



UNIVERSITÀ
DEGLI STUDI
FIRENZE

FLORE

Repository istituzionale dell'Università degli Studi di Firenze

Evolution of disease activity and biomarkers on and off rapamycin in 28 patients with autoimmune lymphoproliferative syndrome

Questa è la Versione finale referata (Post print/Accepted manuscript) della seguente pubblicazione:

Original Citation:

Evolution of disease activity and biomarkers on and off rapamycin in 28 patients with autoimmune lymphoproliferative syndrome / Klemann, Christian; Esquivel, Myrian; Magerus-Chatinet, Aude; Lorenz, Myriam R; Fuchs, Ilka; Neveux, Nathalie; Castelle, Martin; Rohr, Jan; Bettoni da Cunha, Claudia; Ebinger, Martin; Kobbe, Robin; Kremens, Bernhard; Kollert, Florian; Gambineri, Eleonora; Lehmborg, Kai; Seidel, Markus G; Siepermann, Kathrin; Voelker, Thomas; Schuster, Volker; Goldacker, Sigune; Schwarz, Klaus;

Availability:

This version is available at: 2158/1061434 since: 2016-11-14T23:28:41Z

Published version:

DOI: 10.3324/haematol.2016.153411

Terms of use:

Open Access

La pubblicazione è resa disponibile sotto le norme e i termini della licenza di deposito, secondo quanto stabilito dalla Policy per l'accesso aperto dell'Università degli Studi di Firenze (<https://www.sba.unifi.it/upload/policy-oa-2016-1.pdf>)

Publisher copyright claim:

(Article begins on next page)



Evolution of disease activity and biomarkers on and off rapamycin in 28 patients with autoimmune lymphoproliferative syndrome

by Christian Klemann, Myrian Esquivel, Aude Magerus-Chatinet, Myriam R. Lorenz, Ilka Fuchs, Nathalie Neveux, Martin Castelle, Jan Rohr, Claudia Bettoni da Cunha, Martin Ebinger, Robin Kobbe, Bernhard Kremens, Florian Kollert, Eleonora Gambineri, Kai Lehmborg, Markus G Seidel, Kathrin Siepermann, Thomas Voelker, Volker Schuster, Sigune Goldacker, Klaus Schwarz, Carsten Speckmann, Capucine Picard, Alain Fischer, Frederic Rieux-Laucat, Stephan Ehl, Anne Rensing-Ehl, and Benedicte Neven

Haematologica 2016 [Epub ahead of print]

Citation: Klemann C, Esquivel M, Magerus-Chatinet A, Lorenz MR, Fuchs I, Neveux N, Castelle M, Rohr J, da Cunha CB, Ebinger M, Kobbe R, Kremens B, Kollert F, Gambineri E, Lehmborg K, Seidel MG, Siepermann K, Voelker T, Schuster V, Goldacker S, Schwarz K, Speckmann C, Picard C, Fischer A, Rieux-Laucat F, Ehl S, Rensing-Ehl A, and Neven B. Evolution of disease activity and biomarkers on and off rapamycin in 28 patients with autoimmune lymphoproliferative syndrome.

Haematologica. 2016; 101:xxx

doi:10.3324/haematol.2016.153411

Publisher's Disclaimer.

E-publishing ahead of print is increasingly important for the rapid dissemination of science. Haematologica is, therefore, E-publishing PDF files of an early version of manuscripts that have completed a regular peer review and have been accepted for publication. E-publishing of this PDF file has been approved by the authors. After having E-published Ahead of Print, manuscripts will then undergo technical and English editing, typesetting, proof correction and be presented for the authors' final approval; the final version of the manuscript will then appear in print on a regular issue of the journal. All legal disclaimers that apply to the journal also pertain to this production process.

Evolution of disease activity and biomarkers on and off rapamycin in 28 patients with autoimmune lymphoproliferative syndrome

Christian Klemann^{1,2*}, Myrian Esquivel^{3,4*}, Aude Magerus-Chatinet^{3,5}, Myriam R. Lorenz^{6,7}, Ilka Fuchs¹, Nathalie Neveux⁸, Martin Castelle⁴, Jan Rohr^{1,2}, Claudia Bettoni da Cunha⁹, Martin Ebinger¹⁰, Robin Kobbe¹¹, Bernhard Kremens¹³, Florian Kollert¹², Eleonora Gambineri¹⁴, Kai Lehmborg¹⁵, Markus G. Seidel¹⁶, Kathrin Siepermann¹⁷, Thomas Voelker¹⁸, Volker Schuster¹⁹, Sigune Goldacker¹, Klaus Schwarz^{6,7}, Carsten Speckmann^{1,2}, Capucine Picard^{3,4,20}, Alain Fischer^{3,4,21}, Frederic Rieux-Laucat^{3,5}, Stephan Ehl^{1,2}, Anne Rensing-Ehl^{1*} and Benedicte Neven^{4*}

