

Pilot study in patients with congenital low-flow vascular malformation treated with low dose sirolimus

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Abstract

Background

Patients with congenital low-flow vascular malformations (capillary (CM), lymphatic (LM), venous (VM) or combined) may have an impaired quality of life (QoL), due to their symptoms, which include pain, swelling, bleeding, thrombosis, and functional impairment. Unfortunately, current treatment methods are challenging and not always successful. Previous studies have shown that the mTOR-inhibitor sirolimus is an effective treatment for these patients. Target levels of 10–15 ng/ml were well tolerated; however, grade three adverse events were observed (ranged 20–40%).

Methods

A pilot study was performed using a Challenge–Dechallenge–Rechallenge (CDR) design to determine the pharmacodynamics of low target levels of sirolimus (target levels 4–10 ng/ml) in respect of efficacy and adverse events in patients with disabling low-flow vascular malformations without treatment alternatives. The patients received sirolimus over a three-to-six-month period (Challenge), followed by the withdrawal of sirolimus (Dechallenge). If the complaints returned, sirolimus was reintroduced during a twelve month period (Rechallenge). Efficacy was determined on pain (end point of the pilot study) and other symptoms related to the vascular malformation; and adverse events were determined in all phases of the study.

Results

An improvement in symptoms was seen in 92% (n = 11/12) of patients during the Challenge phase. In the Rechallenge phase, a positive response rate of 78% was found (n = 7/9). These response rates are comparable to those found in the literature despite low target levels of sirolimus. However, less serious adverse events were observed with low dose sirolimus, especially bone marrow toxicity and grade III liver toxicity.

Conclusions

This pilot using low dose sirolimus showed high efficacy in patients with therapy resistant and disabling low-flow malformation, with a lower incidence of serious adverse events (especially bone marrow toxicity and grade III liver toxicity). This is extremely relevant to patients with low-flow vascular malformation, as current clinical protocols tend to advise lifelong treatment.

Trial registration

The pilot study was part of a phase III study. Trial registration: EudraCT number: 2016-002157-38 and ClinicalTrials.gov Identifier: NCT03987152, registered 06/14/2019 - Retrospectively registered, <https://clinicaltrials.gov/ct2/show/NCT03987152?term=sirolimus&cond=Vascular+Malformations&cntry=NL&draw=2&rank=1>

Background

Vascular malformations include a heterogeneous group of developmental anomalies of the vascular system, capillaries, veins, arteries, lymphatics or any combination of these vessels may be involved. The International Society for the Study of Vascular Anomalies (ISSVA) classified vascular malformations into the following categories(1): simple (low-flow vascular malformation: capillary (CM), lymphatic (LM), venous (VM) arteriovenous (AVM), combined, vascular malformation of major named vessels, and vascular malformation associated with other anomalies.

Vascular malformations are congenital, however, they can be discovered at any life stage, depending on their size and associated symptoms.

Current treatment options for low-flow vascular malformation may be conservative, with compression bandages, analgesics, anti-inflammatory or anti-coagulation drugs, or more invasive with intralesional sclerotherapy or embolization, and surgery(2). Unfortunately, treatment is challenging and not always successful, and can leave patients with a high clinical burden and subsequently a reduced Quality of Life (QoL)(3). Clinical symptoms that reduce the QoL in patients with low-flow vascular malformations include pain, functional impairment, bleeding, thrombophlebitis, ulceration, infections, and leakage (in LM)(4).

Sirolimus

The PI3/AKT/mTOR pathway plays a pivotal role in low-flow vascular malformation(5). The activation of mammalian Target of Rapamycin (mTOR), stimulates angiogenesis, cell proliferation, and glucose metabolism. Some activating somatic mutations in genes in a target of the mTOR pathway, such as PIK3CA, AKT-1, TEK/TIE-2 and PTEN, in patients with low-flow vascular malformation resulted in the increased activation of mTOR(6–10). Therefore, the inhibition of this pathway in these patients seems a logical approach to treatment.

