80%, a two-sided type I error rate of 5%, we will need to recruit 52 patients. We plan to recruit 55 patients.

Recruitment

The recruitment of children with these malformations is allowed by the fact that all co-investigators belong to tertiary care centers for vascular anomalies and participate in multidisciplinary consultations on lymphatic malformations. Most co-investigators are already involved in the French trial on oral sirolimus PERFORMUS (NCT02509468) for which

recruitment ended in March 2018. Some belong to the French network for research on pediatric dermatology (Groupe de Recherche de la Société Française de Dermatologie Pédiatrique).

Methods: assignment of interventions

Allocation

Sequence generation and allocation concealment mechanism

In this within-person trial, areas of the CMLM will be randomly assigned to either control or experimental group with a 1:1 ratio allocation as per a computer (SAS based) generated randomization schedule. Participants will be randomized using Ennov Clinical©, an online central randomization procedure via e-CRF. To insure allocation concealment, randomization procedure will not be possible until the participant has been recruited into the trial, especially the consent and all eligibility criteria must be collected and met.

Implementation

Allocation sequence will be generated by a statistician not involved in the recruitment or follow-up of participants.

Blinding

Patients, parents, nurses and investigators will be blinded to the treatment allocated to each area of the CMLM during the first step of the study (until week 12, when the primary endpoint will be assessed). To ensure double blinding, both areas will be randomized, and the topical treatments (sirolimus and vehicle) to be applied will have similar packaging. The appearance of the drugs is similar; thus the active drug (topical sirolimus) and vehicle cannot be distinguished at drug allocation. Also, the consistency of the creams is similar.

Topical sirolimus might induce burning or pruritus. However, we do not consider that this side effect would compromise the blinding by allowing the patient to know which area is being treated with the active cream and the vehicle because 1) identifying precisely which area is itching or burning on a CMLM is difficult, 2) itching and burning might be linked to the CMLM itself, and 3) usually, no inflammation is objectively detectable. The allocation table will be kept in an envelope and will be stored in a secure place. Unblinding will be requested for any reason considered essential by the investigating doctor following the procedure determined in advance.

Methods: data collection, management, and analysis

Data collection methods

The table shows data collection according to inclusion and follow-up visits.

Data management

An e-CRF will be developed by using Ennov Clinical© software. The e-CRF will be managed in agreement with INSERM CIC 1415 Standardized Operating Procedures (SOP). Data from investigating centres will be entered by using a secure web site monitored by clinical research associates, and queries will be edited by data managers, in agreement with an *a priori*-specified data-management plan. A blinded review will be performed before locking the database. The database will be locked in agreement with INSERM CIC 1415 SOPs and data will be extracted in a SAS format or other, according to statistical requirements. Raw data will be stored in an XML format.

Statistical methods

Considering the primary outcome, the study is planned as a within-person trial with the specificity that in these types of studies, each patient serves as his/her own control. In this

way we will have a paired sample. The PGA score for the two areas at week 12 will be compared by a Wilcoxon signed rank test if necessary. For secondary outcomes, PGA score, physician assessment of efficacy, patient self-assessment and biological relative changes will be analyzed by the same approach (i.e., paired *t* test or Wilcoxon signed-rank test). Considering efficacy seen on digital photographs, assessed by two independent trained readers, the "lady-tasting-tea" procedure will be used to test whether readers performed better than expected in identifying the area treated by the active treatment.[28] Regarding AEs and systemic passage of sirolimus, descriptive statistics (numbers and percentages) will be calculated.

Methods: monitoring

Data monitoring

A clinical research assistant will be responsible for coordinating the study: the assistant will be responsible for the logistics of and monitoring the study, producing reports concerning its state of progress, verifying that the e-CRFs are updated (request for additional information, corrections, etc.) and transmitting severe AEs to the sponsor. The technician will follow the SOPs.

A data safety monitoring board (DSMB) will be composed of three medical doctors specialized in pharmaco-dermatology and dermatology. The DSMB will be systematically contacted 1) at any time by the sponsor for each case of expected serious adverse reaction or for a suspected unexpected serious adverse drug reaction (SUSAR); 2) before each development safety update report is sent to the French Agency for the Safety of Health Products (ANSM); and 3) if data may change the benefit/risk ratio during the clinical trial.

Harms

All AEs will be monitored until they are completely resolved. The investigator will immediately notify the sponsor of any serious AE. The sponsor will report all SUSARs to the Eudravigilance (European pharmacovigilance database), French health authorities (ANSM), and the investigators within the regulatory time periods for reporting.

Auditing

An audit may be performed at any time by sponsor-appointed people who are independent of those responsible for the study. The investigators agree to comply with the requirements of the sponsor and the relevant authority for an audit or inspection of the study. The audit can apply at all stages of the study, from development of the protocol to publication of results.

