

Study Protocol

**SAFETY, TOLERABILITY AND MECHANISM OF ACTION OF
BOSWELLIC ACIDS (BA) IN MULTIPLE SCLEROSIS (MS) AND
CLINICALLY ISOLATED SYNDROME (CIS):
A MRI-CONTROLLED, MULTICENTER, BASELINE-TO-
TREATMENT, 32-WEEKS, OPEN-LABEL, PHASE IIA TRIAL IN
PATIENTS WITH RELAPSING-REMITTING MULTIPLE
SCLEROSIS OR CLINICALLY ISOLATED SYNDROME**

- SABA -

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- Confidential -

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Herewith, I confirm that I have read the study protocol carefully and declare my consent with it. I will treat and examine the patients in accordance with the study protocol, the national applicable laws, the international guidelines on good clinical practice (ICH-GCP), and the declaration of Helsinki.

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1. Summary

Protocol Title: Safety, Tolerability and Mechanism of Action of Boswellic (BA) Acids in Multiple Sclerosis (MS) and Clinically Isolated Syndrome (CIS):
A MRI-Controlled, multicenter, Baseline-to-Treatment, 32-weeks, open-label, Phase IIa Trial in Patients with Relapsing-Remitting Multiple Sclerosis or Clinically Isolated Syndrome

Short Title: SABA

Study Phase: IIa

Study Design: Phase IIa study consisting of a run-in phase, a dose finding phase, a stabilization phase, a continued treatment phase and a follow-up.

The trial will consist of:

- 12 week **run-in phase** (4 monthly baseline MRIs)
- **Stage 1: Dose finding:** Treatment with Boswellic acid will be initiated by escalating the dose to the maximum individually well tolerated dose within the first 4 weeks of treatment. Dose finding period of up to 4 weeks. Safety with respect to the effect of Boswellic acid (BA) on MS/CIS will be monitored by MRI and clinical assessment. The highest individual well tolerated dose will be used for each patient in Stage 2.
- **Stage 2: Stabilization:** A phase of 4 weeks will be used for adjustment of the dose to a continuously well tolerated dose. Patients not tolerating the dose established in Stage 1 will be treated with their maximum individually well tolerated dose.
- **Stage 3: Treatment:** A phase of 6 months will follow the dose finding and stabilization phases for a total of 8 months treatment. MRI will be used as the primary outcome measure to assess safety and to determine the effect of Boswellic acid on disease activity. The mechanism of action of Boswellic acids will be examined in parallel to the MRI and clinical assessment.
- **Stage 4: Follow-Up:** A phase of 4 months will follow the treatment phase. Patients are offered to continue study drug or to stop study drug after month 8. In either case the final follow-up visit will take place at month 12 including a complete clinical and MRI assessment.

Study rationale: Boswellic acids (BA) are the main components of extracts from the resin of the frankincense tree (*Boswellia serrata*).

These BAs have known anti-inflammatory activities and have been used literally for thousands of years in traditional Eastern and Asian medicine. Recently, the molecular mechanism of action of several specific BAs has been defined as inhibition of cathepsin G, microsomal prostaglandin synthase (mPEGS)-1, and 5-lipoxygenase (5-LO), i.e. factors that are involved in cell migration, inflammation and neurodegeneration in Multiple Sclerosis.

An orally available, antiinflammatory drug, with a good safety and tolerability profile is still an unmet need in MS treatment. Frankincense extracts have already shown this profile in a couple of clinical trials of different autoimmune diseases. The triple inhibition of cathepsin G, mPEGS-1 and 5-LO offers a novel pharmacological approach in the treatment of multiple sclerosis that has not been studied so far.

Here we propose a study design to test the safety, tolerability, efficacy and mechanism/s of action of BA in a Phase IIa proof-of-concept baseline-to-treatment study in relapsing-remitting MS or CIS patients with the standardized frankincense extract BOSWELAN. Dose finding, assessment of tolerability and efficacy on contrast-enhancing MRI lesions will be accompanied by mechanistic studies that will address by ex vivo and in vitro experiments if treatment with the frankincense extract only affects the above enzymes or has further pharmacodynamic effects.

Study Objective: To determine the safety and tolerability of BOSWELAN in subjects with multiple sclerosis or clinically isolated syndrome and to describe the effect of Boswellic acids on the disease activity as assessed by monthly MRI measures.

Number of Subjects:

75 subjects will be enrolled to obtain at least 30 subjects completing 8 months of treatment at a t.i.d. (Ter In Die (*Latin: Three Times A Day*) dose of between 400-1600 mg. It is estimated that 75 patients will undergo baseline MRI assessment in order to identify 30 patients meeting the MRI entry criteria.

Study Population: Clinically isolated syndrome (CIS) or relapsing-remitting Clinically definite MS (CDMS) with an age between 18 - 65 years
Baseline number enhancing lesions 0.5 or greater per month

Safety Parameters and Stopping rules:
treatment

Stage 1 – 4 (dose finding, stabilization, a continued phase and a follow-up):

Dosing will be discontinued in patients showing an increase in total Gd-enhancing lesions beyond 6 standard deviations of the mean lesion frequency during baseline plus 10 lesions. The maximum lesion number allowed during treatment will be 40 Gd-enhancing lesions. The upper margin (40 Gd-enhancing lesions) stems from a systematic review of the numbers and distribution of contrast-enhancing lesions derived from the placebo cohorts of pivotal phase III trials, which was performed by the Sylvia Lawry Center for MS Research (M. Daumer and colleagues). Furthermore, if in the opinion of the investigators there is a change in appearance of enhancing lesions inconsistent with the patient's previous MRIs, e.g. change in average lesion size from small to substantially larger lesions, dosing will be discontinued.

Two clinical safety criteria will also be used as stopping criteria:

Clinical criterion 1- relapses: Two or more confirmed relapses/exacerbations (see section 7.3.2 for definition of relapse/exacerbation) or clinical criterion 2 - progression: Worsening of two or more points on the EDSS scale during the treatment phase, compared to baseline post treatment, confirmed and consistent through three monthly visits.

If dosing had to be discontinued in five patients because of MRI parameters or in more than 1 of the first 4 participants, more than 2 of the first 8 participants, and 3 or more participants in the entire group because of clinical parameters the study will be placed on clinical hold, i.e. treatment with BOSWELAN of all patients already in the study will be discontinued and no additional patients will be recruited until the data from the trial has been reviewed by the safety committee.

Side effect monitoring, blood biochemistry (especially liver enzyme levels), as well as neurological and other clinical parameters will be used to further assess safety.

**Efficacy
Parameters:**

Primary outcome measure

- Number and volume of total Gd-enhancing lesions

Secondary outcome measures

- Number of persisting Gd-enhancing lesions
- Number of new active lesions (new Gd-enhancing lesions +new or enlarging non-enhancing T2 lesions)
- Number of new Gd-enhancing lesions evolving into persistent hypointense lesions
- T2 lesion volume
- T1 hypointense lesion volume
- Number of persisting T1 lesions
- Brain atrophy (brain parenchymal fraction)
- Magnetization Transfer Ratio (MTR)
- Relapse rate

Tertiary outcome measures

- MRS (multi-voxel magnetic resonance spectroscopy)
- Neurological measures: Expanded disability status scale (EDSS), SCRIPPS neurological rating scale (SNRS), MS functional composite (MSFC) consisting of 9-HP-test (Nine-Hole-Peg Test), timed 25 foot walk, paced auditory serial addition test (PASAT), Depression scale (Hospital Anxiety and Depression Scale) and a fatigue scale (Fatigue severity scale according to Flachenecker et al.), Hamburg Relapse Assessment Scale (HARAS)
- Immunological parameters
- Biomarker assessment (Cathepsin G activity and PGE₂ levels)

Patient selection: Subjects with clinically isolated syndrome (CIS) fulfilling MRI inclusion criteria (see below) or clinically definite relapsing remitting multiple sclerosis (RRMS) in an active inflammatory phase who either failed standard treatment by clinical measures or were not eligible for any of the standard treatments (e.g. interferon beta, glatiramer acetate) or, after consultation with an independent neurologist, elected not to start or to continue with any of these treatments. Subjects will be enrolled following completion of all pre-screening procedures. After enrollment, subjects will undergo baseline Gd-enhanced MRIs at 4-week intervals prior to the first dose of study drug.

Statistical Plan: Evaluable populations

The primary outcome in the treatment phase (Stage 3) will first compare mean Gd-enhancing lesion number occurring during the 4 month baseline to mean Gd-enhancing lesion number occurring months 5, 6, 7, 8 on treatment in patients treated with a dose of at least 800 mg t.i.d.