¹Center for Chronic Immunodeficiency (CCI), Medical Center – University of Freiburg, Faculty of Medicine, University of Freiburg, Germany

²Center for Pediatrics, Department of Pediatric Hematology and Oncology, University Medical Center, University of Freiburg, Faculty of Medicine, University of Freiburg, Germany

³INSERM UMR 1163, Laboratory of The immunogenetics of pediatric autoimmune diseases, Paris, France

⁴Unite d'immuno hematologie pediatrique, Hopital Necker-Enfants Malades, Assistance Publique des Hopitaux de Paris, Paris, France

⁵Paris Descartes – Sorbonne Paris Cité University, Imagine Institute, Paris, France

⁶Institute for Transfusion Medicine, University of Ulm, Ulm, Germany

⁷Institute for Clinical Transfusion Medicine and Immunogenetics Ulm, German Red Cross Blood Service, Ulm, Germany

⁸service de Biochimie, Hopital Cochin, Assistance publique Hopitaux de Paris, Paris, France

⁹Department of Pediatric Hematology and Oncology, Hannover Medical School, Germany

¹⁰University Children's Hospital Tübingen, Tübingen, Germany

¹¹Department of Pediatrics, Infectious diseases and Immunity, University Medical Centre Hamburg-Eppendorf, Germany

¹²University Hospital Essen, Dept of Paediatrics III, University of Duisburg-Essen, Germany

¹³Department of Rheumatology and Clinical Immunology, University Medical Centre Freiburg, Freiburg, Germany

¹⁴Department of "NEUROFARBA": Section of Child's Health, University of Florence, Department of Haematology-Oncology: BMT Unit, Department of Fetal and Neonatal Medicine: Rare Diseases, "Anna Meyer" Children's Hospital, Florence, Italy

¹⁵Department of Pediatric Haematology/Oncology, University Medical Centre Hamburg, Germany

¹⁶Div. of Ped. Hematology-Oncology, Dept. of Pediatrics and Adolescent Medicine, Medical University Graz, Graz, Austria

¹⁷Department of pediatric and adolescent medicine, HELIOS Klinikum Krefeld, Germany

¹⁸Hospital for Pediatric Haematology and Oncology, Medical Center Kassel GmbH, Germany

¹⁹Hospital for Children and Adolescents, University of Leipzig, Leipzig, Germany

²⁰Centre d'étude des deficts immunitaires, Hopital Necker-Enfants Malades, Assistance Publique des Hopitaux de Paris, Paris, France

²¹College de France, Paris, France

Statement of equal authors' contribution:

*the first 2 authors CK & ME and the last 2 authors ARE & BN have contributed equally to this work.

Authorship contributions:

C.K., M.E., S.E., A.R-E., and B.N. analyzed data and wrote the manuscript. A.M-C., F.R-L., M.R.L. and K.S. performed genetic investigations. I.F., J.R., C.B.C, M.E., R.K., B.K., F.K., E.G., K.L., M.S., K.S., T.V., V.S., S.G., C.S., A.F. and M.C. provided clinical information. S.E., A.R.-E. and B.N. supervised the project. All authors commented on the manuscript.

Disclosure of Conflicts of Interest: The authors do not report any conflicts of interest.

Running heads: Rapamycin in ALPS

Keywords: ALPS, sirolimus, cytopenia, autoimmunity

Contact information for correspondence:

Benedicte Neven, Unite d'immuno hematologie pediatrique, Hopital Necker-Enfants

Malades, Assistance Publique des Hopitaux de Paris, Paris, France;

benedicte.neven@aphp.fr

OR

Anne Rensing Ehl, Center for Chronic Immunodeficiency, Breisacher Str. 115, 79106

Freiburg, Germany; anne.rensing-ehl@uniklinik-freiburg.de

Word count: 1480 words.

Number of tables: 2

Number of figures: 3

Number of supplemental files: 1

Trial registration: Comité de Protection des Personnes Ile de France Number DC2011-1338 and Freiburg, Germany, German Clinical Trial Register: DRKS00000298.

Acknowledgments:

We thank the patients, their families and referring physicians who made this study possible.

This work was supported by the German Federal Ministry of Education and Research (BMBF 01 EO 0803 grant to the Center of Chronic Immunodeficiency and BMBF 01GM1111B grant to the PID-Net Initiative).