Ethics and dissemination

Research ethics approval

The sponsor and the investigators undertake to conduct this study in compliance with French law no. 2004-806 of August 9, 2004 and following Good Clinical Practice and the Helsinki Declaration (Ethical Principles for Medical Research involving Human Subjects, Tokyo 2004). The study will be conducted in accordance with this protocol. With the exclusion of emergency situations requiring specific therapeutic actions, the investigators will observe the protocol in all respects, particularly in obtaining consent and the notification and follow-up of serious AEs.

The protocol was submitted to the French institutional review board and to ANSM for authorizations.

Protocol amendments

Important protocol modifications will be submitted for approval to the institutional review board of the University Hospital of Tours and will be communicated to coinvestigators.

Consent and assent

Participants will be orally informed of the study and will receive written information and their informed sign consent will be obtained. For children < 18 years old, parents will give their informed signed consent after their child has consented (if able). Children ≥ 16 years old must also consent to use of their data according to article 89 (Regulation [EU] 2016/679 - RGPD).

Confidentiality

During this biomedical research study or when it is completed, the information collected for participants and forwarded to the sponsor by the investigators (or any other specialized staff member involved) will be made anonymous. Under no circumstances will the uncoded names or addresses of the participants concerned appear in any data.

Access to data

The sponsor is responsible for obtaining agreement from all parties involved in the study in order to guarantee direct access (in all sites where the study is being conducted) to source data, source documents and reports, to control their quality and to audit them.

The investigators will make available to people with a right of access according to the legislative and regulatory provisions in force (articles L.1121-3 and R.5121-13 of the French Public Health Act) the documents and individual data strictly necessary for monitoring, carrying out quality control and auditing the biomedical research.

Dissemination policy

INSERM CIC 1415 Tours will analyse the data provided by the study centres. Results will be displayed in a written report that will be submitted to the sponsor. At the end of the analysis, results will be published in ClinicalTrials.gov. The international rules for writing and publication (Vancouver Agreement, February 2006) will be followed.

Patients will be informed, at their request, about the overall results of the study.

SPIRIT

This protocol has been written in accordance with the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines. The SPIRIT checklist is in Additional file 2.

Discussion

This within-person, blinded RCT aims to compare 0.1% topical sirolimus with topical vehicle in CMLMs in children over 6 years and adults. Several case reports have suggested efficacy of this treatment, linked to the anti-lymphangiogenic properties of sirolimus, but this needs to be demonstrated. CMLMs are very rare diseases, and this design allows for reducing the number of patients to be included; indeed, the population is too rare for a parallel-group trial. Furthermore, the location and severity of CMLMs are heterogeneous among patients, and a within-person design, also called split-body design, has the advantage of reducing individual inter-variability. Finally, all patients will receive the experimental treatment, which would not be the case with a parallel-group trial.

The hypothesis of this protocol study is that 0.1% topical sirolimus applied once daily during 12 weeks on CMLM is more efficient than a placebo cream (vehicle), by reducing the thickness of CMLM, oozing, bleeding, impairments and pain. In the literature, modalities of use of topical sirolimus are highly heterogeneous: the product has been used as creams or solutions, with concentrations ranging from 0.015 to 8%. Creams were better tolerated than solutions, and the most frequently used was sirolimus cream 0.1%, all conditions included.[29] This heterogeneity is linked to the fact that topical sirolimus is not marketed, and different formulations are therefore used. Sirolimus has a high molecular weight, 914.17 Dalton,[30] which allows for diffusion limited to the skin. Thus, rapamycin was soluzibilized in a solvent

(Transcutol®) which is an excellent permeation agent that enhances drug diffusion through the skin.[31]

We expect no general side effects and no severe local adverse effects: for topical sirolimus, adverse effects previously described are local and of mild intensity: (irritation, burning sensation, pruritus), reported in one third of cases. We also expect a non-significant blood passage of the drug. Indeed, in previous reports, systemic passage of sirolimus with different concentrations of topical sirolimus in different skin conditions was not or almost not detectable and depends on the quantity of active principle administered and on the surface to be treated.[29] In the present study, if systemic passage of sirolimus is evidenced, the consequence would be a reduction in difference between the two areas, and this would be unfavorable to our expected results.

As current treatments in CMLM are often disappointing and painful (lasers, surgery, sclerotherapy), an individual benefit is expected. If the trial demonstrates the efficacy and safety of the treatment in patients with CMLMs, this will lead to a real change in the management of this rare condition, and 0.1% sirolimus cream would become the first-line treatment.

Trial status

Regulatory applications have been submitted (French institutional review board and ANSM).