In cells, sirolimus binds to the immunophilin FK Binding Protein-12 (FKBP-12), which in turn binds to and inhibits the activation of mTOR. This inhibition results in the obstruction of several signal transduction pathways, thereby inhibiting downstream protein biosynthesis, cell proliferation, and angiogenesis(11, 12). In theory, this should decrease the size of the low-flow vascular malformation or at least inhibit activity and stop further growth. Unfortunately, the inhibition of lymphocyte activation also results in immunosuppression and might therefore be associated with the susceptibility to infections(13).

Several studies have been performed to explore the use of sirolimus as a treatment option in low-flow vascular malformations(14–17). These prospective open-label trials used - high target sirolimus levels of 10–15 ng/ml leading to (partial) response in 85–100% patients(14–17). As these trials were open label trials, the question remains what is the true efficacy of sirolimus and what is natural behavior of the vascular malformation. Ideally, a placebo controlled randomized trial should be performed, however, in respect to the severe clinical burden of the patients and the rarity of the disease, it is difficult to execute. Recently, it has been postulated that a different design can be used in rare diseases to identify true efficacy of a drug(18). This design is based on the concept of Challenge, Dechallenge and Rechallenge (CDR) to proof the efficacy (or adverse events) caused by a single drug. Ideally, a future study should use this design to investigate true efficacy more in detail. Furthermore, more insight in the adverse events of sirolimus used as a single drug can be gained, as most information available so far, is based on adverse events observed in patients using a combination of drugs (e.g. renal transplant patients)(13, 19–23).

The side effects of sirolimus described include oral ulceration, mucositis and stomatitis, interstitial lung disease, diabetes mellitus, hyperlipidemia, hypertriglyceridemia, hypercholesterolemia, gastro-intestinal side effects, angioedema, thrombo-embolic disease, anemia, leucopenia, thrombocytopenia, proteinuria, glomerulonephritis, and lymphedema(13).

Our knowledge regarding long term toxicities of sirolimus is still expanding and makes it necessary to minimize the risk for these long term toxicities. For example, it has been observed that in patients with long-term sirolimus impaired insulin receptor substrate signaling and Akt activation can be found indicating a deterioration of glucose metabolism leading to an increase of development of diabetes(24).

As in most drugs observed, one can imagine that higher levels of a drug increase the risk of developing adverse events of which the most serious and sometimes even fatal complication is sirolimus-associated interstitial pneumonitis(19, 23, 25, 26).

Bee et al. showed that low sirolimus serum levels (< 3 and 6.9 ng/ml) are related to less side effects without compromising efficacy of treatment in patients with diffuse lymphangiomyomatosis(27). Additionally, Kahan et al. showed a significant relation between the occurrence of adverse events (hypertriglyceridemia, hypercholesterolemia, leukopenia and thrombocytopenia) and the steady state concentration value of sirolimus(28). A C_{ss} below 10 ng/L showed no toxic values.

In the pilot study described here, we hypothesized that sirolimus used in low dosages with lower target levels (4–10 ng/ml) than previously described, is equally effective in the treatment of low flow vascular malformations however will lead to less serious adverse events as observed in those treated with high target levels. Treatment with low target levels (4–10 ng/ml) may require longer treatment than with high target levels (> 10 ng/ml), however low dose sirolimus will be more tolerable if less (serious) adverse events occur. This is especially important, as our knowledge regarding long term toxicities of sirolimus is still growing and makes it necessary to reduce the risk for these long term toxicities.

Methods

The pilot study was performed between 2015 and 2017. Patients included in the pilot study had a severe form of congenital vascular malformation, suffering severe pain and/or impairments. No predefined response criteria were used.

All patients included underwent a Challenge-Dechallenge-Rechallenge to determine the efficacy of low dose sirolimus. Primary objectives of the pilot study were to determine the pharmacodynamics of low dose Sirolimus in respect of efficacy and adverse events.

Patients received sirolimus for three to six months in the Challenge phase, after which sirolimus treatment stopped (Dechallenge phase). If complaints (e.g., pain) did not reoccur, the follow-up phase continued for at least one year, during which time the duration of the pain/symptom-free period and any serious adverse events was measured. If pain or other symptoms returned during the Dechallenge phase, a Rechallenge phase of one year of sirolimus treatment was initiated.