A secondary analysis will compare mean Gd-enhancing lesion number occurring over the 4 months of baseline to that occurring during all treatment months on treatment.

Sample size

Based on analyses of a natural history cohort studied with monthly MRIs for a minimum of one year, a baseline-to-treatment designed trial incorporating 4 baseline MRIs and 4 treatment MRIs will require 30 patients to detect a 40% reduction in Gd-enhancing lesions with an alpha of 0.05 (one-sided).

2. Introduction and Study Rationale

Preparations from the olegum resin of *Boswellia serrata* and other *Boswellia* species species have been used in traditional eastern and asian medicine for the management of rheumatism, respiratory diseases and liver disorders from 1500 B.C. but were described only in the 1980ies by Indian scientists to show anti-inflammatory potential in animal models of arthritis (1). The active ingredient of BOSWELAN is 400 mg native *Boswellia serrata* Resin Extract containing the four major present pentacyclic triterpenic acids β - Boswellic Acid (18.5%), Acetyl- β -Boswellic Acid (10.5%), 11-keto- β -Boswellic Acid (KBA, 6.1%) and Acetyl-11-keto- β -Boswellic Acid (AKBA).

For details, please see the BOSWELAN Investigational Brochure.

2.1 Background

Currently approved treatments for MS include the interferon- β (IFN- β) preparations, glatiramer-acetate (GA), natalizumab and mitoxantrone. All of these act as anti-inflammatory agents, and, while moderate neuroprotective activity has been reported for GA, this effect is, however, not proven. Several biologicals including alemtuzumab, daclizumab, rituximab and atacicept as well as a number of orally available agents including cladribine, fingolimod, laquinimod, teriflunomide, and fumaric acid are in late phases of clinical development. Some of the above mentioned drugs have shown substantial efficacy with respect to reduction of MS relapses and/or inflammatory MRI lesions, but it has become clear that several are not only highly active, but also pose a considerable risk for serious adverse events including malignancies, life-threatening infections, secondary autoimmune diseases and death.

Early treatment of MS has become a desired aim in MS management (2), (3), even though patients may experience a benign disease course. Clear prognostic indicators for active disease and rapid disability progression are currently lacking, although some signs and findings such as early pareses and cerebellar involvement as well as poor recovery from first relapse have been linked to poor outcome. In this context a dilemma arises for patients and treaters: they often have to decide to start either early on a highly active treatment (i.e. natalizumab, mitoxantrone, or other), that includes a considerable risk for serious adverse events or on a less active, but often inconvenient treatment like IFN- α preparations or GA with consecutive adherence and withdrawal problems (4), (5). Although a number of oral drugs are now in late phase III testing it becomes already evident that high inflammatory activity induced by immunosuppression might coincide again with severe side effects (6). Even the less active compounds as fumaric acid or others have shown relevant side effects.

In this context the following aspects represent the largest unmet medical needs in MS (those addressed by the frankincense therapy are underlined):

1. Development of orally available drugs for all phases of MS, which act via anti-inflammatory, neuroprotective or neuroregenerative effects
2. Drugs that are more effective and/or have a better safety profile or act via a novel mechanism of action or offer a preferable route of administration over currently available therapies
3. Rational combination therapies that take into account the pathogenetic aspects that underlie MS in the individual patient. These can be combinations of anti-inflammatory with neuroprotective or neuroregenerative approaches, but also combinations of two different anti-inflammatory approaches
4. Symptomatic therapies that improve the leading and frequent symptoms of MS such as fatigue, neurocognitive deficits, spasticity, weakness, bladder/bowel/sexual dysfunction
5. Approaches, biomarkers and algorithms need to be developed that allow the identification early or even prospectively whether a patient will be a responder or non-responder to a specific therapy.

According to our current understanding (see below), BAs are not directly neuroprotective or neuroregenerative, although such activities will be studied further, and it is conceivable that neuroprotection will be achieved indirectly by suppressing neuroinflammation.

Considering the necessity of high acceptance for a safe long-term treatment, MS patients use supplementary dietary and phytotherapeutic approaches even though the evidence for their usefulness is at best weak (7). A recently started trial of add-on green tea extract (epigallocatechin-gallate as proposed active substance) to GA has raised high expectations, and recruitment of patients has been easy at our centers.

2.2 Known and proposed mechanisms of action of the frankincense extract and BA

Preparations from the oleum resin of *Boswellia serrata* and other *Boswellia* species have been used in traditional eastern and asian medicine from 1500 BC, but were described only in the 1980ies by Indian scientists to show anti-inflammatory potential in animal models of arthritis (1). Following these observations the induction of a broad range of immunmodulatory and antiinflammatory effects by boswellic acids (BAs) could be proven by several independent research groups (7).

Ammon and colleagues demonstrated that BAs inhibit 5-Lipoxygenase (5-LO) (8), and subsequently Werz et al. (manuscript submitted) could show that the beta-BAs inhibit the serine protease cathepsin G (Cat G) and the microsomal prostaglandin E2 synthase (mPGES)-1 specifically. The pharmacological strategy of dual inhibition of cathepsin G and mPGES-1 or even inhibition of three key enzymes in the arachidonic acid pathway by dual mPGES-1/5-LO or even triple Cat G/mPGES-1/5-LO inhibitors is novel and has thus far not been described for any compound. Importantly, in contrast to the traditionally used non-steroidal anti-inflammatory drugs (NSAIDs) and coxibs that suppress the formation of all prostaglandins by inhibition of cyclooxygenases, these new compounds selectively inhibit the formation of PGE₂ and do not inhibit the synthesis of prostacyclin, which is a prominent safety aspect. Elevated levels of PGE₂ have recently been described in the Cerebrospinal fluid (CSF) of MS patients (9). Observations of a regulatory role in the glutaminergic, neurotransmission of PGE₂ (10) are raising the question of whether BAs might even show neuroprotective effects.

The dual inhibition of 5-LO and the mPGES-1 might be a second relevant mechanism in approaching MS. The relevant classes of synthetic small molecule compounds with this mechanism of action are: indol-3-carboxylates, α-substituted pirinixic acid derivatives, and α-substituted thiophenylether derivatives. The latter two activate the peroxisome proliferators-activated receptor (PPAR)-γ, for which a multitude of activities that are beneficial in Experimental autoimmune encephalitis (EAE), the mouse model of MS, and potentially also in MS including blocking IL-12/IL-23 family members and thus Th1- and Th17 development, immune cell extravasation, NMDA-mediated excitotoxicity and others (11) have been shown.

Cathepsin G (Cat G) participates in killing and digestion of pathogens, but is also involved in tissue remodelling at inflammatory sites by increasing matrix metalloproteinase (MMP) activity. The chemotactic activity of Cat G appears to play a role in synovial inflammation in rheumatoid arthritis via attracting neutrophils, and reduced Cat G activity is a mechanism of host immune defense evasion during tuberculosis. The chemokine CXCL5 is significantly downregulated in immunomodulatory treated MS-patients (12), and Cat G increases the acitivity of the CXCL5 and CCL15, which are important chemoattractants of monocytes and neutrophils (13). Cat G differentially modulates the antigen processing of myelin basic protein (MBP) in various APCs (B cells, dendritic cells) (14). Inhibition of Cat G resulted in failure to process MBP, a central autoantigen of MS (15), although the relevance of these data for MS is currently not clear to the applicants. Finally, Cat G has been shown to activate specifically pro-inflammatory cytokines IL-1 and TNF-α (16).

5-Lipoxygenase (5-LO) catalyzes two reactions in the formation of proinflammatory leukotrienes and is involved in the TNF- α -induced expression of CXCL9, CXCL10 and CXCL11, which are all ligands for the Th1 chemokine receptor CXCR3. 5-LO inhibitors downregulate CXCR3 ligands. Blocking 5-LO in wild-type mice delayed the onset and reduced the severity of EAE, but did not reduce the production of Th1- and Th17 cytokines (17). In contrast, EAE is exacerbated in constitutive knockouts of 5-LO suggesting that cell-type or tissue-specific inhibition is beneficial, but outweighed by global lack of the enzyme (18). Of particular interest here is the finding that 5-LO is among the most highly differentially expressed genes in the brains of MS patients and animals with EAE (19) and also its upregulation in the brains of patients with Alzheimer's disease (20) indicating a central role in the neuroinflammatory processes in both diseases. The latter notion is supported by the observation that 5-LO and FLAP in monocytes and microglia participate in the generation of products that are toxic for neurons (21-23). As a further indication of an involvement of 5-LO in MS, several reports have found elevated leukotriene (LTB4) concentrations in the CSF of MS patients (24). Finally, the administration of omega-3 poly-unsaturated fatty acids lowers the expression of 5-LO (25).