Abbreviations

AEs: Adverse Events

ANSM: Agence Nationale de Sécurité du Médicament et des produits de santé

CMLM: Cutaneous Microcystic Lymphatic Malformation

DLQI: Dermatology Life Quality Index

DSMB: Data Safety Monitoring Board

e-CRF: Electronic Case Report Form

HPLC: High Pressure Liquid Chromatography

ISSVA: International Society for the Study of Vascular Anomalies

mTOR: mammalian Target Of Rapamycin

PGA: Physician Global Assessment

RCT: Randomized Controlled Trial

SAEs: Severe Adverse Events

SOP: Standardized Operating Procedures

SUSAR: Suspected Unexpected Serious Adverse Reaction

VAS: Visual Analog Scale

VM: Vascular Malformation

Declarations

Ethical Approval and Consent to participate

Regulatory applications have been submitted (French institutional review board and ANSM).

Written and oral informed consent is obtained from all participants before enrolment.

Consent for publication

We have obtained informed consent for publication of the dataset from patients at the point of recruitment to the trial. All patient details will be fully anonymous.

Competing interests

The authors declare no competing interests.

Funding

The study is funded by the French Ministry of Social Affairs and Health (French National Program of Clinical Research [PHRC-N], 2017). No industry support is involved.

Authors's contributions

SL, AM, GL, DG, DH, AL, VG and BG conceived of the study. OB, DB, LG, PV, JMH, SB, CC, SB, CD, CD, SM, LM, SL and BM participated in its design and coordination. AC and BG perform the statistical calculations. SL, AM, BG and AC will write the manuscript. All authors will read and approve the final manuscript.

Acknowledgements

We are indebted to Mrs Frédérique Musset, Anne Rebion and Michèle Carriot, Clinical Investigation Center of CHU Tours; Mrs Estelle Boivin from the Délégation à la Recherche Clinique et à l'Innovation, University Hospital Centre (CHU) Tours. We also deeply thank Dr Hélène Bourgoin, Pharmacy of CHU Tours, and Dr Sandy Vrignaud, Pharmacy of CHU Angers, for their technical help. We thank Dr Bénédicte Lebrun-Vignes, Assistance-Publique-Hôpitaux de Paris; Dr Laurence Le Cleach, Assistance-Publique-Hôpitaux de Paris; and Dr Romain Gabeff, CH La Roche sur Yon, for being members of the Data Safety Monitoring Board of TOPICAL.

Additional files

Additional file 1. Informed consent

Additional file 2. SPIRIT checklist

References

1. Wassef M, Blei F, Adams D, Alomari A, Baselga E, Berenstein A, et al. Vascular anomalies classification: recommendations from the International Society for the Study of Vascular Anomalies. Pediatrics. 2015;136:e203–14.

2. Kennedy TL, Whitaker M, Pellitteri P, Wood WE. Cystic hygroma/lymphangioma: a rational approach to management. The Laryngoscope. 2001;111:1929–37.

3. Smith RJH. Lymphatic malformations. Lymphat Res Biol. 2004;2:25–31.

4. Schulte-Merker S, Sabine A, Petrova TV. Lymphatic vascular morphogenesis in development, physiology, and disease. J Cell Biol. 2011;193:607–18.

5. Eliasson JJ, Weiss I, Høgevold HE, Oliver N, Andersen R, Try K, et al. An 8-year population description from a national treatment centre on lymphatic malformations. J Plast Surg Hand Surg. 2017;51:280–5.

6. Acord M, Srinivasan AS, Cahill AM. Percutaneous treatment of lymphatic malformations. techniques in vascular and interventional radiology. 2016;19:305–11.

7. Erikçi V, Hoşgör M, Yıldız M, Örnek Y, Aksoy N, Okur Ö, et al. Intralesional bleomycin sclerotherapy in childhood lymphangioma. Turk J Pediatr. 2013;55:396–400.

8. Defnet AM, Bagrodia N, Hernandez SL, Gwilliam N, Kandel JJ. Pediatric lymphatic malformations: evolving understanding and therapeutic options. Pediatr Surg Int. 2016;32:425–33.

9. Bagrodia N, Defnet AM, Kandel JJ. Management of lymphatic malformations in children. Curr Opin Pediatr. 2015;27:356–63.

10. Lerat J, Mounayer C, Scomparin A, Orsel S, Bessede J-P, Aubry K. Head and neck lymphatic malformation and treatment: Clinical study of 23 cases. European Annals of Otorhinolaryngology, Head and Neck Diseases. 2016;133:393–6.

11. Xue Q, Nagy JA, Manseau EJ, Phung TL, Dvorak HF, Benjamin LE. Rapamycin inhibition of the Akt/mTOR pathway blocks select stages of VEGF-A164 driven angiogenesis, in part by blocking S6Kinase. Arterioscler Thromb Vasc Biol. 2009;29:1172–8.

12. Desai N, Heenan S, Mortimer PS. Sirolimus-associated lymphoedema: eight new cases and a proposed mechanism. Br J Dermatol. 2009;160:1322–6.