Letter to the Editor

Chronic benign lymphoproliferation and autoimmune cytopenias are the main features requiring treatment in *FAS* mutant patients with autoimmune lymphoproliferative syndrome (ALPS)^{1,2}. Successful use of the mTOR inhibitor rapamycin has initially been reported in the treatment of refractory cytopenia in 3 ALPS-*FAS* patients³. The remarkable efficacy as a second-line agent for this indication was confirmed in a recent prospective study including further 9 ALPS-*FAS* patients⁴. Here, we analyze aspects of rapamycin therapy that have so far not been addressed including first versus second line therapy, comprehensive biomarker responses and the consequences of stopping rapamycin by reporting our experience in 28 ALPS-*FAS* patients.

We performed a retrospective survey of ALPS patients enrolled into research protocols in Paris, France (DC2011-1338) and Freiburg, Germany (DRKS00000298). Patients were included if they fulfilled NIH diagnostic criteria of ALPS⁵ with genetical confirmation, and had received rapamycin for >6 months. Lymphoproliferation was defined as enlarged spleen or lymphadenopathy (>= 2 lymph nodes in >= 2 sites enlarged for >= 3 months). Autoimmune cytopenia required autoantibodies or documented response to immunosuppression. In patients not previously treated with steroids or immunosuppressive drugs, rapamycin was regarded as first line therapy. IVIG was not considered immunosuppression. Rapamycin was initiated at 1-2.8 mg/m²/d aiming for plasma levels of 2-10 ng/ml. Treatment responses were evaluated at 6-9 months and at last follow up. Complete remission (CR) was defined as normal blood counts with platelets >100,000/mm³, absent splenomegaly (palpable <2cm) and lymphadenopathy and cessation of immunosuppression including steroids. Partial remission (PR) was defined as persistent symptoms but >= 50% decrease in spleen size and/or in a reference lymph node and cessation of steroids without relapse of cytopenia.

Of 28 patients, 19 had heterozygous germline *TNFRSF6* mutations, one had a homozygous mutation; 8 had somatic mutations (Suppl. Table 1). Median age at disease onset was 4 (0-26) years (Table 1). The indication for rapamycin was lymphoproliferation in 8, lymphoproliferation and autoimmunity in 18, and autoimmunity in 2 patients. Overall, 19 patients had autoimmune cytopenia (16 in 1 lineage, mostly AIHA, 2 in 2 lineages, 1 in 3 lineages). Before rapamycin, 7 patients had no previous therapy, 2 had received IVIG. In these 9 patients, rapamycin was considered first-line therapy (Table 1). It was initiated as monotherapy in 8 patients and in P21 together with steroids and IVIG. The remaining 19 patients had received up to 6 lines of previous therapies for a median of 3.7 (0.3-13) years. Two patients had undergone hematopoietic stem cell transplantation. Both had disease relapses due to graft rejection (P1) and mixed chimerism (P14). In these patients, rapamycin was considered second-line therapy (Table 1). At initiation of rapamycin, 3/19 had no response to previous treatment, 13 were in PR and 3 had responded, but had side effects (Table 1). Except for two cases, ongoing therapy was maintained (steroids=9, steroids+6MP=2, 6MP=4, AZA=3, MMF=1). Rapamycin was introduced at a median dose of 2 (1-2.8) mg/m². After 6-9 months of treatment, 22 patients (79%) were in CR and 6 (21%) in PR. Rapamycin add-on therapy led to CR in 94% (17/18). The other immunosuppressive drugs were discontinued in all patients within 6 months, except for one with PR (P13). In that patient, who had presented with severe AIHA, rapamycin resolved the splenomegaly and stabilized, but did not fully control AIHA. Nevertheless, corticosteroids could be terminated. First-line rapamycin induced CR in 4 and PR in 5 patients (Table 1, suppl. Fig. 1). The incomplete treatment response was associated with documented poor compliance in 2 patients (P22 and P28). The other 3 PR patients were satisfied with the response achieved and no dose escalation or alternative therapies were attempted. Importantly, rapid stabilization of cytopenias was achieved among all 5 patients who received rapamycin as first line therapy for AIHA or ITP. At last follow up, 26 patients were still on rapamycin. P28 developed IgG4-related disease while on rapamycin and it was decided to stop treatment, P22 stopped because of feeling “unwell” on the medication. Median treatment duration was

2.8 (0.5-5.6) years. All 23 patients with initial CR maintained CR (Table 1). Rapamycin dose was reduced in 14/22 patients where this information was available. At last follow up, serum levels were available in 10 patients and CR was maintained in all 6 patients at levels between 2-5 ng/ml.

