The SYNBioSe Study
A proof-of-concept study involving synergetic B-cell imunnomodulation in patients with refractory systemic lupus erythematosus

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A proof-of-concept study in refractory SLE patients to assess the immunological consequences of a combination treatment with rituximab (anti-CD20) and belimumab (anti-BAFF) to achieve long-term B-cell depletion. The immunological and clinical...

Ethical review	Approved WMO
Status	Recruitment stopped
Health condition type	Autoimmune disorders
Study type	Interventional

Summary

ID

NL-OMON42194

Source
ToetsingOnline

Brief title
Synergetic B-cell immunomodulation in SLE

Condition

- Autoimmune disorders

Synonym
systemic lupus eryhtematosus

Research involving
Human

Sponsors and support

Primary sponsor: Leids Universitair Medisch Centrum
Source(s) of monetary or material Support: Nierstichting;ZonMW

Intervention

Keyword: B-cell, systemic lupus erythematosus

Outcome measures

Primary outcome

In this proof-of-concept study the primary objective is to assess whether a combination treatment of rituximab and belimumab will lead to a sustained reduction of pathogenic autoantibodies and thereby inhibition of NET formation. Therefore, the primary endpoints are:

- a sustained reduction of pathogenic autoantibodies, in particular anti-dsDNA autoantibodies, at 24 weeks after treatment start

- an inhibition of the formation of NETs at 24 weeks after treatment start

Secondary outcome

The main secondary objective is to evaluate the effects of long-term B-cell depletion which will involve assessments of the clinical response correlated with immunological parameters. To this end, the relevant study parameters will be evaluated after 4 weeks (short term), 24 weeks (intermediate term) and 104 weeks (long-term). The secondary endpoints measured at these times are:

- the effects on the reduction of pathogenic autoantibodies, in particular anti-dsDNA autoantibodies

- the inhibition of the formation of NETs

- seroconversion of pathogenic autoantibodies, in particular anti-dsDNA

autoantibodies

- the normalization of complement usage, i.e. C3/C4 levels and C1Q-binding

Other secondary objectives are to evaluate:

- the safety and feasibility of the combination treatment according to the WHO

toxicity criteria

- the clinical response of refractory SLE patients upon long-term B-cell

depletion by:

* a reduction in SLEDAI scores, no new BILAG A involvement and the SLE

responder index

* in case of lupus nephritis: the number of partial and complete renal

responders

* the number of moderate or severe flares and renal flares

Study description

Background summary

Introduction
Systemic lupus erythematosus (SLE) affects predominantly young women with childbearing potential (20-40 years) and inflammation can occur in virtually every organ, including kidneys, lungs, heart or brain1. The disease course in SLE patients is typically characterized by frequent flares, requiring immunosuppressive treatment. SLE patients have an increased mortality with 1 of 8 patients dying within 8 years of follow-up, which is 2,5 times higher as the general population 2. Moreover, it is estimated that 50-60% of all SLE patients develop lupus nephritis, one of the more severe manifestations, in the first 10 years after diagnosis. These data emphasize the impact that SLE has in a young patient population when, once diagnosed, means lifelong medical treatment and a significant reduction of life expectancy. It also illustrates the need for developing better means to prevent and treat the sequelae of SLE.
Current, evidence-based treatment modalities for SLE consist of

immunosuppressive treatment with high dose corticosteroids, cyclophosphamide or mycophenol mycophenolate acid, that non-specifically target the immune system to reduce inflammation 3. Side-effects of these treatment strategies are (opportunistic) infections in the short term and risk for malignancy and cardiovascular disease in the long-term. Treating SLE patients with biologicals is an attractive alternative because biologicals specifically target the immune system by blocking cytokines or deplete one specific cell population, thereby reducing the risk for infections or malignancies as compared to conventional immunosuppressants. Furthermore, the scarce treatment options underscore the need to exploit new therapeutic possibilities for SLE patients who frequently experience a flare of the disease. Recent advances in the knowledge of the pathophysiology of SLE have identified the new immunological mechanism of NETosis which potentially is at the heart of SLE pathogenesis. Therefore, we set up the present study to investigate whether this process can be successfully targeted in refractory SLE patients.