13. Kasap B. Sirolimus in pediatric renal transplantation. Pediatr Transplant. 2011;15:673–85.

14. Strychowsky JE, Rahbar R, O'Hare MJ, Irace AL, Padua H, Trenor CC. Sirolimus as treatment for 19 patients with refractory cervicofacial lymphatic malformation. Laryngoscope. 2018;128:269-276.

15. 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 Dermatol. 2017;153:39–48.

16. 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 Dermatol. 2018;154:773–80.

17. Çalışkan E, Altunel CT, Özkan CK, Tunca M. A case of microcystic lymphatic malformation successfully treated with topical sirolimus. Dermatol Ther. 2018;e12673.

18. Ivars M, Redondo P. Efficacy of topical sirolimus (rapamycin) for the treatment of microcystic lymphatic malformations. JAMA Dermatol. 2017;153:103–5.

19. García-Montero P, Del Boz J, Sanchez-Martínez M, Escudero Santos IM, Baselga E. Microcystic lymphatic malformation successfully treated with topical rapamycin. Pediatrics. 2017;139.

20. Leducq S, Vrignaud S, Lorette G, Herbreteau D, Dubée V, Martin L, et al. Topical rapamycin (sirolimus) for treatment of cutaneous microcystic lymphatic malformation of the gluteal area. Eur J Dermatol. 2018. Accepted.

21. Zaballos P, del Pozo LJ, Argenziano G, Karaarslan IK, Landi C, Vera A, et al. Dermoscopy of lymphangioma circumscriptum: A morphological study of 45 cases. Australas J Dermatol. 2018;59:e189-e193.

22. Jha AK, Lallas A, Sonthalia S. Dermoscopy of cutaneous lymphangioma circumscriptum. Dermatol Pract Concept. 2017;7:37–8.

23. Bouguéon G, Lagarce F, Martin L, Pailhories H, Bastiat G, Vrignaud S. Formulation and characterization of a 0.1% rapamycin cream for the treatment of Tuberous Sclerosis Complex-related angiofibromas. International Journal of Pharmaceutics. 2016;509:279–84.

24. Long CC, Finlay AY. The finger-tip unit--a new practical measure. Clin Exp Dermatol. 1991; 16:444-7.

25. Breuer K, Braeutigam M, Kapp A, Werfel T. Influence of pimecrolimus cream 1% on different morphological signs of eczema in infants with atopic dermatitis. Dermatology (Basel). 2004;209:314–20.

26. Garduno J, Bhosle MJ, Balkrishnan R, Feldman SR. Measures used in specifying psoriasis lesion(s), global disease and quality of life: a systematic review. J Dermatolog Treat. 2007;18:223–42.

27. Nadal M, Giraudeau B, Tavernier E, Jonville-Bera A-P, Lorette G, Maruani A. Efficacy and safety of mammalian target of rapamycin inhibitors in vascular anomalies: a systematic review. Acta Derm Venereol. 2016;96:448–52.

28. Falissard B, Milman D, Cohen D. A generalization of the «lady-tasting-tea»

procedure to link qualitative and quantitative approaches in psychiatric

research. Int J Stat Med Res. 2013;2:88-93.

29. Leducq S, Giraudeau B, Tavernier E, Maruani A. Topical use of mammalian target of rapamycine inhibitors in dermatology: a systematic review with meta-analysis. 2018. Submitted.

30. 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. Br J Dermatol. 2013;169:1314–8.

31. Mura P, Faucci MT, Bramanti G, Corti P. Evaluation of transcutol as a clonazepam transdermal permeation enhancer from hydrophilic gel formulations. Eur J Pharm 2000;9:365-372.

Tables

Table 1. Schedule of enrolment, interventions, and assessments

	STUDY PERIOD					
	Screening	Inclusion	Follow up			
	V0	**D1 (V1)**	**W6 (V2)**	**W12 (V3)**	**W20 (V4)**	**M12 (V5)**
TIMEPOINT	*14 to 28 days just before treatment initiation*	-	+/- 3 days	+/- 3 days	+/- 3 days	+/- 5 days
DRUG ADMINISTRATION		<——————once daily——————>				
ENROLLMENT						
Eligibility screening	X					
Informed consent	**X**					
Control of inclusion and non-inclusion criteria	X					
Preparations ordering	**X**					
Randomization		**X**				
Physical examination		X	X	X	X	X
Height and weight		X				
Vital signs(cardiac frequency, arterial pressure)		X	X	X	X	X
Photographs		X		X		
Location and mesure of the CMLM	X					
Definition of the 2 areas		X				
Urinary pregnancy test for women***	X	X	1 X / month until S20		X	