As described^{3,6}, DNT cells rapidly decreased upon rapamycin treatment, but only 7/25 patients (33%) reached normal percentages (Fig. 1A). IL-10 levels decreased, but remained >20 pg/ml in 14/23 patients (61%) (Fig.1C). sFASL decreased, but only 2/28 patients reached normal values (Fig. 1D). Significant biomarker decreases were observed in both first and second-line treated patients. VitB12 levels remained elevated in 16/23 (69%) patients. While 6/19 patients with second line treatment showed a drop in VitB12 levels, 7/19 showed an increase (Fig. 1B). All patients with rapamycin first line treatment showed a decrease (Fig. 1 B). Among 22 patients in CR, two had complete normalization and 5 had close to normal values for DNT cells, IL-10 and sFASL, while 15 patients still had elevated biomarkers. Biomarker responses were not statistically different between PR and CR patients. Thirteen patients experienced mild adverse effects, including mouth ulcers (n=4), mild proteinuria (n=2), increased blood pressure (n=2) and transient skin rash, *Helicobacter Pylori* associated gastritis, transient liver enzyme elevation concomitant to herpes zoster and EBV infection in one patient each. In 6 patients, rapamycin was temporarily stopped due to non-compliance (n=3), complete remission (n=2) or elevated blood pressure (n=1). All 6 had rapid relapse of lymphoproliferation and two relapsed with autoimmune cytopenias, accompanied by rapid re-augmentation of biomarkers (Fig. 2A, B). Five patients reinitiated treatment. All had the same response as during the first treatment episode (Fig. 2A).

Our results provide novel, practically relevant insights on rapamycin therapy in ALPS-FAS based on the pioneering original observations by Teachey et al.^{3,7}. First, we confirm the efficacy and safety of rapamycin by reporting 28 additional ALPS-FAS patients, all of whom responded to treatment. Our results also confirm the good tolerability within the treatment

period of up to 6 years. Nevertheless, long-term side effects of rapamycin can be relevant and careful monitoring is required. Second, we present the first data on ALPS-FAS patients receiving rapamycin as first-line therapy. We were not only able to stop all other treatments in patients who had received several lines of prior therapy, but we also achieved excellent responses using rapamycin as the first and single agent. This included rapid control of cytopenia and lymphoproliferative manifestations. Nevertheless, immediate stabilization of autoimmune cytopenia cannot be expected and may require initial concomitant treatment. We propose to consider rapamycin as a first-line treatment in ALPS-FAS patients. In genetically proven cases, there is a clear biological rationale for rapamycin. Lymphoproliferation in ALPS is not just due to an accumulation of DNT cells that cannot die, but these cells and their single positive precursor cells⁸ are highly proliferative *in vivo*^{6,9}. This proliferative activity is associated with hyperactive mTOR signaling⁶. Blocking proliferative activity and induction of apoptosis in DNT cells and their precursors are two non-mutually exclusive explanations for the impressive effect of rapamycin on lymphoproliferation^{6,7}. The rapid relapse of disease after stopping treatment indicates that the cells giving rise to DNT cells are either not fully eliminated or are rapidly regenerated once targeted mTOR inhibition is stopped. How rapamycin abrogates autoimmune manifestations remains unclear. Fas deficient B cells escape germinal center selection and undergo enhanced somatic hypermutation¹⁰. Rapamycin could either directly affect B cell signaling or survival and/or it could indirectly influence germinal center function by decreasing DNT and IL-10. While mTOR inhibition represents targeted molecular treatment for ALPS, this is less clear in patients with autoimmunity and lymphoproliferation in the context of other diseases clinically resembling ALPS-FAS. Although there are other immunodeficiencies characterized by mTOR activation (e.g. activated PI3K delta syndrome¹¹), the rapamycin response in these diseases is much more variable⁴. At present, we therefore advocate first-line rapamycin only in ALPS-FAS patients. Third, we present new observations relevant for the guidance of long-term therapy. Rapamycin levels <5 ng/ml were sufficient to maintain disease control in most patients. However, stopping rapamycin was associated with rapid relapse in all cases. Based

on these observations, we currently start with 2 mg/m²/d and adapt this up to 10 ng/ml. Once full remission has been achieved, we titrate down to 2-5 ng/ml. Because at least the lymphoproliferative manifestations tend to attenuate with age, the necessity of life-long treatment remains unclear. Another open issue of long-term treatment is whether rapamycin reduces or increases the risk of lymphoma. While biomarkers have proven useful for establishing a diagnosis of ALPS-FAS¹²⁻¹⁴, their value in guiding therapy appears limited. Although a significant decrease was observed, CR was accompanied by biomarker normalization in no more than a third of the patients. In summary, our results further establish rapamycin as an excellent targeted therapy for ALPS-FAS patients and provide support for using it as a first-line agent in this disease.