Sirolimus was administered orally using a start dose of 0.8 mg/m² twice a day for children, and 1 mg twice a day for adults. This starting dose is based on the pharmacokinetic properties of sirolimus. During the treatment period, therapeutic drug monitoring was performed. Target trough levels of sirolimus between 4–10 ng/ml were used, based on laboratory and clinical studies(28–30).

Since sirolimus has immunosuppressive properties, cotrimoxazole as a Pneumocystis Jiroveci Pneumonia prophylaxis was prescribed. Recent guidelines suggest that this prophylaxis is not necessary, citing no clear evidence of increased infection, however, at the time this study was performed these guidelines were not available(31). Adverse events were assessed according to the Common Terminology Criteria for Adverse Events (CTCAE).

An MRI is only made in a subset of patients before and after each phase as this outcome measure was not prospectively defined for this pilot study. Therefore the evaluation of the effect of sirolimus on the size of the vascular malformation will be only possible in these patients. In case there was secondary material that was obtained after surgery, stored in the HECOVAN biobank it will be used for analyses. Comprehensive targeted Next Generation Sequencing screen using Unique Molecular Identifiers with a technical sensitivity of 1% mutant alleles was performed for frequently mutated positions using Single Molecule Molecular Inversion Probes(6).

The end point of the pilot study was pain reduction, therefore response was assigned to sirolimus based on pain reduction during the first period (Challenge phase), after which the patients stopped taking sirolimus (Dechallenge). When pain or other complaints returned, sirolimus was re-administered (Rechallenge).

Results

Twelve patients (aged between 1 and 50; 8 M, 4 F) were included in the pilot study. The clinical characteristics of the patients are shown in Supplemental Table S1. All patients had an otherwise

untreatable form of a low-flow vascular malformation. All patients had a multiple sclerotherapy treatments and some had a partial resections in the past. These treatments were not possible or successful anymore. The initial treatment duration was at least three months (Challenge phase). At the end of the Challenge phase, eleven patients experienced pain reduction; of whom five became entirely pain free (pretreatment pain scores: 6–10); only one patient experienced no change in complaints. Pain reduction was achieved between one week and five months after start of sirolimus (mean: seven weeks). Table 1 summarizes the results per vascular malformation type.

Table 1
Summary of clinical and MRI responses in the Challenge and Rechallenge periods.

Vascular malformation type	Challenge (n = 12)		Rechallenge (n = 9)	
	Response after six months of sirolimus	Vascular malformation size as assessed using MRI	Response after 12 months of sirolimus	Vascular malformation size as assessed using MRI
VM (n = 2)	Improvement of symptoms (pain, QoL) (2)	- Decrease (1) - No MRI (1)	Pain free (1) Not restarted (1)	- Decrease (1) - Not restarted (1)
LM (n = 5)	Improvement of symptoms (pain, QoL, leakage, infections, decrease of clinical size of LM, etc.) (5)	- No change (2) - Decrease (2) - No MRI (1)	- Improvement of symptoms (pain, QoL, leakage, decrease of clinical size of LM, etc.) (2) - No change (1) - Not restarted (1) - Restarted with mTOR inhibitor (1)	- No change (1) - Decrease (1) - Not restarted (1) - Restarted with mTOR inhibitor (1) - Unknown (1)
Lymphangiomatosis (n = 1)	No change (1)	Decrease (1)	No change in symptoms (1)	Increase (1)
LVM (n = 2)	Improvement of symptoms (pain, clinical size of LVM) (2)	- No change (1) - No MRI (1)	- Pain reduction (2) - Clinical size reduction LVM (1)	- No change (1) - Unknown (1)
KTS (n = 2)	Improvement of symptoms (pain, QoL) (2)	- No change (1) - No MRI (1)	Improvement of symptoms (pain, clinical size reduction) (2)	- No change (1) - Unknown (1)