Microsomal Prostaglandin E2 synthase 1 (mPGES-1) is induced by the proinflammatory cytokine IL-1 β via nuclear factor kappa B (NF κ B) (26) and is the terminal enzyme in the synthesis of prostaglandin E₂. mPGES-1 expression is regulated among other factors by the transcription factor HIF-1a, which is highly expressed in the early demyelinating lesions and normal appearing white matter of MS patients (27). The upregulation in synovial tissue of rheumatoid arthritis patients implicates mPGES-1 in the local inflammatory response of this autoimmune disease (28). mPGES-1-deficient animals show a markedly reduced tissue inflammation in autoimmune models, e.g. collagen-induced arthritis (29), and the induction of mPGES-1 in brain endothelial cells has been linked to the pyretic response (30). Mattson et al. recently reported elevated levels of PGE₂ in the CSF of MS patients, although they did not observe a correlation with clinical signs of MS (9).

From the above, the single, dual, or triple inhibition of Cat G, 5-LO and mPGES-1 by BAs appears a promising and novel treatment strategy in MS. Supporting this hypothesis, BAs were able to ameliorate the disease course of EAE (31). Pharmacologic studies have shown (32), Werz et al. 2009, in press), that the relevant plasma levels for a safe inhibition of cathepsin G and mPGES-1 are reached in vivo with low to moderate doses of the only standardized frankincense extract (BOSWELAN), which is the extract to be used in this study.

In summary, the use of BAs or the standardized frankincense extract of BOSWELAN appear highly promising for the treatment of relapsing-remitting MS for the following reasons:

1. Novel mechanism of action by single- and dual inhibition of three enzymes that appear to play a role in neuroinflammation in general and also in MS, Cat G, 5-LO and mPGES.

2. Oral availability, very favourable safety profile that is known for a long time from applying BAs in man. Pharmacokinetic data have shown that in vivo active concentrations that are required for the inhibition of the above enzymes can be reached by the standard doses of frankincense extract, i.e. at 3 x 400 mg/day.
3. Well known biomarkers for the pharmacodynamics of BAs, i.e. Cat G Cat G (and PGE₂) activity that will be useful for dose finding and responder profiling.
4. Based on its mechanism and good tolerability well suited for combination therapy approaches.

An additional consideration is the recent realization that most if not almost all currently approved drugs do not act on single targets, but rather have a spectrum of effects and affect multiple biological pathways or several steps in single pathways (33). Accordingly, it has been proposed to apply network pharmacology approaches that capture the entire spectrum of effects of novel treatments and that it is more desirable to identify compounds and therapies that hit several rather than single and highly specific targets (33, 34). Along these lines, BAs appear an attractive new group of drugs since they inhibit several inflammatory mediators at the same time. The frankincense extract BOSWELAN may be ideally suited for investigating the proof-of-concept of this approach since it combines a number of BAs.

2.3 Proposed Study

For the proposed study, we will use a well-established design that has been developed at the Neuroimmunology Branch and the Laboratory of Diagnostic Radiology Research at the National Institutes of Health (35). The design is a MRI-controlled, baseline to treatment, cross-over Phase IIa study (36-38). Since clinical efficacy in MS can only be judged by large-scale phase IIb and III trials which require large patient numbers followed over long time periods, MRI measures are considered the best validated biomarkers and are the recommended outcome measure for mechanism of action-oriented phase II clinical trials (35, 39). Use of MRI measures are ideal for correlating inflammatory activity in the CNS with immunological mechanisms that mirror the mechanism of action of the study therapy, in small cohorts of patients (36). Obtaining serial and longitudinal MRI studies also serve as a sensitive safety measure. This approach has already been used for phase I/II trials of interferon-beta, an altered peptide ligand of myelin basic protein peptide 83-99, transforming growth factor-beta, insulin-like growth factor and interleukin-2 antagonist (40-44).

This trial design offers major advantages: (1) Since each patient's baseline will be compared to the treatment of the same individual, such trials can be performed with sufficient statistical power even with small patient numbers, i.e. 10-15 patients (35, 37); (2) The combination of clinical, MRI and immunological parameters (eg. frequency and phenotype of myelin reactive T cells) provides a maximum of information as to the safety, efficacy and mechanism of action of experimental treatment approaches and is therefore ideal at this stage.

The proposed study will examine the effect of Boswellic Acids in MS and CIS, and includes 4 phases: (1) an individual dose finding phase of 4 weeks, followed by (2) a 4 weeks stabilization phase, (3) a treatment phase of additional 6 months and a follow-up after another 4 months. Patients with CIS or clinically definite RRMS (according to the McDonald criteria) will be enrolled.

Stage 1

All patients have 4 baseline MRIs. Only patients with an average of at least 0,5 Gd-enhancing lesions during Stage 1 will be included to the treatment phase. Patients eligible based on MRI criteria will begin a 4 week dose escalation to examine the safety of BOSWELAN and to determine the maximal well tolerated dose. A safety MRI will be done after 4 weeks.

Stage 2

The individual well tolerated dose from Stage 1 will be used for each patient in a subsequent 4-week stabilization phase during which the dose will be observed for continuous safety and toleration. Afterwards this individual maximal well tolerated dose will be used in Stage 3 of the study for further 6 months. Patients not tolerating the minimum dose of 800mg t.i.d. will be excluded from the study.

Stage 3

In Stage 3 of the study, patients will be treated with their individual well tolerated dose of BOSWELAN as assessed in Stage 2 subsequently for six months. The minimum dose for patients to be considered evaluable in the primary statistical analysis will be 800mg t.i.d..

The primary outcome will assess the change in mean Gd-enhancing lesion number from the baseline to that observed over months 5, 6, 7 and 8 since a delay in treatment effect is expected. In addition to MRI evaluation, patients will have monthly neurological evaluations and immunological assessment.

Stage 4

After completing Stage 3 patients are offered to continue their BOSWELAN dose for 4 months or to stop drug intake after month 8. In any case patients are seen for clinical and MRI-follow-up at month 12 at the study center.

2.4 Preclinical Studies

The pharmacology of the frankincense extracts has been examined in multiple studies *in vitro* and *in vivo*. With respect to its toxicology, frankincense extracts have been studied comprehensively for all relevant toxicity endpoints. The LD50s in mg/kg after oral application were found to be: >2000mg/kg for rats, mice, doses of 2000 mg/kg orally or intraperitoneal have not caused death in rats, mice and monkeys, high doses have reportedly not yielded significant effects on behavior or clinical, hematological, biochemical and pathological data (1, 45).

Single dose toxicity studies were performed in mice, rats and dogs by oral, i.m. and i.p. application. The acute toxicity was above 5000 mg/kg b.w. for the rat by oral administration (Laboratory of Pharmacology and Toxicology, Hamburg, Germany (LPT), Investigator's Brochure for BOSWELAN.

Subacute toxicity studies with an alcoholic extract of salai-guggal in rabbits over three months, and chronic toxicity studies in rats and monkeys over six months, have found no toxic effects of boswellic acids at high doses (45). A 26-week toxicity study in rats and a 9-month toxicity study in dogs were conducted at LPT according to the current ICH- and GLP-guidelines. In the 26-week chronic toxicity study in Sprague-Dawley rats, Boswellia extract was administered orally at dose levels of 300, 900 and 2700 mg/kg b.w./day. At 2700 mg/kg b.w./day substance-related mortality was noted. Hence, the dose level of the high-dosed male rats was reduced to 1800 mg/kg b.w./day from test week 15 onwards. None of the rats treated and also none of the surviving rats then treated with the low or intermediate dose showed any clinical signs of toxicity. The macroscopical inspection of the prematurely deceased high-dosed animals revealed a reduction in the size of the thymus. The relative liver weight of the intermediate and high-dosed rats was increased in a dose-related way (increase of 25 – 75% in comparison to controls).

In conclusion there were indications for a liver toxic influence of Boswellic acids at very high doses (46), at these high doses there was also a decreased urine volume and general body weight reduction observed.

In the 9-month chronic toxicity study in Beagle dogs, Boswellia extract was administered orally at dose levels of 300, 900 and 2700 mg/kg b.w./day. Due to the marked increased aP values in the intermediate and high dose groups, the dose levels were reduced to 100, 300 and 900 mg/kg b.w./day from test week 20 onwards. Moreover the increased levels of total bilirubin and total cholesterol are considered to be substance-related. The severity of the changes decreased after reduction of the dose levels from test week 20 onwards and all the above-mentioned changes had nearly disappeared at the end of the 4-week recovery period.