Anti-DNA autoantibodies and NETosis in SLE
Systemic lupus erythematosus (SLE) is a prototypic systemic autoimmune disease in which the loss of tolerance to nucleic acids (=DNA) and their binding proteins results in the generation of autoantibodies (e.g. anti-DNA, anti-chromatin or anti-histone autoantibodies) that initiate and propagate tissue-damaging inflammation involving almost every organ system 4. SLE can present itself by a wide array of symptoms ranging from skin eruptions and arthritis to life-threatening renal and cerebral involvement. Not only the presence of autoantibodies against nuclear components implies that the humoral immune system plays an important role in the pathophysiology, but also, the recent approval of belimumab, a human antibody targeting the B-cell survival cytokine BAFF (B-cell activating factor), for treatment in SLE patients 5;6.
Because autoantibodies against nuclear components are a central hallmark in the diagnosis and prognosis of SLE, much research has focused on unraveling the mechanisms underpinning the generation of these pathogenic autoantibodies. Two crucial steps have been shown defective in SLE patients responsible for the generation of autoantibodies: first, defects in B-cell tolerance have been identified during B-cell selection in bone marrow (at the transition of early immature B-cells to the immature B-cells) and in the peripheral lymphoid tissues (at the transition of transitional B-cells to mature B-cells) 7.
Second, in SLE patients components of nuclear material, usually residing intracellular and therefore not exposed to the immune system, have been found in the extracellular space providing a crucial opportunity for the formation of SLE-specific autoantibodies. Currently, three, not mutually excluding, mechanisms are proposed for the presentation of nuclear autoantigens to the, already tolerance defective, immune system in SLE: a) aberrant clearance of apoptotic 8 and necrotic 9 cells can lead to the exposure of human DNA components to the immune system 10; b) viral infections, often associated with the initiation of flares 1, expose the immune system of lupus patients to circulating viral RNA and DNA components; and c) recently, neutrophil extracellular trap (NET) formation by neutrophils, recruited to infection

sites, is a crucial source of extracellular human DNA components 11;12. The latter mechanism has been the most recent breakthrough in lupus research with potential therapeutic consequences, as further explained below.
The formation of NETs by neutrophils has been described in 2004 showing the formation of an extracellular structure that consists of threads of nuclear chromatin-DNA 11. NETs were shown to be decorated by antimicrobial peptides (AMPs) providing a highly immunogenic structure capable of killing invading pathogens 13. This process is called NETosis, sound-related to *apoptosis* because neutrophils die after extracellular disposing NETs. The relevance of NET formation for SLE has only very recently come to the attention 12;14;15 when whole genome analysis observed that SLE patients over-expressed granulopoiesis-specific genes (the so called *granulocyte signature*) and IFN-regulated genes (the so called *IFN signature*) 16. The phenotypic expression of the granulocyte signature was traced down to the presence of an abnormal subset of neutrophils, so called low-density granulocytes (LDGs) in the peripheral blood of SLE patients. Subsequently, it was found that LDGs display an enhanced capacity to form NETs in comparison to normal density SLE-derived and control neutrophils 15. Eventually, Garcia-Romo et al. found that neutrophils of SLE patients underwent NETosis upon stimulation by immune complexes from a well-known SLE-specific anti-nuclear antibody, anti-ribonucleoprotein antibody (anti-RNP)12. Moreover, these NETS were able to mount a secondary immune response through TLR7/9-mediated activation of plasmacytoid dendritic cells. Taken together, enhanced NETosis triggered by autoantibodies in SLE is at the heart of SLE disease pathogenesis.