Blood sample for tolerance*		X		X	X	
Seric level of sirolimus****			X	X	X	
Cutaneous effects			X	X	X	
Adverse events			X	X	X	
Delivery of nursery/tracking notebook		X	X	X		
INTERVENTIONS**:						
Delivery of topical sirolimus		**X**	**X**	**X**		
Delivery of vehicle Treatment returns		**X**	**X** X	X	X	
ASSESSMENTS:						
PGA score		X	X	X	X	X
Dermatological quality of life scale-DLQI		X			X	X
VAS for self-assessment of pain		X			X	X
VAS for assessment of efficacy by the investigator on oozing, bleeding, erythema, thickness		X		X	X	X
VAS for self-assessment of global efficacy				X	X	X
VAS for self-assessment on functional and esthetic impairments		X			X	X

* Blood count, ionogram, liver function (ASAT, ALAT, gamma-GT), renal function (creatinine), lipids (cholesterol, triglycerides), glycemia. Local analysis.

** Application of both topical preparations (intervention or inactive comparator) by a liberal nurse

*** If detectable sirolimus rate: monthly pregnancy test for 3 additional months.

**** If CMLM ≥ 30*30 cm and/or ≥900 cm^2, a blood sample for evaluation of systemic passage of sirolimus (residual serum level of sirolimus) will be performed at week 16

Figures

Figure 1. Example of a cutaneous microcystic lymphatic malformation of the leg: the investigator will define two areas of similar size and severity by using a ruler, with a separation area of at least 2 cm wide between both areas (five points will be drawn on the patient to define the two areas to apply the product in a reproducible way by the nurse and to avoid inter-area contamination).

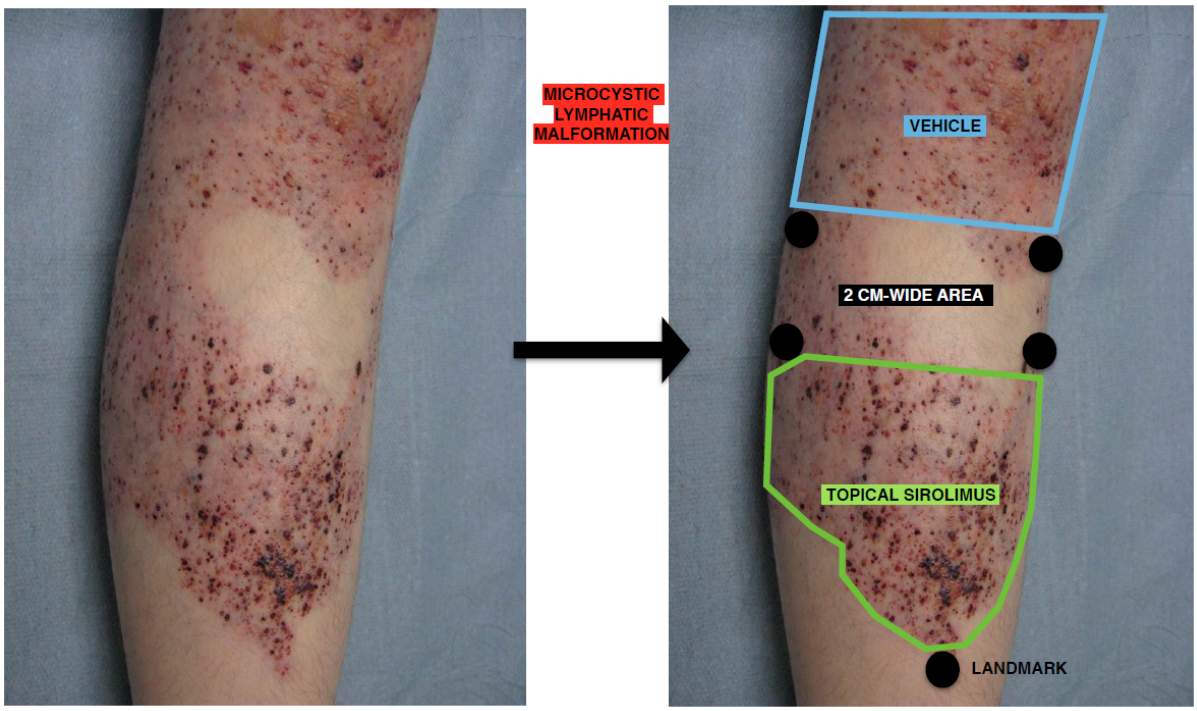
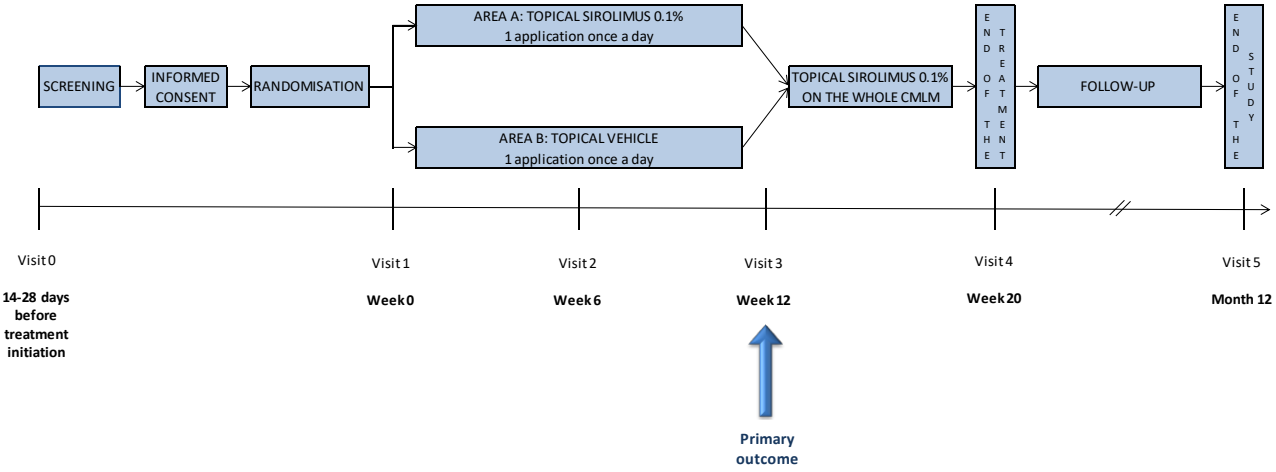