For assessing the genotoxic and carcinogenic potential studies were carried out by the Cytotest Cell Research and the GSF-Forschungszentrum für Umwelt und Gesundheit. Studies were performed for gene mutations in bacteria: (*Salmonella typhimurium* strains [CCR, 1987a]), gene mutations in eukaryotic systems (HPRT test in V79 cells [GSF, 1990]), chromosomal aberrations in human lymphocytes: *in vitro* [CCR, 1991] and chromosomal aberration test in bone marrow cells of the Chinese hamster [CCR, 1987b]. No mutagenic potential was observed for Boswellia extract [CCR, 1987a+b, 1991; GSF, 1990].

Carcinogenicity studies have not yet been considered necessary to be performed, as no reasons, e.g. chemical structure, previous demonstration of a carcinogenic potential in the product class that is considered relevant to man, or toxicological reasons including mutagenicity results, make such studies necessary. In addition, a cell transformation assay using Syrian hamster embryo (SHE) cells gave no indication for a carcinogenic potential [CCR, 1987c].

Since December 2014 estragole, a phenylpropene and natural organic genotoxic compound in various trees and plants like basil, anise, bay and also boswellia extracts has received new attention as shown by the recently drafted and still discussed public statement on the use of herbal medicinal products containing estragole (EMA/HMPC/137212/2005 Rev 1). Several studies in rodents have congruently established the genotoxic carcinogenicity of estragole in single substance studies (47-49).

Likewise metabolic activation and DNA binding occur also in human experimental systems in vitro. However it remains currently unclear if and to which extent these observations can be translated to humans as data from clinical studies are lacking and if the ingestion or application together with other compounds like in herbal extracts (50, 51) has comparable mutagenic effects. The EMA drafts and previous animal studies state that the mechanism of action of genotoxicity and carcinogenicity is dose-dependently conveyed by the production of reactive metabolites, primarily the sulfate conjugate of 1'-hydroxy estragole, which subsequently binds to DNA with possible genotoxic and carcinogenic potential (52).

Estragole is also used as flavoring agent in food products and has been approved by the US Food and Drug Administration (FDA), although the daily intake by consumers can only be estimated due to varying data on flavored and naturally estragole-containing food. Human data are missing but are estimated to lie between 0.5 to 5 mg estragole exposure daily.

The expert panel of the Flavor and Extract Manufacturers Association concluded in 2002 that dietary exposure to estragole from spice consumption does not pose a significant cancer risk to humans because several studies clearly established that profiles of metabolism, metabolic activation and covalent binding were dose dependent at high levels but diminished markedly at lower levels of exposure (49). The metabolic and genotoxic effects diminish markedly at low levels of exposure as described in the EMA guideline of 2005 (53).

Rodent studies show that genotoxic and carcinogenic events are probably minimal in the dose range of 1-10 mg/kg body weight in mice which is equivalent to 0,14 mg/kg – 1,43 mg/kg body weight in humans. This dose is approximately 100-1000 times higher than the anticipated human exposure to estragole by non-pharmaceutical ingestion. There are currently no regulatory established concentration limits for estragole in the area of medicinal products. The estragole doses in the study medication BOSWELAN have been shown to be below 0,14 mg/kg body weight. Yet these doses are significantly higher than the anticipated human exposure by ingestion, which will be pointed out in the patient information.

Embryotoxicity studies were conducted in rats and rabbits at LPT. Testing of the effect on fertility, reproductive capacity and peri- and postnatal development was not conducted since there was no indication that the reproductive organs had been affected by the test substance.

In rat embryotoxicity study the no-observed-effect level (NOEL) for dams was 300 mg Boswellia extract/kg b.w./day p.o.. In dams at 900 and 2700 mg/kg b.w./day a dose-related moderately reduced body weight and a reduction in food consumption were noted. There were no signs of embryotoxicity. The no-observed-effect level for the fetuses was above 2700 mg/kg b.w./day.

In conclusion, there were no signs of embryotoxicity including malformations, variations and/or retardations at any of the tested dose levels, even at materno-toxic doses [LPT, 2000f].

In the embryotoxicity study in rabbits, Boswellia extract was administered orally at dose levels of 50, 150, 450 and 1350 mg/kg b.w./day from the 6th to 20th day of pregnancy. The high dose of 1350 mg/kg b.w./day was included in this study as the dose of 450 mg/kg b.w. resulted in no clear maternal toxicity.

In conclusion, the test substance possessed no teratogenic properties. A marginal embryotoxicity was noted only at the materno-toxic dose of 1350 mg/kg b.w./day [LPT, 2000g].

2.5 Pharmacokinetics in humans

Twelve hundred milligrams of boswellia resulted in plasma concentrations of 10-32 µl M of 11-keto-β-boswellia acid and 18-20 µl M acetyl-11-keto-β-boswellic acid, measured 2-3 hours following administration (54).

A small pilot bioavailability study after administration of the Boswellia serrata soft gelatine capsules BOSWELAN (Medeon GmbH) in six healthy subjects resulted in a maximal peak concentration of 180.9 ± 57.59 ng/ml for KBA and of 34.8 ± 19.19 ng/ml for AKBA within the first two hours after administration with a half-time of 2.45 ± 0.44 hours for KBA and of 1.14 ± 0.24 hours for AKBA.

Food effect

In a recently completed Phase I, open, randomised, cross-over pharmacokinetics study plasma levels of KBA and AKBA after a single oral morning dose of 800 mg Boswelan after either a standardized breakfast or after an overnight fasting period (fasting) were assessed. Plasma levels were about twofold higher under "with meal" conditions. From a different study about 200 fold higher plasma concentrations were achieved by βBA and αβBA , compared to KBA.

2.5.1 Clinical Studies Assessing the Safety, Tolerance and Efficacy

BAs are generally believed to be safe. BA-containing extracts have already been used in several randomised placebo-controlled clinical phase II- and -III studies. BAs have shown beneficial effects in rheumatoid arthritis, asthma bronchiale and osteoarthritis (evidence level B and C). BAs were well tolerated (55) based on altogether 7 reported adverse events (3 cases of local skin reaction, 1 case of diarrhea, 1 case of gastral intolerance) in 233 patients who received BA extracts. Similarly, in a recent review about placebo-controlled studies with BAs in patients suffering from autoimmune diseases (i.e. asthma, rheumatoid arthritis, Crohn's and others) adverse events were described in max. 5% of BA-treated patients, mostly presenting as gastrointestinal intolerance, i.e. nausea, acid reflux, mild gastrointestinal upset, diarrhoea (56). No allergic or hypersensitivity to BAs have been reported in the available

literature.

A double-blind placebo-controlled Phase II study with BOSWELAN in inflammatory bowel disease has recently been conducted. In the recent interims-analysis of 76 patients (2 groups of 38 patients each with BOSWELAN vs. placebo), no significant no serious adverse events were reported.

With respect to the clinical use of BAs, more than 500 patients (with different inflammatory, mainly autoimmune diseases) have been treated, and no serious adverse events have been reported from any of the clinical trials (56).

High doses of frankincense extracts up to 4800mg/d have been well tolerated in a couple of well documented cases and studies in cancer patients, including children (45,46,54,55).

In addition to pharmacokinetic and animal toxicity data BA extracts (BOSWELAN) show an excellent safety profile even up to doses of 3600mg/d (2400mg/d) (BOSWELAN, see Investigator's brochure). Regulatory permission based on the toxicology data for the standardized frankincense extract BOSWELAN has already been granted from the BfArM for the above mentioned Phase II study in Crohn's disease. Data concerning the treatment of MS with BA are currently not available, and approval of BOSWELAN or any other frankincense extract for the treatment of MS has not yet been granted, but is expected to be granted without any major anticipated problems.

The most common complaints in trial have been skin irritations and dermatitis (55-57). In 38 patients with Crohn's disease, treated for one year with 800 mg t.i.d., no serious adverse events were observed.

For any further information on toxicology, preclinical studies, and pharmacokinetics please refer to the Expert Safety Review on *Boswellia serrata* Extracts (7th March 2011) and the CMC Review for Boswelan® (14th March 2011).

2.5.2 Dose justification

In conclusion from the abovementioned studies we deduce as minimum effective dose 800mg t.i.d., which is sufficient to induce plasma levels of AKBA *in vivo* that have previously been shown to inhibit Cathepsin G *in vitro*. The other two enzymes that are relevant to be inhibited by boswellic acids in the context of MS, namely 5-LO and mPGE1 require higher doses between 1-10µM of AKBA for inhibition, and consequently higher doses of BA extracts are probably needed to achieve these effects. 4800 mg/d (1600 mg t.i.d.) is the highest dose that we are planning to administer in this study because frankincense extracts have been reported to be safe and well tolerated up to this dose (45,46,54,55) and due to their higher efficacy that has been shown in studies of cerebral malignancies.