Targeting NETosis in SLE
From a pathophysiological role, therapeutically interfering with the process of NETosis in SLE is new. Recent studies have shown that the enhanced NET formation correlates with disease severity such as renal involvement 17;18. It has also been shown that NETs induce complement activation and are responsible for the usage of C1q, C3 and C4 in active SLE 18. These studies are part of the increasing evidence that suggest neutrophils are contributors to the pathogenesis of SLE flares. Therefore, reducing NETosis in SLE has therapeutic potential.
Because neutrophils form a crucial 1st line of defense in the human immune system, non-selective targeting of neutrophils (by blocking their activity or depleting their numbers) is not a desirable approach. However, because immune complexes formed with SLE-specific autoantibodies trigger neutrophils to form NETs, targeting the production of these autoantibodies by plasma cells is a plausible treatment strategy. There are currently no trials using biologicals to target autoreactive plasma cells in SLE. However, we previously showed that disease-specific autoantibody production is susceptible to long-term B-cell depletion through a 6-monthly fixed retreatment protocol with rituximab in patients with rheumatoid arthritis 19. Rituximab in SLE patients is currently only warranted in an off-label setting because a recent randomized trial did not show superiority over placebo 20;21. Also, on a pathophysiological level, B-cell depletion alone is not likely to interfere with the basic pathologic

mechanisms described above. On the contrary, B-cell depletion leads intrinsically to a rise in B-cell survival factor (BAFF or Blys)22 where, physiologically, increased BAFF levels are triggers for the bone marrow of patients to regenerate new B-cells. Thus, in SLE patients treated with rituximab the increased BAFF levels can lead to repopulation of autoreactive B cell clones 23;24. Noteworthy is that the need for repetitive treatment with rituximab in many autoimmune diseases is already undisputed 25. Therefore, in order to induce long-term B-cell depletion in SLE patients the combined treatment of rituximab with belimumab (which blocks BAFF) is a more potent and logical therapy. In mice, this combination treatment of rituximab and belimumab led to a more profound B-cell depletion due to the additional depletion of rituximab-resistant B-cells and CD20 negative plasmablasts/-cells 26. In SLE patients, add-on therapy with belimumab has been proven effective as steroid-sparing maintenance treatment 5. These findings led to the present study involving a proof-of-concept study in refractory SLE patients to assess the immunological consequences of a combination treatment with rituximab (anti-CD20) and belimumab (anti-BAFF).

Study objective

A proof-of-concept study in refractory SLE patients to assess the immunological consequences of a combination treatment with rituximab (anti-CD20) and belimumab (anti-BAFF) to achieve long-term B-cell depletion. The immunological and clinical monitoring of refractory SLE patients include the quantification of NETosis, the kinetics of B-cell and plasma cell depletion and SLE-specific autoantibody levels. As secondary goals, this study will evaluate the safety and feasibility of this combination treatment and assess the clinical response.

Study design

This is a single-center, non-randomized, phase 2A, proof-of-concept study to evaluate the effects of a combination treatment with rituximab and belimumab. This combination therapy is designed to induce long-term B-cell depletion to achieve significant reduction of autoantibody-mediated immune complexes. In addition to standard therapy, SLE patients will receive 2 infusions of rituximab 1000 mg on day 0 and 14 (week 2) and belimumab on day 28 (week 4) , 42 (week 6) and 56 (week 8), then every 28 days. The primary endpoint is at 24 weeks after which an extended follow-up will take place, for subjects continuing belimumab, until 104 weeks after treatment start.

Rituximab and Belimumab will be administered intravenously according to the manufacturer*s instructions. All subjects will continue standard therapy during the study period. Clinical and immunological parameters will be assessed every 8-12 weeks. The study medication is not blinded for patients nor physicians. The study intends to include 15 refractory SLE patients.

Intervention

Rituximab
Patients will be intravenously treated with Rituximab 1000mg on day 0 and day 14. Before every infusion of Rituximab patients will receive intravenous methylprednisolon 100mg together with oral acetaminophen 1000 mg and intravenous Tavegil 2 mg.