Figure 2. Flow-chart of the study



Additional file 1. Informed consent

Formulaire de consentement de participation à une Recherche interventionnelle sur la personne humaine :
Adultes participant à l'étude

Je soussigné(e) ***Nom :*** …… ***Prénom :*** ……

Adresse : ……

……

Déclare avoir pris connaissance des informations orales et écrites (*lettre d'information v1-A du 19/07/2018*) qui m'ont été transmises pour une **participation à l'étude clinique TOPICAL** « *Évaluation de l'efficacité et de la tolérance du sirolimus topique 0.1% dans les malformations cutanées lymphatiques microkystiques : essai thérapeutique de phase II, randomisé, en double aveugle, en comparaison intra individuelle versus véhicule* » organisée par le CHRU de Tours qui en est le Promoteur, autorisée par l'ANSM (n° XXXX) et le CPP XX (n°XXXX).

J'ai reçu du médecin investigateur, toutes les informations nécessaires concernant l'objectif de cette recherche, son déroulement ainsi que mes conditions de participation, mes droits, les bénéfices attendus, les contraintes et les risques prévisibles. J'ai pu poser toutes les questions que je voulais et j'ai reçu les réponses adaptées. J'ai bénéficié d'un temps de réflexion suffisant entre ces informations et le présent consentement. J'ai également compris que je pouvais refuser de participer à cette étude sans que cela ne porte préjudice à la suite de ma prise en charge. Compte tenu des informations qui m'ont été transmises,

- J'accepte librement et volontairement de participer à cette étude,

- J'accepte que les données enregistrées à l'occasion de cette étude puissent faire l'objet d'un traitement informatisé par le promoteur ou pour son compte dans les conditions prévues par la Loi Informatique et Libertés et par le Règlement Européen 2016/679. J'ai été informé(e) de tous mes droits concernant mes données personnelles selon les modalités décrites dans la lettre d'information en vigueur,

- Je certifie être bénéficiaire d'un régime de sécurité sociale.

Conformément à la loi, le CHRU de Tours, promoteur de cette étude, a souscrit une assurance de responsabilité auprès de la compagnie SHAM afin de couvrir tout préjudice corporel ou toute incapacité que pourrait entraîner cette étude.

Partie à remplir par le participant à l'étude	***Partie à remplir par le médecin investigateur***
Nom et prénom : _____	Nom et prénom : _____
Date : ____ / ____ / _____ Signature :	Date : ____ / ____ / _____ Signature :

En deux exemplaires : original pour le médecin investigateur et un exemplaire pour le participant

Formulaire de consentement de participation à une Recherche interventionnelle sur la personne humaine : **Mineurs participant à l'étude**

Parent (1) : Je soussigné(e),
Nom : **……** *Prénom :* **……**
Adresse : **……**
……

Parent (2) : Je soussigné(e),
Nom : **……** *Prénom :* **……**
Adresse (à compléter si différente de (1)) : **……**
……

Merci de cocher seulement si applicable : ☐ **Certifie être le seul titulaire de l'exercice de l'autorité parentale**

De l'enfant *Nom :* **……** *Prénom :* **……** **Né(e) le :** ___ / ___ / _____

Déclarent avoir pris connaissance des informations orales et écrites (*lettres d'information v1-B du 19/07/2018*) qui nous ont été transmises pour une **participation de notre enfant à l'étude clinique TOPICAL** « *Évaluation de l'efficacité et de la tolérance du sirolimus topique 0.1% dans les malformations cutanées lymphatiques microkystiques : essai thérapeutique de phase II, randomisé, en double aveugle, en comparaison intra individuelle versus véhicule* » organisée par le CHRU de Tours qui en est le Promoteur, autorisée par l'ANSM (n° XXXX) et le CPP XX (n°XXXX).