The minimal no observable adverse effect level (NOAEL) as documented in the toxicology studies translates to the following human equivalent doses (HEDs):

Table 1: Translation of minimal NOAEL doses from rat / dog to man

Species	NOAEL/LTD mg/kg	mg/kg	mg/m2
Rat	300	300	1770

	600	600	3540
	900 (LTD)	900	5310
Man/Rat		6.8	253
		13.7	506
		20.5	759
Dog	100	100	2000
	300 (LTD)	300	6000
Man/Dog		27	1000
		81	3000

Source: Guidance for Industry. Estimating the Maximum safe Starting Dose in Initial Clinical Trials for Therapeutics in Healthy Adult Volunteers. US CDER, July 2005, Pharmacology and Toxicology

The doses used with *Boswellia serrata* extract formulations in man to date range from approximately 1200 mg to 4800 mg per day divided over 3 daily doses. Clinical experience indicates that, dependent on the indication, optimal daily doses range between 3600 mg and 4800 mg/day in the acute phase or during initiation of treatment (below also referred to as higher, previously applied dose range) and between 1200 mg and 2400 mg/day (below also referred to as intermediate dose range) during maintenance therapy.

Therefore, the highest applied daily human dose (3600 mg – 4800 mg, approx. 70 kg b.w.) is between 51,4 - 68,5 mg/kg and 17,1 - 34,3 mg/kg for the maintenance doses based on the conversion standards as provided by the FDAs “Guidance for Industry. Estimating the Maximum safe Starting Dose in Initial Clinical Trials for Therapeutics in Healthy Adult Volunteers”, US CDER, July 2005, Pharmacology and Toxicology.

These daily doses, which have already been applied in man, are considerably above the NOAEL and even the lowest toxic dose (LTD) in the rat, above the NOAEL in the dog, but below the LTD in dog in repeated dose chronic toxicity. The rat appears to be the most sensitive model in repeated dose chronic toxicity. However, in safety pharmacology studies in the rat single doses up to 2700 mg were largely unproblematic, while earlier toxicological studies (1) demonstrated a cloudy swelling of liver and kidneys with doses up to 10 times the human dose. In chronic toxicology studies in rats the LTD was mainly determined by a significant increase of liver weight. Significant biochemical, hematological and histopathological changes were only encountered at high doses. In the dog toxicity was limited to the liver without any further changes, including histopathology, in any other system at any dose level

At the initial 900 mg/kg dose in dogs a moderate increase in total bilirubin, total cholesterol and alkaline phosphatase (AP) was observed. None of these findings were significant compared to control. While bilirubin was normalized, cholesterol and AP dropped by 50%-65% upon dose reduction to 300 mg/kg, the dose that was originally defined as the LTD for this group, with complete reversibility during recovery. Therefore we deem it appropriate to ask, whether a conversion to HED based on guideline standards is meaningful for extract compositions, in particular *Boswellia serrata*. The guideline standards apply in principle to single molecules with a defined pharmacological activity.

Boswellia serrata resin extracts basically contain the following substances: essential oils (more than 80 different compounds including mono-, di- and triterpenes), resin acids, ether-insoluble components (neutral and acidic

polysaccharides) and ether-soluble compounds (higher terpenes and boswellic acids; 56, 57). Boswellic acids have been identified as the main active moieties contained in *Boswellia seirata* oleogum resin.

Besides the possibility that the various active moieties contained in the extract, the boswellic acids in particular, may not yield the same bioavailability, in different species. In consequence, this may lead to differing pharmacokinetic, and, as a consequence, probably also pharmacodynamic profiles. Generally, it has to be considered that such an extract contains large quantities of lipophilic components, both pharmacologically active and inactive, which may be preferentially stored in the liver following first-pass (and subsequently eventually in e.g. kidney and bone marrow).

Therefore, the initial changes encountered at the LTD levels in rat and dog may be related to the volume i.e. the relative weight of lipophilic material applied rather than the dose intensity of the active moieties contained therein.

If the relative weight of the doses of native extract applied is considered, the upper limit of the dose range based on previously applied doses and projected in man of 52-69 mg/kg compared with the NOAEL and LTD in rat provides a "therapeutic window" ratio of at least 8, respectively 13.

Compared with the NOAEL and LTD in dogs, these ratios are at least 2 and 4 respectively. The ratios increase considerably if the intermediate dose range is considered. However, the safety margin is effectively much wider as the daily dose in man is split into 3 doses/day.

From a toxicological point of view, *Boswellia serrata* extract appears to possess at least a margin of safety as wide as e.g. mesalazine, if not even wider. Evidence of renal papillary necrosis was found for mesalazine in dogs already at 60 mg/kg b.w./day treated for 12 months (56). Mesalazine is used at a higher therapeutic dose of approx. 64 mg/kg b.w./day in man.

In conclusion, based on standard conversion rates, the previously applied and projected doses in man provide a narrow therapeutic window of safety compared to the NOAEL and LTD established in rats and dogs. In contrast a comparison based on relative weight of native extract of *Boswellia serrata* that is given provides a considerable safety margin. On the basis of the above consideration and the existing data from application of *Boswellia serrata* extracts at higher dose levels in humans we believe that a carefully conducted dose escalation from intermediate to higher dose levels is justified and safe.

3. Objectives and Outcome Measures

3.1 Primary Objective

The primary objective of this study is to determine the safety and tolerability of BOSWELAN in subjects with CIS or RR multiple sclerosis using side effect monitoring MRI (primary MRI safety parameter: mean total number of Gd-enhancing lesions), clinical, and laboratory measures.

3.2 Secondary Objective

The secondary objective of this study is to determine the effect of BOSWELAN therapy on disease activity in CIS or RR multiple sclerosis as measured by MRI

activity. In addition, the effect of BOSWELAN on immunological function will be assessed.

The primary efficacy outcome measure of this study will be the mean total number and the volume of Gd-enhancing lesions based on a comparison of 4 baseline MRIs and 4 treatment MRIs (months 5-8) in patients of this study who tolerated a dose of at least 800 mg t.i.d.

Secondary outcome measures will be:

1. Number of new active lesions (new Gd-enhancing lesions plus new non-enhancing or enlarging T2 lesions). Number of persisting Gd-enhancing lesions. Number of new Gd-enhancing lesions evolving into persistent T1 hypointense lesions. T2 lesion volume (fast spin echo sequence), T1 hypointense lesion volume, Number of persisting T1 lesions, Magnetization Transfer Ratio (MTR), brain parenchymal fraction as measure of atrophy
2. Relapse rate

Tertiary outcome measures will be:

1. Magnetic resonance spectroscopy (MRS). Multivoxel magnetic resonance spectroscopy will be performed once during the baseline and twice during the treatment phase (months 4 and 8) in three patients (those with the highest EDSS).
2. Expanded disability status scale (EDSS), SCRIPPS neurological rating scale (SNRS), MS functional composite (MSFC) consisting of 9-HP-test (Nine-Hole-Peg Test), timed 25 foot walk, paced auditory serial addition test (PASAT), and in addition a depression scale (Hospital Anxiety and Depression Scale) and a fatigue scale (Fatigue severity scale according to Flachenecker), Hamburg Relapse Assessment Scale (HARAS).

Immunological measures:

A number of immunological measures will be studied to determine the in vivo immunomodulatory effect, in particular the influence on T helper 1 (Th1) and Th2 cytokines,

Biomarker assessments:

Cathepsin G and mPEGS-1 serum levels will be analyzed at every visit starting from baseline (month 0).

4. Study Outline

Months -3 - 0	Month 1	Month 2	Months 3-8	Months 9-12
Baseline MRI Lesion frequency of 0.5 or greater	Dose escalation to treatment dose Monthly MRI	Stabilization of dose Monthly MRI	Primary treatment phase Monthly MRI Assessmet of individual treatment effect	Follow up of patients until Month 12 and MRI; optional for patients: washout or treatment continuation

5. Study Population

5.1 Number of Subjects

30 patients meeting the MRI entry criteria of an average of up to 2 enhancing lesions per month during baseline. It is expected that 75 patients otherwise meeting the entry criteria will need to be screened by MRI to identify 30 study patients.