	Challenge (n = 12)		Rechallenge (n = 9)	
Total	Improvement of symptoms (11; 92%)	- No change (4; 33%)	- Improvement of symptoms (pain, QoL, leakage, decrease of clinical size of LM, etc.) (7; 78%)	- No change (3; 33%)
	No change (1; 8%)	- Decrease (4; 33%)	- No change (2; 22%)	- Decrease (2; 22%)
		- No MRI (4; 33%)	- Not restarted (2; 22%)	- Increase (1; 11%)
			- Restarted with mTOR inhibitor (1; 11%)	- Not restarted (2; 22%)
				- Restarted with mTOR inhibitor (1; 11%)
				- Unknown (3; 33%)

VM: venous malformation, LM: lymphatic malformation, LVM: lymphatico-venous malformation, KTS: Klippel Trenaunay Weber syndrome.

During the Dechallenge phase, ten patients had a return of pain/symptoms with a duration range of 10 days to 4 months. Nine patients restarted the sirolimus treatment shortly. In all patients, reasons for the re-administration of sirolimus were return of severe pain or other symptoms, such as leakage in the case of a lymphatic malformation or clear evidence of growth of the vascular malformation in the time period sirolimus was stopped. (see Supplemental Table S2).

Three patients did not restart with sirolimus in the Rechallenge phase despite the positive response primarily observed. Reasons were a loss of energy (patient 6), logistic reasons (patient 12), or the initiation of a different mTOR inhibitor (everolimus) (patient 7) at a nearby hospital. The patient that switched to a different mTOR inhibitor showed a significant reduction in complaints again. A substantial relationship between sirolimus and the amelioration of symptoms was confirmed during the Rechallenge phase in seven patients by a substantial reduction of complaints after they resumed taking sirolimus. This indicates the efficacy of sirolimus at a low dose.

All patients experienced adverse events when taking sirolimus. The most frequent adverse events that were likely related to sirolimus treatment were: aphthous stomatitis grade I (50% of patients) and menstrual disorder grades I-II (female patients only; 75%). No patients experienced grade III bone marrow toxicity in the pilot study (Table 2); however, two patients (16.7%) experienced grades I-II bone marrow toxicity. One adult patient developed hypophosphatemia that rapidly recovered after stopping sirolimus. One patient (patient 4) experienced a grade III sepsis due to an infected lymphatic cyst. Reasons for a temporary stop (several days) of sirolimus were: due to vaccination (n = 1), interventional radiology

(bleomycin sclerotherapy n = 2), decannulation (n = 1), and adverse events: infections (n = 13) of which n = 1 sepsis, menstrual disorder (n = 2), aphthous stomatitis (n = 1), general malaise (n = 1), elevated liver enzymes (n = 1). Therapy-limiting adverse events were seen in two patients: one patient had due to a grade II increase of liver enzymes after five months sirolimus treatment (Challenge phase), and one patient had a grade II menorrhagia after 36 months sirolimus treatment (Rechallenge phase). All adverse events were resolved by interrupting treatment, and there have so far been no reported long-term adverse events (median follow-up three years; range 1–5 years).

Table 2
Adverse events observed in our pilot study compared to other studies.

Adverse events attributable to sirolimus	Grade of toxicity	Pilot study n = 12	Adams et al. n = 57	Hammer et al. n = 19	Nguyen et al. Review
Blood/bone marrow toxicity	Grade I and II	2 (17%)	Grade II or higher: 30 (49%)	1 (5%)	11–76%
	Grade \geq III	0 (0%)	16 (27%)	1 (5%)	
Gastro-intestinal toxicity (e.g. mucositis)	Grade I and II	9 (75%)	Grade II or higher: 33 (55%)	19 (100%)	3–19%
	Grade \geq III	0 (0%)	2 (3%)	2 (11%)	
Metabolic/laboratory toxicity	Grade I and II	4 (33%)	Grade II or higher: 12 (20%)	0 (0%)	20–64%
	Grade \geq III	0 (0%)	2 (3%)	0 (0%)	
Infection	Grade I and II	6 (50%)	Grade II or higher: 9 (15%)	6 (32%)	Unknown
	Grade \geq III	0 (0%)	1 (2%)	0 (0%)	
Endocrine toxicity	Grade I and II	3 (25%)	Unknown	Unknown	20–27% (Diabetes mellitus)
	Grade \geq III	0 (0%)	Unknown	Unknown	
Dermatology toxicity	Grade I and II	3 (25%)	5 (8%)	7 (36.9%)	Unknown
	Grade \geq III	0 (0%)	Unknown	1 (5.3%)	
Neurologic toxicity	Grade I and II	4(33%)	Unknown	12 (63%)	Unknown
	Grade \geq III	0 (0%)	Unknown	0 (0%)	
Pulmonary/upper respiratory toxicity	Grade I and II	7 (58%)	Grade II or higher: 1 (2%)	1 (5%)	Unknown
	Grade \geq III	0 (0%)	1 (2%)	0 (0%)	