References

1. Fisher GH, Rosenberg FJ, Straus SE, et al. Dominant interfering Fas gene mutations impair apoptosis in a human autoimmune lymphoproliferative syndrome. *Cell*. 1995;81(6):935-946.
2. Rieux-Laucat F, Le Deist F, Hivroz C, et al. Mutations in Fas associated with human lymphoproliferative syndrome and autoimmunity. *Science*. 1995;268(5215):1347-1349.
3. Teachey DT, Greiner R, Seif A, et al. Treatment with sirolimus results in complete responses in patients with autoimmune lymphoproliferative syndrome. *Br J Haematol*. 2009;145(1):101-106.
4. Bride KL, Vincent T, Smith-Whitley K, et al. Sirolimus is effective in relapsed/refractory autoimmune cytopenias: results of a prospective multi-institutional trial. *Blood*. 2016;127(1):17-28.
5. Oliveira JB, Bleesing JJ, Dianzani U, et al. Revised diagnostic criteria and classification for the autoimmune lymphoproliferative syndrome (ALPS): report from the 2009 NIH International Workshop. *Blood*. 2010;116(14):e35-40.
6. Volkl S, Rensing-Ehl A, Allgauer A, et al. Hyperactive mTOR pathway promotes lymphoproliferation and abnormal differentiation in autoimmune lymphoproliferative syndrome. *Blood*. 2016;128(2):227-238.
7. Teachey DT, Obzut DA, Axsom K, et al. Rapamycin improves lymphoproliferative disease in murine autoimmune lymphoproliferative syndrome (ALPS). *Blood*. 2006;108(6):1965-1971.
8. Rensing-Ehl A, Volkl S, Speckmann C, et al. Abnormally differentiated CD4+ or CD8+ T cells with phenotypic and genetic features of double negative T cells in human Fas deficiency. *Blood*. 2014;124(6):851-860.
9. Sneller MC, Straus SE, Jaffe ES, et al. A novel lymphoproliferative/autoimmune syndrome resembling murine *lpr/gld* disease. *J Clin Invest*. 1992;90(2):334-341.
10. Janda A, Schwarz K, van der Burg M, et al. Disturbed B-lymphocyte selection in autoimmune lymphoproliferative syndrome. *Blood*. 2016;127(18):2193-2202.
11. Angulo I, Vadas O, Garcon F, et al. Phosphoinositide 3-kinase delta gene mutation predisposes to respiratory infection and airway damage. *Science*. 2013;342(6160):866-871.
12. Caminha I, Fleisher TA, Hornung RL, et al. Using biomarkers to predict the presence of FAS mutations in patients with features of the autoimmune lymphoproliferative syndrome. *J Allergy Clin Immunol*. 2010;125(4):946-949 e946.
13. Magerus-Chatinet A, Stolzenberg MC, Loffredo MS, et al. FAS-L, IL-10, and double-negative CD4- CD8- TCR alpha/beta+ T cells are reliable markers of autoimmune lymphoproliferative syndrome (ALPS) associated with FAS loss of function. *Blood*. 2009;113(13):3027-3030.
14. Rensing-Ehl A, Janda A, Lorenz MR, et al. Sequential decisions on FAS sequencing guided by biomarkers in patients with lymphoproliferation and autoimmune cytopenia. *Haematologica*. 2013;98(12):1948-1955.

Table 1: Treatment history and rapamycin response of individual patients

A) Pretreated patients with second-line rapamycin treatment

Patient	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
age of onset	1m	12m	5y	4m	2m	12y	11y	9y	7m	10m	3.4y	14y	3y	2m	4y	18m	6m	6m	4y
age start IS	1m	7y	7y	4m	2m	12y	11y	9y	3y	10m	3.4y	14y	3y	2m	4y	18m	4y	16y	8y
steroids	gray			gray	gray	gray	gray	gray		gray	gray	gray	gray	gray	gray	gray	gray	gray	gray
MMF													gray						gray
6MP	gray	gray	gray	gray	gray	gray		gray	gray	gray				gray	gray				
AZA	gray	gray		gray	gray	gray	gray							gray					gray
IVIG	gray			gray			gray				gray	gray	gray			gray		gray	
HSCT	gray													gray	gray				
anti-CD20	gray			gray	gray	gray	gray						gray	gray	gray				
others	gray				gray									gray	gray				
response ¹	gray	gray	tox	gray	gray	gray	gray	gray	gray	tox	gray	gray	gray	gray	tox	tox	tox	gray	tox
age start rapa [yrs]	13	16	13	6	5	13	14	10	6	1.9	4	14	5	7	5	1.6	6	17	14
rapa response (6 mo) ¹	black	black	black	black	black	black	black	black	black	black	black	black	gray	black	black	black	black	black	black
rapa response (last FU) ¹	black	black	black	black	black	black	black	black	black	black	black	black	gray	black	black	black	black	black	black
rapa side effects ²					gray		gray	gray	gray	gray						gray	gray	gray	