5.2 Inclusion Criteria for Pre-Treatment Screening

To be eligible for entry into this study, candidates must meet the following criteria at the time of enrollment. If a washout period becomes necessary, these criteria have to be assessed again at the beginning of the MRI baseline period. An additional assessment of the inclusion criteria follows before the beginning of the treatment (day 1 of stage 1)

- Between the ages of 18 and 65 years, inclusive*
- Females and Males (as no specific gender-related differences are expected, no specific gender distribution is planned. See GCP-V § 7 (2) Nr. 12)
- Subjects with a clinically isolated syndrome (high risk of conversion to MS) as well as subjects with clinically definite relapsing-remitting according to published criteria (50)
- Diseases with similar clinical neurological symptoms (e.g. lues, borreliosis, kollagenosis or vasculitis) have been excluded by differential diagnostics
- Subjects able of giving informed consent
- Signed informed consent
- EDSS score between 0.0 and 5.5, inclusive.

- Patients are clinically stable, i.e. without relapse and not having received steroids within 30 days prior to inclusion
- Patients have either failed standard treatment (interferon beta, glatiramer acetate) by clinical measures or were not eligible for any of the standard treatments available or opted not to start or to continue with any of these treatments
NOTE: The decision not to start or not to continue with any of the standard treatments has to be made by the patient after discussion with an independent neurologist not involved in this study and has to be signed in the informed consent.

* Individuals above 65 years of age and minors will not be included in the study since no experience about the side effect profile of BOSWELAN exists for minors and since MS patients with > 65 years of age are extremely rare, and the disease at this age is often mitigated by other concomitant diseases, e.g. cardiovascular disease.

5.3 Eligibility Criteria for Initiating Therapy

To be eligible to proceed to the treatment phase of the study (Stage 1-4), subjects must have an average of at least 0.5 Gd-enhancing lesions per month over the 4 month pre-treatment baseline period.

Subjects must not have a relapse during 30 days before initiation of treatment. If a relapse occurs during the last 30 days of the pre-treatment baseline period and eligibility MRI criteria are fulfilled, beginning of the treatment (day 1) is delayed for at least so many days that treatment starts not earlier than 30 days after the relapse and not earlier than 60 days in case i.v. corticosteroids had been given.

5.4 Exclusion Criteria

Candidates will be excluded from study entry (pre-treatment screening) if any of the following exclusion criteria exist at the time of enrollment:

Medical History

Abnormal screening/pre-treatment blood tests will be carefully evaluated in any case by the responsible physician. Blood tests exceeding any of the limits defined below will definitely lead to exclusion from the study:

- ALT (SGPT) or AST (SGOT) > three times the upper limit of normal
- Total white blood cell count < 3,000/mm³
- Platelet count < 85,000/mm³
- Creatinine > 1.5 mg/dl

- Serology indicating active hepatitis B or C infection or other chronic liver disease
- Positive pregnancy test, or breast-feeding female
- Nausea/vomiting as a frequent complaint
- History or signs of immunodeficiency
- Concurrent, clinically significant (as determined by the investigator) cardiac, immunological, pulmonary, neurological, renal, and/or other major disease

Treatment History

If prior treatment was received, the subject must have been off treatment for the required period prior to first investigational drug dose (see Table 2).

Table 2: Restrictions on pre-treatments

Agent	Time required off agent prior to enrollment
Glatiramer acetate (Copaxone™), Interferon beta (Betaferon™, Avonex™, Rebif™)	12 weeks
IV Ig, Azathioprine (Imurek™), Methotrexate, Cyclophosphamide (Cytoxin™), Mitoxantrone, plasma exchange, Cyclosporine, oral myelin, Cladribine, natalizumab, and other immunosuppressive treatments	24 weeks
Corticosteroids, ACTH	8 weeks

Prior treatment with any other investigational drug or procedure for MS will be evaluated individually by the investigators.

Miscellaneous

- History of alcohol or drug abuse within the 5 years prior to enrollment
- Female subjects who are not post-menopausal or surgically sterile who are not using an highly effective method of birth control. Highly effective is defined as having a failure rate of <1%. Written documentation that the subject is post-menopausal or surgically sterile must be available prior to study start
- Unwillingness or inability to comply with the requirements of this protocol including the presence of any condition (physical, mental, or social) that is likely to affect the subject's returning for follow-up visits on schedule
- Previous participation in this study
- Participation in other pharmaceutical trials during this study or 3 months before
- Patients hospitalized due to juridical or legal regulation
- Known hypersensitivity to BA
- Known contraindications for MRI examinations including hypersensitivity to gadolinium, severe renal insufficiency, a mechanical heart valve or any kind of metallic implants
- Patients with difficulties swallowing up to 12 capsules per day should not be recruited for screening.

6. Study Medication, Description and Allocation

Study drug must be stored in a secure location.

6.1 Study Drug

BOSWELAN will be provided as gelatine capsules containing 400 mg of the frankincense extract.

6.2 Enrollment Procedure

Subjects will be enrolled following completion of inclusion and exclusion evaluation, and enrollment will be documented at this point in the patient's file. The patient identification number will serve as identifier for each patient.

6.3 Drug Accountability

Accurate records will be kept with respect to documenting dates and amount of study drug received, to whom dispensed (subject-by-subject accounting), and

accounts of any study drug accidentally or deliberately destroyed. All unused capsules must be saved for drug accountability.

7. Therapy

7.1 Treatment Schedule

BOSWELAN is provided as capsules containing 400 mg of the frankincense extract.

Patients must be advised to swallow the capsules with a full glass of liquid before or during a meal.

Definition of “not tolerated”: Persisting moderate adverse events at the next dosing time point or whenever the investigator feels that the dose has to be (temporarily) reduced or dosing be omitted.

Definition of “well tolerated”: No or mild adverse events which have resolved before the next dose. Since the definition of the highest well tolerated dose will in most cases be done retrospectively after first moderate or severe adverse events occurred, establishing the highest well tolerated dose will include at least one dose above this dose level. Whenever the definition of “not tolerated” is fulfilled, the dose will be reduced until the “well tolerated” dose is established.

“Dose” is defined as any daily dosing regimen which also allows for different number of capsules at the 3 dosing time points per day.

BOSWELAN will be titrated up to the maximum well tolerated dose or to a maximum of 1600 mg t.i.d (whatever occurs first). The dose titration period will occur over 28 days (stage 1). After determining the maximum well-tolerated dose, each patient will be continued on this dose for another 28 days for dose tolerability and stabilization (stage 2), followed by additional 6 months of treatment (stage 3). Subsequent adjustments to the dose will be made as necessary. Patients are offered to continue drug intake after completing stage 3 during the follow-up stage.

The titration rules for the dose finding period stage 1 of the study are as follows:

A dose increase will be performed only if the previous dose was sufficiently tolerated, i.e. no adverse event or only mild adverse event(s), which has resolved at the next dosing time point.

If an increase in dose is not tolerated, the dose will be temporarily reduced. There should be no more than 3 temporary dose reductions during the dose escalation phase, i.e. after a third dose reduction the dose will be fixed at that or a lower well tolerated dose level.

7.2 Dose Titration Scheme

The dose unit for the following titration scheme has been set as 400 mg/capsule. Dosing is at morning, midday and evening with at least 6 hours between doses.

BOSWELAN will be titrated up to the highest well tolerated dose or to a maximum of 3 x 1600mg during Stage 1.

The dose titration period will be up to 28 days. The dose reached at that time point should be the maximum dose that is well tolerated.

Titration scheme: The dose unit has been set as 400 mg/capsule.

Days	Dosing schedule	Special measures
0	1-0-0	
1:	1-1-1	phone contact on Day 1 before morning dose
2-3	1-1-2	
4-6:	2-1-2	phone contact on Day 4 before morning dose
7-9:	2-2-2	
10-12:	2-2-3	
13-15:	3-2-3	phone contact on day 13 before morning dose
16-18:	3-3-3	
19-21:	3-3-4	
22-24:	4-3-4	
25-28:	4-4-4	

It is expected that adjustments to this schedule will be needed in the event that side effects occur or if dose reductions are necessary. However, patients should remain on each new dose for a minimum of 3 days before the next increase.

Any re-increase of dose after a dose reduction due to an adverse event requires consultation by phone with either the treating physician or the study nurse. In case of a serious adverse event (SAE), dosing will be stopped until the SAE has resolved. Dosing will then resume at a reduced dose level. If dosing is stopped for greater than 2 weeks, the study medication will be stopped in that patient and the patient will be withdrawn prematurely from the study.

Any intolerance of moderate or severe degree must be reported by the patient to either to the treating physician or a study nurse by phone.

The first dose will be administered in the respective Clinical Center. Each patient will contact the study nurse on days 1 (before morning dose), 4 (before morning dose), and on day 13 (before morning dose). Patients will be seen at the center

on days 0 and day 28 and the morning dose will be administered at the center on these days (in case of participating in the pharmacokinetic sub-study).