Adverse events attributable to sirolimus	Grade of toxicity	Pilot study n = 12	Adams et al. n = 57	Hammer et al. n = 19	Nguyen et al. Review
Interstitial lung disease	Grade I and II	0 (0%)	Unknown	Unknown	4–17%
	Grade ≥ III	0 (0%)	Unknown	Unknown	
Musculoskeletal/soft tissue	Grade I and II	1 (8%)	0 (0%)	0 (0%)	Unknown
	Grade ≥ III	0 (0%)	0 (0%)	0 (0%)	
General symptoms (for example hypertension/wound healing)	Grade I and II	1 (8%)	Cardiac general: 0 (0%)	20 (105%)	Angioedema (2.2–15%), urologic (12%)
	Grade ≥ III	0 (0%)	Cardiac general: 0 (0%) Constitutional symptoms: 0 (0%)	0 (0%)	
Lymphedema	Grade I and II	0 (0%)	Grade II or higher: 4 (7%)	Unknown	6.4–12%
	Grade ≥ III	0 (0%)	1 (2%)	Unknown	

Adverse events observed in our pilot study and those reported in the studies performed by Adams et al. and Hammer et al. and Nguyen et al.'s review. Adams et al.: patients with various complex vascular anomalies (including vascular tumors), target levels: 10–15 ng/ml (15). Hammer et al: patients with a vascular malformation using target levels: 10–15 ng/ml (17). The table showed a 105% percentage due to categorizing and summarizing patients with general symptoms. Nguyen et al.'s review of sirolimus in solid organ transplantation identified a wide array of adverse effects (13).

After the Challenge phase, an MRI was performed in eight out of twelve patients. In four of the eight patients in which an MRI was performed, a visual size reduction of the low-flow vascular malformation was found. Stable disease was observed in the remaining four patients.

MRIs were also performed in six of the nine patients who underwent the Rechallenge phase. Two patients showed a decrease in vascular malformations size compared to baseline as assessed before each treatment phase.

Genetic testing of the vascular malformation was no performed routinely, however, in three out of four patients, DNA diagnostics on tissue revealed a PIK3CA mutation. In the other patient, no genetic aberrations could be found with our vascular anomalies panel (6) (Supplemental Table S1).

Case Presentation

To illustrate the efficacy of lower target levels of Sirolimus, patient 10 is described in more detail. This patient, was a two years old female at the start of treatment and suffered of a severe macroglossia due to a lymphatic malformation (Fig. 1). A life-threatening situation in the first months after birth led to the need for a tracheal cannula to guarantee an open airway for a longer time period. Before start of sirolimus, patient had a substantial reduced QoL. In the first weeks after the start of the sirolimus treatment, a rapid decrease in the size of the low-flow vascular malformation was observed. The sirolimus target levels ranged between 4.4 and 6.0 ng/ml during this period. After six months of treatment, an MRI was performed to quantify the response to sirolimus, and a clear reduction in the lymphatic malformation was observed. During a six weeks Dechallenge period, the decision was made to restart sirolimus treatment, because of the increase of tongue volume, noduli and nodus tongue. After restarting, the vascular malformation further reduced in size and, at the age of three years, after using sirolimus for 21 months, bleomycin sclerotherapy, and a tongue resection, the cannula was removed.