B) Patients with first-line rapamycin treatment

Patient	20	21	22	23	24	25	26	27	28
age of onset	3.7y	2.3y	12m	10m	2.0y	8m	22m	2.5y	26y
age start rapa	4y	4y	15y	12m	14y	12m	6y	8y	26y
rapa response (6 mo) ¹	gray	black	gray	black	black	gray	black	gray	gray
rapa response (last FU) ¹	gray	black	off	black	black	gray	black	black	off
rapa side effects ²	gray			gray			gray		black

Table 1: A) Patients #1-19 were pre-treated as indicated by gray boxes. ¹Response to treatment: white - no response, gray - partial remission, black - complete remission. „tox“ indicates necessity to stop the drug due to side effects. ²Side effects of rapamycin: white - none, gray – moderate, black – requiring stop. Age in years (y) or months (m), immunosuppressive treatment (IS), mycophenolate mofetil (MMF), 6-mercaptopurine (6MP), azathioprine (AZA), intravenous IgG (IVIG), hematopoietic stem cell transplantation (HSCT), anti-CD20 monoclonal antibodies (anti-CD20), rapamycin (rapa), follow up (FU).

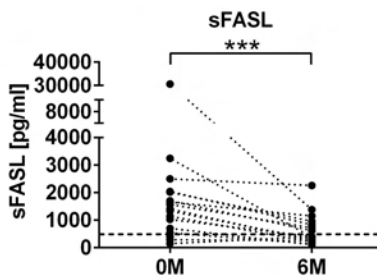
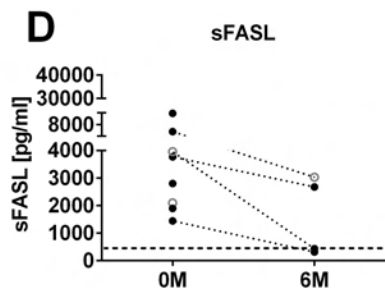
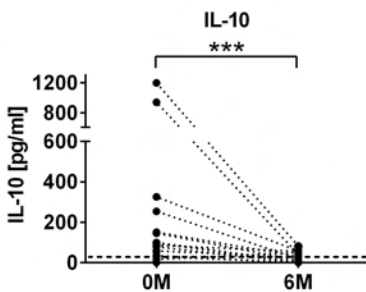
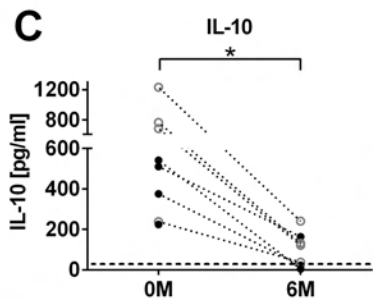
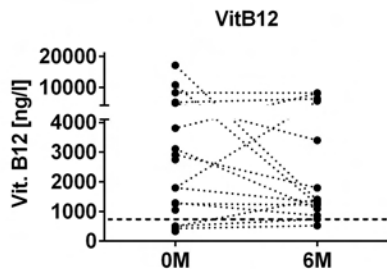
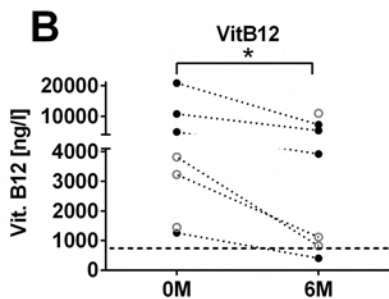
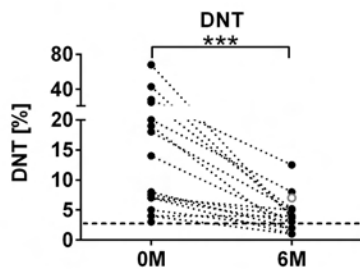
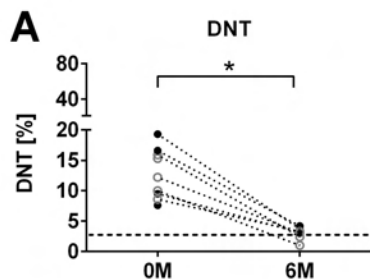
Fig. 1: Biomarker responses at 6 months in patients receiving rapamycin as first-line or second-line treatment

The percentage of DNT cells among CD3+TCR $\alpha\beta$ + lymphocytes (A), the serum levels of VitB12 (B), IL-10 (C) and sFASL (D) were determined before rapamycin therapy and 6(-8) months after initiation of treatment in patients who received rapamycin as first-line therapy (left panels) or after prior immunosuppressive treatment (right panels). Patients with complete remission are depicted in black, partial remissions are indicated by open gray circles. Analyses were performed using PRISM-software (GraphPad software, San Diego, USA). Populations were compared using the Wilcoxon matched-pairs signed rank t-test. $p < 0.05$ was considered significant and indicated by asterisk (* $p < 0.05$; ** < 0.01 , *** < 0.001 , **** < 0.0001).