Treatment of adverse events is at the investigator's discretion.

7.3 Discontinuation of Treatment

If patients are not able to swallow the capsules with water capsules might be put in warm water and solved which takes about 10-15 minutes. As the extract is liquid and the capsule water-solvable this procedure guarantees that the whole amount of the extract is ingested. There is no indication from the manufacturer Alpinia that this procedure will lead to changed pharmacokinetics. However, to avoid heterogeneity within the treated cohort this procedure will be restricted to single cases.

7.3.1 Subject Withdrawal from the Study

A subject *must* permanently discontinue study drug treatment and *must* be withdrawn from the study prematurely for any of the following reasons:

The subject becomes pregnant. Treatment must be *immediately* discontinued. The pregnancy must be reported to the study center (study coordinator/treating physician/PI) immediately. Information about the subject, the subject's pregnancy, the outcome of the pregnancy, and the status of the infant at 8 to 12 weeks of age will also be collected.

The subject desires to discontinue study drug treatment under this protocol. Patients have the right to withdraw from the study at any time and for any reason. The investigator also has the right to withdraw patients from the study in the event of an intercurrent illness, incompliance after a prescribed procedure, protocol violations, administrative reasons, other reasons.

The subject receives or wishes to start any restricted medication listed in table 2 except corticosteroids.

At the discretion of the investigator for medical reasons or for non-compliance.

The study will be prematurely terminated for an individual if an exclusion criteria occurs during the study.

Dosing in stage 1 has to be discontinued in case of a SAE. If dosing is stopped for greater than 2 weeks, the study medication will be stopped in that patient and the patient will be withdrawn prematurely from the study.

The reasons for discontinuation of the study drug must be recorded in the subject's Clinical Center patient file and the respective case report forms (CRFs).

Patients discontinuing dosing will be followed for an additional 2 months. If discontinuation was because of a serious adverse event, the patient will be followed until the event is resolved or stabilized, but not shorter than 2 months.

7.3.2 Stopping Rules for Individual Patients and for the Study

7.3.2.1 Stopping Rules for Individual Patients

MRI Safety Criteria

Regarding stopping rules, we apply stipulations that we have previously applied in other trials of this kind (39, 40). Dosing will be discontinued in patients showing an increase in total Gd-enhancing lesions beyond 6 standard deviations of the mean lesion frequency during baseline plus 10 lesions. The maximum lesion number allowed, before treatment will be stopped, is 40 Gd-enhancing lesions. The upper margin (40 Gd-enhancing lesions) stems from a systematic review of the numbers and distribution of contrast-enhancing lesions derived from the placebo cohorts of pivotal phase III trials, which was performed by the Sylvia Lawry Center for MS Research (M. Daumer and colleagues). Furthermore, if in the opinion of the investigators there is a change in appearance of enhancing lesions inconsistent with the patient's previous MRIs, e.g. change in average lesion size from small to substantially larger lesions, dosing will be discontinued.

Clinical Safety Criteria

Two clinical safety criteria will also be used as stopping criteria for each individual patient:

Clinical criterion 1 - relapses: Two or more confirmed relapses/exacerbations. A relapse/exacerbation is defined as the appearance of any new symptom or worsening of previous symptoms associated with significant changes in signs lasting longer than 24 hours and not accompanied by a rise in temperature or infection ("pseudoattack" modified after Schumacher (51)).

Single paroxysmal events (such as a tonic spasm) do not constitute a relapse, but multiple episodes occurring over not less than 24 hours do.

Treatment of relapses will be conducted according to the recommendations of the German Neurological Society (DGN)(section 7.5.3).

or

Clinical criterion 2 - progression: Worsening of two or more points on the EDSS scale for 3 months during the treatment phase, compared to baseline in the first 3 months before treatment. For patients with an EDSS score of 5.5 at study entry, a worsening of one point on the EDSS scale for 3 months during the treatment phase will lead to halt of study treatment.

Patients, who discontinue study medication due to one of the above mentioned stopping rules, will be advised to start on one of the established disease-modifying treatments according to the recommendations of the German Neurological Society (DGN).

7.3.2.1 Stopping Rules for the Study

MRI Safety Criteria

The trial will be put on hold if the following MRI safety concerns arise:

1. Increase in total CELs beyond 6 standard deviations, plus 10 lesions, of the individual mean lesion frequency during baseline. This criterion applies to patients with low baseline inflammatory MRI activity.
2. The maximum number of new lesions allowed at one MRI exam in an individual patient before the trial will be put on hold is 40 lesions.
3. If in the opinion of the investigators, there is occurrence of atypical non MS-like lesions or MRI lesions that are unusual for the individual patient dosing will be discontinued and the patient will be prematurely withdrawn from the study. The latter consideration is up to the judgement of the trial neuroradiologist and, if such concerns should arise, will be discussed with the DSMB within 48 hours of the respective MRI scan.

Clinical Safety Criteria

The entire trial will be put on hold if the following clinical safety concerns occur:

1. Relapses: Two or more relapses/exacerbations during the treatment phase (stage 1-3) in the first 3 months post treatment in more than 1 of the first 4 participants, more than 2 of the first 8 participants, and 3 or more participants in the entire group.
2. Progression: Worsening of two or more points on the EDSS scale, during the treatment phase, compared to baseline in the same number of patients as under (1). The change in EDSS must be confirmed and consistent through three monthly visits.
3. All serious adverse events and adverse events will be reported within 3 months to the data safety monitoring board (DSMB), which will decide on the discontinuation of the study.

If dosing had to be discontinued in five patients because of MRI parameters or in the number of patients as in 1 of clinical safety criteria because of clinical parameters the study will be placed on clinical hold, i.e. treatment with BOSWELAN of all patients already in the study will be discontinued and no additional patients will be recruited until the data from the trial has been reviewed by the DSMB.

If any safety issue leads to concerns to continue treatment with BOSWELAN in the MS or CIS patients of this study or if any safety issue of other clinical studies with BOSWELAN raises such concerns, the study will be put on hold or be terminated. The study DSMB will review the safety and efficacy data on a regular basis.

7.4 Compliance

A record of the dose titration and of deviation from prescribed treatment will be kept in the patients' file and in the respective CRFs.

7.5 Concomitant Therapy

Concomitant therapy will be continued throughout the study. Drugs used as concomitant therapy and dose should be kept unchanged throughout the study whenever possible. Treatment of side effects, concurrent diseases, and dose adaptations of baseline concomitant therapy are generally allowed but have to be documented accordingly. Concomitant therapy will be documented in the patients' file and in the respective CRFs.

7.5.1 Symptomatic Therapies

Symptomatic therapy, such as treatment for spasticity, depression, bladder dysfunction, or fatigue is not restricted, but should be optimized as early as possible during screening/pre-treatment in an attempt to maintain consistent treatment for the duration of the study.

7.5.2 Therapies not allowed during Treatment

Any of the drugs mentioned in table 2 except corticosteroids are not allowed during the study participation. Before any treatment except the study drug is initiated, the patient or her/his outside physician should contact the study center to seek advice, if any safety issue might arise. This does not hold true for emergency cases.

7.5.3 Treatment of Relapses on Scheduled or Unscheduled Visits

Patients having a clinically significant relapse/exacerbation will be treated according to the recommendations of the German society for neurology (DGN) with 1000 mg methylprednisolone, given once a day via i.v. infusion for 3 days unless there are valid reasons for not treating or for alternative schedules. In case of a relapse each patient has to give written informed re-consent before continuing the study irrespective of his/her choice to receive methylprednisolone. In the case of methyprednisolone administration, a subsequent MRI examination will be postponed for at least 28 days, while treatment with BOSWELAN and study visits will be continued according to the study protocol.

7.5.4 Recording Concomitant Medication

Any medication and any non-drug procedure or therapy utilized from the start of study drug treatment until the completion of the study must be recorded in the subject's case report forms (CRFs) independent of the continuation of treatment with the study drug.

7.6 Treatment after study termination

Patients will be offered to visit the study centre in individually agreed intervals.

8. Schedule of Events, Tests, and Evaluations (see Study Flow Chart in the appendix)

Unless otherwise specified, the tests and evaluations described below for the screening visit must be performed within 7 days prior to the subject's first MRI in order to determine subject eligibility.

9. Efficacy Assessments

9.1 MRI Efficacy Assessments

See section 3.1 and 3.2

If a therapy with corticosteroids becomes necessary due to a relapse/exacerbation, the MRI has to be performed before the beginning of the corticosteroid therapy (but at least 28 days after the preceding MRI) or at least 28 days after the end of the corticosteroid therapy. The following MRIs have to be delayed accordingly.