Belimumab
Patients will be intravenously treated with Belimumab 10mg/kg on day 28, day 42 and day 56. Thereafter, patients will receive Belimumab 10mg/kg every 4 weeks. No pre-medication is administered

Study burden and risks

There may be a benefit for the subjects participating in this study. The present study will only include SLE patients who will have no other treatment options due to the refractoriness of their disease, intolerance to conventional therapies or cumulative toxicity of current treatment. The use of rituximab with belimumab can ameliorate disease activity with a possible reduction of infectious complication as compared to conventional intensive immunosuppressive treatment.
The risks related to study participation lies predominantly in the side effect profile of the biologicals used, as extensively described in §6.4. At study entry a renal biopsy is performed to diagnose refractory renal disease associated with proliferative lupus nephritis. A renal biopsy will also differentiate from other causes such as chronic glomerulosclerosis or extensive chronic damage which is a relative contraindication to aggressive immunosuppressive therapy because of a small change of regaining kidney function. In most cases, the renal biopsy will likely already be performed as part of routine clinical evaluation of an patient with the suspicion of a refractory lupus nephritis.

Contacts

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Trial sites

Listed location countries

Netherlands

Eligibility criteria

Age
Adults (18-64 years)
Elderly (65 years and older)

Inclusion criteria

Subjects enrolled in the study must meet the following inclusion criteria:
1) age 18 years,
2) ACR diagnosis of SLE (1997 revised criteria, see appendix 1)
3) Severe SLE flare at screening (see also section 5.2.3.2.), defined as a situation in which 1 or more of the following criteria are met:
- Increase in SLEDAI (SLE Disease Activity Index) with 12 or more points
- New or worse SLE-related activity of major organs, i.e.: CNS-SLE (includes NPSLE), vasculitis, nephritis, pericarditis and/or myocarditis, myositis, thrombocytopenia < 60, hemolytic anemia < 4.4mmol/L (<=7.0g/dL).
4) Refractory disease, defined as persisting or progressive disease activity (SLEDAI > 6 points) despite conventional immunosuppressive treatment and 1 or more of the following criteria:
- failure of the initial induction treatment at six months, for which a switch to another induction therapy regime has already been carried out;
- intolerance or contraindication for cyclophosphamide and mycophenolate mofetil (MMF);
- exceeding a cumulative dose of 15 gram of cyclophosphamide;
- a second relapse within two years after start of the initial induction therapy
- a relative contraindication for high-dose oral or intravenous (iv) prednisone, such as avascular osteonecrosis, previous psychosis on corticosteroids, osteoporosis and/or severe obesity (BMI *35 kg/m2).
5) ANA seropositivity, as defined by a positive ANA-titer * 1:80, before and at screening :
- Positive test results from 2 independent time points within the study screening period; OR
- One positive historical test result and 1 positive result during the screening period.
Historical documentation of a positive test of ANA (eg, ANA by HEp-2 titer, ANA by ELISA)

must include the date of the test.
6) Anti-DNA seropositivity, as defined by a positive anti-dsDNA serum antibody * 30 IU/mL, before and at screening:
- Positive test results from 2 independent time points within the study screening period.
- One positive historical test result and 1 positive result during the screening period.
Historical documentation of a positive test of anti-dsDNA (eg, anti-dsDNA by Farr assay or ELISA) must include the date of the test.
7) Immune-complex mediated complement usage, as defined by:
- a low C3 serum level * 0.9 g/L; OR
- a low C4 serum level * 95 mg/L; OR
- a reduced activation of the classical pathway < 75%
8) Female subjects are eligible to enter the study if she is:
- Not pregnant or nursing
- Of non-child-bearing potential (i.e. after hyseterectomy, postmenopausal, bilateral ovariectomy or documented bilateral tubal ligation or other permanent female sterilization procedure)
- Use of effective contraception:
* Complete abstinence from intercourse from 2 weeks prior to administration of the 1st dose of study agent until 16 weeks after the last dose of study agent (Sexual inactivity by abstinence must be consistent with the preferred and usual lifestyle of the subject. Periodic abstinence (e.g. calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception); OR
* Consistent and correct use of 1 of the following acceptable methods of birth control for 1 month prior to the start of the study agent, during the study, and 16 weeks after the last dose of study agent:
o Oral contraceptive, either combined or progestogen alone
o Injectable progestogen
o Implants of levonorgestrel or etonogestrel
o Estrogenic vaginal ring
o Percutaneous contraceptive patches
o Intrauterine device (IUD) or intrauterine system (IUS) with <1% failure rate as stated in the product label
o Male partner sterilisation (vasectomy with documentation of azoospermia) prior to the female subject's entry into the study, and this male is the sole partner for that subject. For this definition, *documented* refers to the outcome of the investigator's/designee*s medical examination of the subject or review of the subject's medical history for study eligibility, as obtained via a verbal interview with the subject or from the subject*s medical records.
o Double barrier method: condom and occlusive cap (diaphragm or cervical/vault caps) plus spermicidal agent (foam/gel/film/cream/suppository)
* These allowed methods of contraception are only effective when used consistently, correctly and in accordance with the product label. The investigator is responsible for ensuring subjects understand how to properly use these methods of contraception.
* Female subjects using mycophenolate mofetil (MMF) should be made aware that MMF affects the metabolism of oral contraceptives and may reduce their effectiveness. As such, women receiving MMF who are using oral contraceptives for birth control should employ an additional method (e.g., barrier method).