Fig. 2: Effect of treatment discontinuation

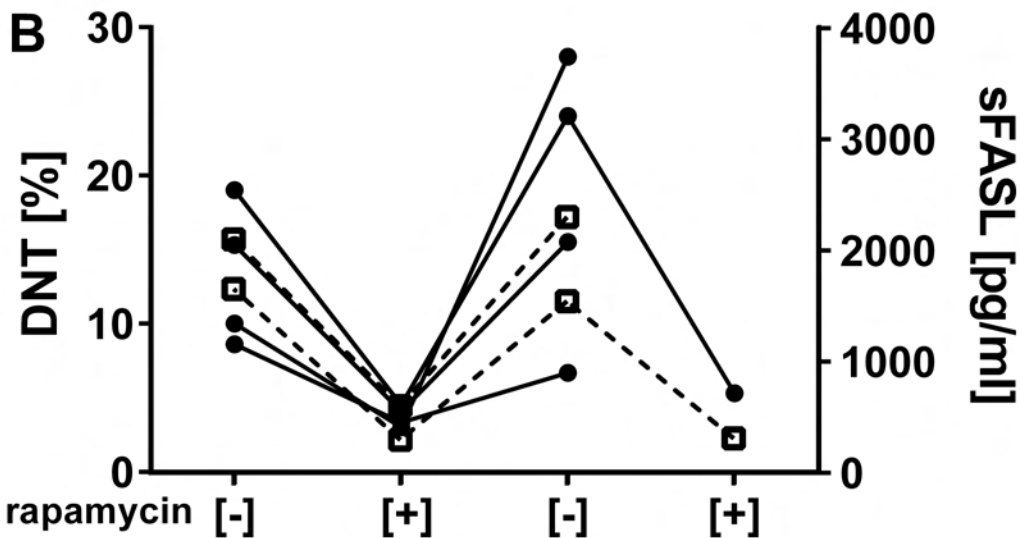
Summary of observations in 6 patients who discontinued rapamycin treatment. A) Status of rapamycin treatment is indicated by [-]/[+]. The spleen size is indicated as follows: - not palpable; + palpable but < 5 cm; ++ > 10 cm or up to umbilicus. Additional lymphadenopathy is indicated by $^{\circ}$, cytopenia by * .

B) Percentages of DNT cells (left y-axis, black circles) and sFASL levels (right y-axis, open boxes/broken lines) at different stages of therapy.



A

Rapamycin	[-]	[+]	[-]	[+]
P11	++ ^{○*}	-	+ ^{○*}	-
P17	++ [○]	-	++ [○]	-
P22	++ [*]	+	++	off
P23	+ [*]	-	+ [*]	-
P25	++	+	++	+
P28	○		+ [○]	-