Nous avons reçu du médecin investigateur qui suit notre enfant, toutes les informations nécessaires concernant l'objectif de cette recherche, son déroulement ainsi que les conditions de participation de notre enfant, nos droits, les bénéfices attendus, les contraintes et les risques prévisibles. Nous avons pu poser toutes les questions que nous voulions et nous avons reçu les réponses adaptées. Nous avons bénéficié d'un temps de réflexion suffisant entre ces informations et le présent consentement. Nous avons également compris que nous pouvions refuser que notre enfant participe à cette étude sans que cela ne porte préjudice à la suite de sa prise en charge.

Compte tenu des informations qui nous ont été transmises,

- **Nous acceptons librement et volontairement que notre enfant participe à cette étude,**

- **Nous acceptons que les données enregistrées à l'occasion de cette étude puissent faire l'objet d'un traitement informatisé par le promoteur ou pour son compte dans les conditions prévues par la Loi Informatique et Libertés et par le Règlement Européen 2016/679. J'ai été informé(e) de tous mes droits concernant mes données personnelles selon les modalités décrites dans la lettre d'information en vigueur. Nous avons été informés que si notre enfant a 16 ans ou plus, ce dernier doit également donner son consentement pour le traitement informatisé de ses données.**

- **Nous certifions être bénéficiaires d'un régime de sécurité sociale.**

Conformément à la loi, le CHRU de Tours, promoteur de l'étude, a souscrit une assurance de responsabilité auprès de la compagnie SHAM afin de couvrir tout préjudice corporel ou toute incapacité que pourrait entraîner cette étude.

Partie à remplir par les parents (ou titulaire de l'exercice de l'autorité parentale)	
Date : ____ / ____ / _____	Signature :
Date : ____ / ____ / _____	Signature :
Partie à remplir par le participant mineur de 16 ans ou plus	
Date : ____ / ____ / _____	J'ai bien été informé(e) de l'étude (*lettre d'information v1-C*) et j'accepte le traitement informatisé de mes données. Signature :

Partie à remplir par le médecin investigateur	
Nom, Prénom : ……	Signature :
Date : ____ / ____ / _____	

En deux exemplaires : original pour le médecin investigateur et un exemplaire pour les parents

Additional file 2: SPIRIT checklist

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	ItemNo	Description	Page Number on which item is reported
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	59
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	61
	2b	All items from the World Health Organization Trial Registration Data Set	-
Protocol version	3	Date and version identifier	61
Funding	4	Sources and types of financial, material, and other support	81
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	81
	5b	Name and contact information for the trial sponsor	81

	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	81
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	81
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	64-65
	6b	Explanation for choice of comparators	64-65
Objectives	7	Specific objectives or hypotheses	65
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	65-66
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	67
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	67-68

Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	68-69
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	66, 68-69
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	66, 68-69
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	68-69
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	69-71
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	72
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	72
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	72-73
Methods: Assignment of interventions (for controlled trials)			
Allocation:			

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	73
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	73
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	73
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	73-74
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	73-74
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	74
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	-

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	74
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	74-75
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	-
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	74-75
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	75
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	-
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	75-76
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	76

Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	76
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	76
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	76-77
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	77
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	81
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	-
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	-
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	77

	31b	Authorship eligibility guidelines and any intended use of professional writers	-
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	-
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Additional file 1
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

IV- CONCLUSION GÉNÉRALE ET PERSPECTIVES

L'utilisation des inhibiteurs de mTOR par voie topique est croissante en dermatologie, avec une efficacité démontrée sur les angiofibromes faciaux liés à la sclérose tubéreuse de Bourneville. Leur tolérance est globalement bonne, avec uniquement des effets secondaires locaux, principalement irritatifs, décrits. Le passage systémique du sirolimus est faible ou absent.

D'autres indications semblent prometteuses mais nécessitent des essais randomisés pour confirmer leur efficacité. L'essai randomisé, en *split body*, étudiant le sirolimus topique 0,1% versus placebo dans les malformations lymphatiques cutanées microkystiques pourrait permettre de modifier la prise en charge de cette pathologie chronique, qui est sans traitement de référence actuellement. Du fait de l'absence de produit commercialisé du sirolimus topique, la crème sera préparée par le CHU d'Angers via la plateforme PPRIGO (Production Pharmaceutique pour la Recherche Institutionnelle du Grand Ouest). La formulation a été développée par le service de Pharmacie du CHU d'Angers, sa stabilité et sa conservation ont été étudiées et publiées (Bouguéon et al., 2016).