Exclusion criteria

4.2. Exclusion criteria
Subjects will be excluded from participation if they meet any of the following exclusion criteria:
1) Active pregnancy, as proven by a positive urine beta-HCG test or a positive serum beta-HCG
2) Significant B-cell depletion (peripheral B-cell counts < 60x10E6)
3) Significant hypogammaglobulinemia (IgG < 4.0 g/L) or an IgA deficiency (IgA < 0.1 g/L)
4) Immunization with a live vaccine 1 month before screening
5) Active infection at time of screening, as follows:
- Hospitalization for treatment of infection within previous 2 months of day 0 of the study
- Use of parenteral (intravenous of intramuscular) antibiotics (including anti-bacterials, anti-virals, anti-fungals or anti-parasitic agents) within previous 2 months of day 0 of the study
- Serological evidence of active viral hepatitis defined as: patients positive for HbsAg test or HBcAb or a positive hepatisits C antibody
6) Have a historically positive HIV test or test positive at screening for HIV
7) Have a history of a primary immunodeficiency
8) Have a history of an anaphylactic reaction to parenteral administration of contrast agents, human or murine proteins or monoclonal antibodies
9) Have any other clinically significant abnormal laboratory value in the opinion of the investigator
10) Have current drugs or alcohol abuse or dependence
11) Have an active malignant neoplasm or one in the history of the last 5 years
12) Have evidence of serious suicide risk including any history of suicidal behavior in the last 6 months and/or any suicidal ideation in the last 2 months or who, in the investigator*s opinion, pose a significant suicide risk

Study design

Design

Study phase:	2
Study type:	Interventional
Masking:	Open (masking not used)
Control:	Uncontrolled
Primary purpose:	Basic science

Recruitment

NL

Recruitment status:	Recruitment stopped
Start date (anticipated):	19-05-2014
Enrollment:	15
Type:	Actual

Medical products/devices used

Product type:	Medicine
Brand name:	belimumab
Generic name:	benlysta
Registration:	Yes - NL intended use
Product type:	Medicine
Brand name:	rituximab
Generic name:	mabthera
Registration:	Yes - NL intended use

Ethics review

Approved WMO Date:	09-05-2014
Application type:	First submission
Review commission:	METC Leiden-Den Haag-Delft (Leiden)
Approved WMO Date:	12-05-2014
Application type:	First submission
Review commission:	METC Leiden-Den Haag-Delft (Leiden)
Approved WMO Date:	06-06-2014
Application type:	Amendment
Review commission:	METC Leiden-Den Haag-Delft (Leiden)
Approved WMO Date:	08-10-2014
Application type:	Amendment
Review commission:	METC Leiden-Den Haag-Delft (Leiden)
Approved WMO Date:	04-02-2015

Application type:	Amendment
Review commission:	METC Leiden-Den Haag-Delft (Leiden)

Study registrations

Followed up by the following (possibly more current) registration

No registrations found.

Other (possibly less up-to-date) registrations in this register

No registrations found.

In other registers

Register	ID
EudraCT	EUCTR2014-000488-42-NL
CCMO	NL48136.058.14