Suppl. Table 1: Patient cohort

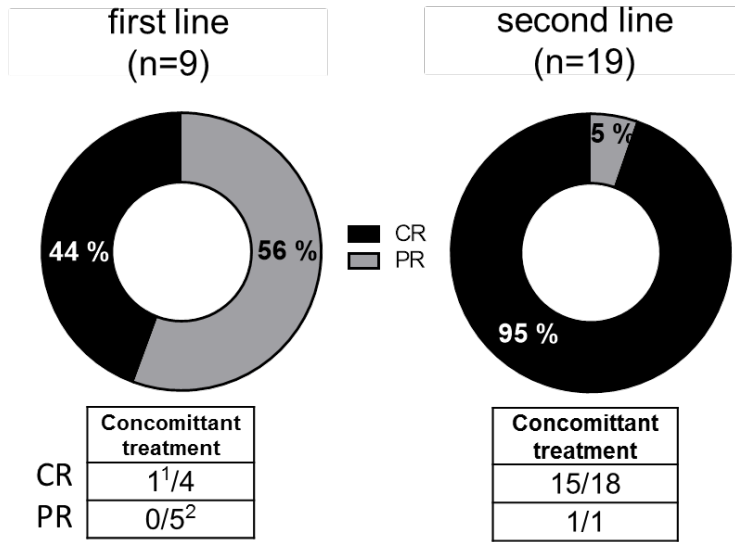
Pt #	Gender	Genetic	Mutation	Protein level	FAS Domain	Age of onset [yrs]	Clinical presentation	Status at last follow up
1	F	FAS Htz	Ex 9 c.688-689dup	p.Ser230fs Arg Ter12	IC	0,0	LP/AI (bone infiltration)	Remission
2	M	FAS Htz	Ex 9 c.709 G>C	p.Ala237Pro	IC	1,0	LP/AI (pancytopenia)	Remission
3	F	FAS Htz	Ex 7 c.586 C>T	p.Gln196Ter	IC	5,0	LP	Remission
4	M	FAS Htz	Ex 8 c.671 T>G	p.Leu224Ter	IC	0,3	LP- AI(AIHA)	Remission
5	M	FAS Htz	Ex 8 c.652-2A>T	P.Glu218fsMet Ter3	IC	0,2	LP/AI(AIHA)	Remission
6	M	FAS Htz	Ex 9 c.794A>G	p.Asp265Gly	IC	12,0	LP/AI(AIHA)	Remission
7	M	FAS Htz	Ex 6 c.506-3C>T	p.Gly169_Trp189del	TM	11,0	AI (ITP)	Remission
8	F	FAS Htz	Ex 9 c.748C>T	p.Arg250Ter	IC	9,0	LP	Remission
9	F	sFAS	Ex 9 c.812_814del	p.Ala271del	IC	0,6	LP	Remission
10	F	sFAS	Ex9 c.768C>G	p.Arg250Gly	IC	0,8	LP/AI(AIHA)	Remission
11	M	sFAS	Ex 9 c.815 A>G	p.Glu272Gly	IC	3,4	LP/AI (ITP)	Remission
12	F	FAS Htz	Ex 6 c.506-2 A>G	p.Gly169_Trp189del	TM	14,0	AI (AIHA)	Remission
13	F	FAS htz	Ex 7 c.613_617del	p.Glu205fsProTer4	IC	3,0	LP/AI(AIHA)	PR
14	F	FAS hmz	Ex 4 c.327T>G	p.Cys143Arg	EC	0,2	LP/AI(AIHA)	Remission
15	M	FAS htz	Ex 4 c.410_411del	p.Ser137fsTyrTer3	EM	4,0	LP/AI(AIHA)	Remission
16	F	FAS Htz	Ex 3 c.275_276delCA	p.Thr92ArgfsTer13	EC	1,6	LP/AI(AIHA/ITP/NTP)	Remission
17	F	sFAS	Ex 9 c.712G>T	p.Gly238Ter	IC	0,5	LP	Remission
18	M	FAS Htz	Ex 9 c.779A>G	p.Asp260Gly	IC	0,5	LP	Remission
19	M	FAS Htz	Ex 6 c.536T>G	p.Leu179Arg	TM	4,0	LP/AI(AIHA)	Remission
20	M	sFAS	IVS7 c.652-2A>T	Splice site	IC	3,7	LP/AI(AIHA)	PR
21	M	sFAS	IVS7 c.652-1G>A	Splice site	IC	2,3	LP/AI(AIHA)	Remission
22	M	sFAS	Ex 8 c.657_658delAG	p.Val220GlyfsTer6	IC	1,0	LP/AI(ITP)	off test
23	F	sFAS	Ex 9 c.778G>T	p.Asp260Tyr	IC	0,8	LP/AI(Anemia)*	Remission
24	M	FAS Htz	Ex 4 c.405T>A	p.Cys135Ter	EC	2,0	LP/AI(AIHA)	Remission
25	F	FAS Htz	Ex 8 c.676_+1G/C	p.Pro217fs	IC	0,7	LP	PR
26	M	FAS Htz	Ex 3 c.471_474delGACA	p.Thr76fs	EC	1,8	LP/AI(AIHA)	Remission
27	F	FAS Htz	Ex 6 c.568G>T	p.Val190Leu	TM	2,5	LP	Remission
28	M	FAS Htz	Ex 9 c.792T>G	p.Asn264Lys	IC	26,0	LP	off test, PR with MMF

Suppl. Table 1: Table of patient characteristics, mutations, and clinical manifestations.

M=male, F=female; IC=intracellular, TM=transmembrane, EC=extracellular; LP=lymphoproliferation, AI=autoimmunity, AIHA=autoimmune hemolytic anemia, ITP=immune thrombocytopenia, NTP=autoimmune neutropenia, PR=partial remission.

*P23 suffered from anemia requiring transfusion, but Coombs-test was negative.

Suppl. Fig. 1: Clinical rapamycin response at 6 months



Suppl. Fig. 1: Clinical response to rapamycin 6 months after treatment initiation.

Pie-charts show percentages of complete and partial remission of patients receiving rapamycin as a first line treatment (left) or second line treatment (right).¹The patient received steroids concomitantly to rapamycin initiation. ²Two of these five patients had documented malcompliance.