Photographs of the patient (Fig. 1) show a clinical reduction in the volume of the low-flow vascular malformation in the submandibular and neck regions after only four weeks of sirolimus treatment. A further reduction is seen after eleven months of treatment with sirolimus. MRI images also show a reduction in volume at six and twelve months post treatment initiation (Fig. 2). The possible related adverse events this patients experienced were intermittent aphthous stomatitis, upper airway infections, tonsillitis, and elevation of triglycerides.

The results of patient 10 (Supplemental Table S1 and S2) in the pilot study are in line with our hypothesis that low target levels of sirolimus are sufficiently effective and lead to fewer and less severe adverse events.

Discussion

We present the results of a pilot study to determine the efficacy and safety of low dose sirolimus on low-flow vascular malformations. A positive response of 92% (n = 11/12) in the Challenge phase was achieved, and in the Rechallenge phase a response of 78% (n = 7/9) was achieved. Two patients did not experience a reduction in symptoms after restarting the treatment. In one patient, a positive response was seen in the Challenge phase but not during the Rechallenge phase. This could be attributed to resistance in the Rechallenge phase, or to a placebo effect during the Challenge phase; however, DNA diagnostics were not performed in this patient, so the genetic basis and resulting sensitivity of the vascular malformation could not be explored. In the other patient lacking a positive response in the Rechallenge phase; DNA diagnostics revealed no mutations. A hypothesis of these non-responders may be a mutation in a different pathway; for example the RAS/BRAF/MAPK/ERK pathway which stimulates angiogenesis also(32). However, clinical appearance did not correspond with a mutation in this pathway. Another possible theory is that the duration of six or twelve months is not long enough for those patients who are potential late responders.

Our data show a comparable response rate to that of the literature despite our lower target level (85% Adams, 20–80% Nadal et al. versus 78–83% in the pilot study(15, 33)).

Less serious adverse events in bone marrow toxicity were seen in this pilot study (0%) compared to other high dose clinical studies: 0% versus Adams et al. (27%), Schena et al (13.4–36.3%) in renal allograft recipients using target trough levels of 8–20 ng/mL(21) and Nguyen et al: (review, 11%-76%)(13) (see Table 2). A significant relationship was seen between steady state concentration (C_{ss}) values of sirolimus and the occurrence of thrombocytopenia and leukopenia by Kahan et al(28). Taken together, these observations support our hypothesis that reduction of the target trough concentration range from high target level 10–15 ng/ml to low target level 4–10 ng/ml does not decrease efficacy but improves tolerance.

Challenge-Dechallenge-Rechallenge-designs are typically used for single-subject clinical trials to investigate efficacy or verify causality when an adverse drug reaction is suspected(18). As vascular malformations are rare and heterogenous, the use of a CDR design offers the opportunity to generate interpretable data on efficacy and safety by analyzing each patient as their own control. We consider this design to be suited to investigate the efficacy of new treatments in those rare diseases, for which randomized clinical trials are less feasible due to the low numbers of patients affected and double blind randomized repeated rechallenges (as implemented in 'n = 1' trials) are not possible due to carry-over effects(34).

During this pilot study a low target level of sirolimus (4–10 ng/ml) is being used in comparison with previous high target level studies (10–15 ng/ml). Parker et al. used a very low target level sirolimus of 2–6 ng/ml in 39 PIK3CA-Related Overgrowth Spectrum (PROS) patients. This study suggests that even very low target level sirolimus can modestly reduce overgrowth; a significant reduction of -7.2% was seen in the volume change of affected tissues(35).

We observed a clear clinical response during the Challenge phase using the CDR concept. After stopping the sirolimus treatment, all patients showed a relapse of complaints and regrowth of the vascular malformation; however, the Rechallenge with sirolimus again reduced the complaints, indicating the effectiveness of sirolimus treatment.

The results of the pilot study have led to the development of a nationwide clinical trial, which is currently enrolling patients. This trial investigates whether low-dose sirolimus is effective for alleviating symptoms including pain, QoL, and lesion volume with an expected lower incidence of serious adverse events compared to high target levels (especially bone marrow toxicity and grade III liver toxicity, which at least have not been observed in the pilot study). The methods of the ongoing clinical trial are based on the data obtained in this pilot study.