D'autres protocoles de recherche sur les malformations lymphatiques, coordonnés par notre équipe de recherche, sont en cours ou en développement : PERFORMUS (traitement par sirolimus des malformations vasculaires de bas débit, volumineuses et compliquées, chez l'enfant : essai thérapeutique de phase 2, multicentrique, PHRCN 2014) dont le recrutement est achevé et le suivi se poursuit ; et TOPGUN (efficacité d'un traitement par sirolimus des malformations lymphatiques microkystiques linguales buccales, légères à modérées, chez l'enfant à partir de 6 ans et l'adulte, Appel d'Offre Interne, CHRU Tours 2018) dont la rédaction du protocole est en cours de finalisation.

Après avoir réalisé le travail de revue de la littérature, j'ai participé à la rédaction du protocole de recherche TOPICAL, coordonnée par le Pr Maruani et avec l'aide de l'équipe du Centre d'Investigation Clinique (CIC) - INSERM 1415 de Tours. J'ai ensuite effectué un semestre au cours de mon internat au sein du CIC, qui m'a permis de participer à la rédaction de protocoles et à la réalisation d'analyses statistiques.

J'ai débuté en Octobre 2018 un Master 2 de Modélisation en Pharmacologie Clinique et Épidémiologie (Universités de Nantes et Rennes), avec comme projet de Master 2 la réalisation d'un algorithme décisionnel pour la planification d'un essai évaluant un traitement topique.

Ainsi, la participation à la rédaction d'un protocole de recherche dans le cadre de ma thèse et mon stage au CIC m'ont permis d'acquérir les bases théoriques et pratiques nécessaires à la recherche clinique et m'ont confortée dans mon attrait pour ce domaine.

Leducq Sophie
Pages : 102
Tableaux : 2
Figures : 5
Annexes : 4

Résumé :
Rationnel. Les malformations lymphatiques microkystiques cutanées (MLMC) sont des maladies rares de l'enfant et de l'adulte. Elles se manifestent par des amas de vésicules à contenu clair ou hématique et peuvent se compliquer de suintement et saignement réguliers. Actuellement, les traitements pour les MLMC sont décevants et leur prise en charge est difficile. Le sirolimus appartient aux inhibiteurs de mTOR (*mammalian Target Of Rapamycin*) ; mTOR est une sérine/thréonine kinase, impliquée notamment dans l'angio/lymphangiogenèse. Le sirolimus topique, qui est efficace et bien toléré pour le traitement des angiofibromes faciaux liés à la sclérose tubéreuse de Bourneville, a été récemment rapporté comme efficace pour le traitement de 3 cas de MLMC. L'objectif de cette étude est d'évaluer l'efficacité et la tolérance d'un traitement par sirolimus topique 0,1% pendant 12 semaines dans les MLMC chez les adultes et enfants, versus crème placebo (véhicule).
Méthodes. Il s'agit d'une étude de phase 2 multicentrique, en split body, randomisée, contrôlée, en double aveugle visant à inclure 50 patients âgés de plus de 6 ans, qui ont une MLMC primaire. La MLMC sera divisée en 2 zones égales de sévérité équivalente sur lesquelles sera appliqué le sirolimus topique 0,1% ou le véhicule pour 12 semaines. A la fin des 12 semaines, l'ensemble de la MLMC sera traité par le sirolimus topique pour 8 semaines supplémentaires. Le critère de jugement principal consistera en l'évaluation de l'amélioration de la zone de la MCLM traitée par sirolimus topique 0,1% comparativement à la zone traitée par le véhicule, par un investigateur (en aveugle), en utilisant le score PGA (*Physician Global Assessment*), à 12 semaines. La qualité de vie, les suintements et saignements, le retentissement fonctionnel et esthétique seront évalués en critères secondaires. Le passage systémique du sirolimus sera évalué par un dosage sanguin du sirolimus à 6, 12 et 20 semaines.
Discussion. Pour les patients atteints de MCLM, pathologie rare et sans traitement satisfaisant à ce jour, le sirolimus topique pourrait être une option thérapeutique non invasive et bien tolérée. Si l'étude démontre l'efficacité et la bonne tolérance du sirolimus topique 0,1% chez les patients ayant une MCLM, il pourrait alors devenir le traitement de première intention et conduire ainsi à un réel changement dans la prise en charge de cette pathologie.

Mots clés : Malformation lymphatique microkystique cutanée, lymphangiectasie, sirolimus, rapamycine, inhibiteur de mTOR, topique, malformation vasculaire

Jury :

Président du Jury :	Professeur Patrice DIOT
Directeur de thèse :	Professeur Annabel MARUANI
Membres du Jury :	Professeur Bruno GIRAUDEAU
	Docteur Valérie GISSOT
	Docteur Anne LE TOUZE
	Professeur Mahtab SAMIMI

Date de soutenance : 15 Novembre 2018