Conclusion

This pilot using low dose Sirolimus showed high efficacy in patients with therapy resistant and disabling low-flow malformation, with a lower incidence of serious adverse events (especially bone marrow toxicity and grade III liver toxicity). This is extremely relevant to patients with low-flow vascular malformation, who are likely to require lifelong treatment for their condition.

Declarations

Ethics approval and consent to participate

The pilot study was approved as non – Medical Research Involving Human Subjects Act (nWMO study) the Research Ethics Committee (CMO Regio Arnhem-Nijmegen – Institutional Review Board) in the Netherlands. The study was performed in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

Consent for publication

Informed consent was obtained in all patients before start. Patient and her parents gave their consent for publication and image.

Availability of data and materials

The datasets supporting the conclusions of this article is(are) included within the article (and its additional files.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

All authors read and approved the final manuscript.

VH writing the manuscript and analysed and interpreted the patient data, GR co-conducted the pilot study, substantively revised the manuscript, CvdV substantively revised the manuscript, PdL substantively revised the manuscript, CvdH substantively revised the manuscript, WK substantively revised the manuscript, LSK substantively revised the manuscript, MtL: conducted the pilot study, analysed and interpreted the patient data, contributor in writing the manuscript and responsible as principal investigator for the execution of the pilot study. All authors read and approved the final manuscript.

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Figures



A



B



C



D

Figure 1

Photographs obtained from patient 10 throughout the treatment period during the pilot study, showing the changes in the submandibular- and neck-localized lymphatic malformation. 1a Five months before start of sirolimus 1b After five months of sirolimus 1c After eleven months of sirolimus 1d Current clinical situation: after partial tongue resection and bleomycin sclerotherapy

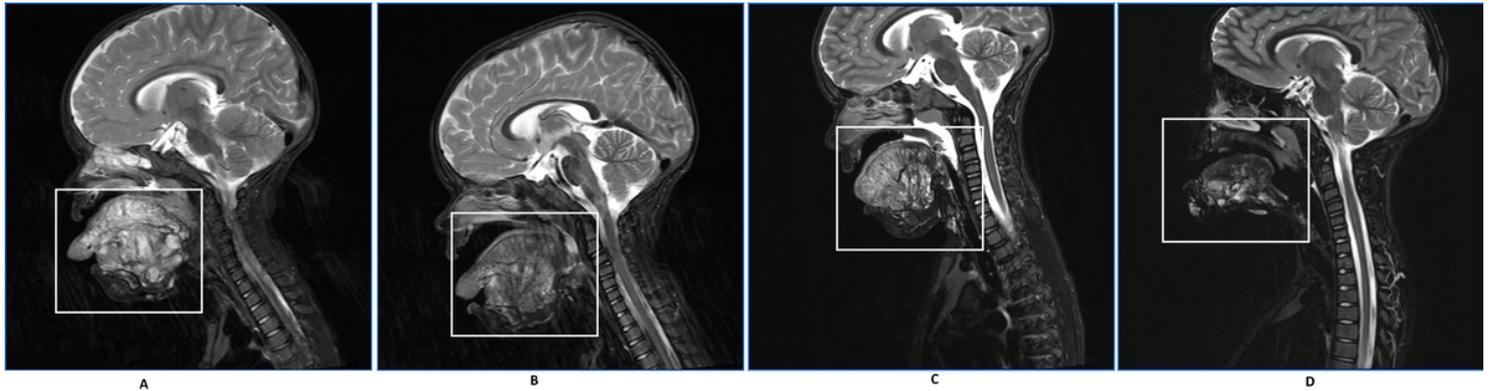


Figure 2

T2-weighted MRI images obtained from patient 10 throughout the treatment period during the pilot study, showing the changes in the submandibular- and neck-localized lymphatic malformation. The white boxes indicate the location of the lymphatic malformation. 2a Before start of sirolimus treatment 2b After six months of sirolimus treatment 2c Before restarting sirolimus treatment after a partial tongue resection and bleomycin sclerotherapy 2d After twelve months of sirolimus during the Rechallenge phase

Supplementary Files

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