10 SCHEDULE OF EVENTS

10.1 Tests and evaluations

Unless otherwise specified, tests and evaluations described below for the screening visit must be performed within seven days prior to the patient's first MRI. Blood samples should be drawn as early in the day as possible, preferably at the same time of day to avoid diurnal variation and before any steroids are taken in order to avoid depletion of lymphocytes by these agents.

In the event of an MS relapse, a MRI scan will be obtained prior to initiation of steroid therapy. The next scheduled MRI scan will be delayed until at least 28 days after the end of steroid therapy. Subsequent MRI scans will occur at regularly scheduled patient visits.

Additional non-pharmaceutical studies carried out at the discretion of the investigator when deemed appropriate for optimal management of the patient must also be reported.

See Study Schedule Flow Chart (App. A) for summary of tests and evaluations by time-point.

Month -3 (Screening Visit)

Unless otherwise specified, the tests and evaluations described below must be performed within seven days prior to the patient's first MRI in order to determine

patient eligibility:

- A complete medical history.
- A complete MS history.
- Inclusion and exclusion criteria.
- A complete physical examination.
- Vital signs and body weight.
- Haematology: see 11.3.
- Blood chemistries: see 11.3.
- Hepatitis B and C Screening or Check for recent Hepatitis B and C Screening: see 11.3.
- Urinanalysis: see 11.3.
- Urine pregnancy test for women of child-bearing potential.
- Recording of concomitant medication.
- EDSS, SNRS, MSFC.
- HAQUAMS, MSIS29, HADS, Fatigue Severity Scale, HARAS for each Relapse within the last 12 months
- Cranial MRI.

Month -2 (\pm 7 days)

- Monitoring of adverse events.
- Vital signs and body weight.
- Urine pregnancy test for women of child-bearing potential.
- Recording of concomitant medication.
- Cranial MRI.
- EDSS, SNRS

Month -1 (\pm 7 days)

- Monitoring of adverse events.
- Vital signs and body weight.
- Urine pregnancy test for women of child-bearing potential.
- EDSS, SNRS, MSFC.
- Recording of concomitant medication.
- Cranial MRI.

Month 0 (\pm 7 days)

- Cranial MRI (within 7 days prior to study drug administration).
- Inclusion and exclusion criteria.
- Monitoring of adverse events.
- Complete physical examination.
- Vital signs and body weight.
- Urine pregnancy test for women of child-bearing potential.
- Haematology: see 11.3.
- Blood chemistries: see 11.3.
- Urinalysis: see 11.3.
- Immunological measures.
- Biomarker Assessment.
- EDSS, SNRS, MSFC.
- HAQUAMS, MSIS29, HADS, Fatigue Severity Scale
- Recording of concomitant medication

- Study drug administration followed by a 2 hour observation period

Day 1 (\pm 1 days)

- Monitoring of adverse events (Telephone call).

Day 4 (\pm 2 days)

- Monitoring of adverse events (Telephone call).

Day 13 (\pm 2 days)

- Monitoring of adverse events (Telephone call).

Month 1 (\pm 7 days)

- Monitoring of adverse events.
- Complete physical examination.
- Vital signs and body weight.
- Haematology: see 11.3.
- Blood chemistries: see 11.3.
- Urinalysis: see 11.3.
- Immunological measures.
- Biomarker Assessment.
- Urine pregnancy test for women of child-bearing potential.
- EDSS, SNRS, MSFC.
- HAQUAMS, MSIS29, HADS, Fatigue Severity Scale
- Recording of concomitant medication
- Study drug administration
- Cranial MRI.

Month 2 (\pm 7 days)

- Monitoring of adverse events.
- Complete physical examination.
- Vital signs and body weight.
- Haematology: see 11.3...
- Blood chemistries: see 11.3..
- Urinalysis: see 11.3..
- Urine pregnancy test for women of child-bearing potential.
- Recording of concomitant medication
- Cranial MRI.
- EDSS, SNRS

Month 3 (\pm 7 days)

- Monitoring of adverse events.
- Complete physical examination.
- Vital signs and body weight.
- Haematology: see 11.3.
- Blood chemistries: see 11.3..
- Urinalysis: see 11.3..
- Urine pregnancy test for women of child-bearing potential.
- Immunological measures.
- Biomarker Assessment.

- EDSS, SNRS, MSFC.
- HAQUAMS, MSIS29, HADS, Fatigue Severity Scale
- Recording of concomitant medication.
- Cranial MRI.

Month 4 (± 7 days)

- Monitoring of adverse events (Telephone call).
- EDSS (Telephone call)

Month 5 (± 7 days)

- Monitoring of adverse events.
- Vital signs and body weight.
- Haematology: see 11.3..
- Blood chemistries: see 11.3..
- Urinalysis: see 11.3..
- Urine pregnancy test for women of child-bearing potential.
- Recording of concomitant medication.
- Cranial MRI.
- EDSS, SNRS, MSFC.
- HAQUAMS, MSIS29, HADS, Fatigue Severity Scale

Month 6 (± 7 days)

- Monitoring of adverse events.
- A complete physical examination.
- Vital signs and body weight.
- Haematology: see 11.3..
- Blood chemistries: see 11.3..
- Urinalysis: see 11.3..
- Urine pregnancy test for women of child-bearing potential
- EDSS, SNRS, MSFC.
- HAQUAMS, MSIS29, HADS, Fatigue Severity Scale
- Recording of concomitant medication.
- Cranial MRI.

Month 7 (± 7 days)

- Monitoring of adverse events.
- Vital signs and body weight.
- Haematology: see 11.3..
- Blood chemistries: see 11.3..
- Urinalysis: see 11.3..
- Urine pregnancy test for women of child-bearing potential.
- EDSS, SNRS, MSFC.
- Recording of concomitant medication.
- Cranial MRI.

Month 8 (± 7 days)

- Monitoring of adverse events.
- A complete physical examination.
- Vital signs and body weight.
- Haematology: see 11.3..

- Blood chemistries: see 11.3..
- Urinalysis: see 11.3..
- Urine pregnancy test for women of child-bearing potential.
- Immunological measures.
- Biomarker Assessment.
- EDSS, SNRS, MSFC,
- HAQUAMS, MSIS29, UNDS.
- Recording of concomitant medication.
- Cranial MRI.

* Optional for patients choosing to continue treatment with BOSWELAN:

- **Month 9 (\pm 7 days) ***
 - Monitoring of adverse events (Telephone call)
 - EDSS (Telephone call)
- **Month 10 (\pm 7 days) ***
 - Monitoring of adverse events (Telephone call).
 - EDSS (Telephone call)
- **Month 11 (\pm 7 days) ***
 - Monitoring of adverse events (Telephone call).
 - EDSS (Telephone call)

Extension: Month 12 (\pm 7 days) / Early Termination

- Monitoring of adverse events.
- A complete physical examination.
- Vital signs and body weight.
- Haematology: see 11.3..
- Blood chemistries: see 11.3..
- Urinalysis: see 11.3..
- Urine pregnancy test for women of child-bearing potential.
- Immunological measures.
- Biomarker Assessment.
- EDSS, SNRS, MSFC.
- HAQUAMS, MSIS29, HADS, Fatigue Severity Scale
- Recording of concomitant medication.
- Cranial MRI.

Unscheduled Visits / Relapse

In case of Relapse mandatory

- Monitoring of adverse events.

- Vital signs and body weight
- Recording of concomitant medication.
- Cranial MRI
- EDSS, SNRS, MSFC.
- HAQUAMS, MSIS29, HADS, Fatigue Severity Scale
- HARAS
- Other procedures as indicated by the investigator

Relapse follow-up

- Monitoring of adverse events.
- Vital signs and body weight
- Recording of concomitant medication.
- EDSS, SNRS, MSFC.
- HAQUAMS, MSIS29, HADS, Fatigue Severity Scale
- HARAS
- Other procedures as indicated by the investigator

All other unscheduled visits including unscheduled drug dispensation

- Monitoring of adverse events.
- Recording of concomitant medication.
- Other procedures as indicated by the investigator

11. Safety Assessments

11.1 Clinical Safety Assessments

Clinical safety will be assessed by neurological examination including standardized rating scales for multiple sclerosis (EDSS (59), SNRS (60), MSFC (61)), relapse rate, general physical examination, and measurement of vital signs (temperature, heart rate, and blood pressure). Adverse events will be collected throughout the study.

11.2 MRI Safety Assessment

see section 7.3.2

11.3 Laboratory safety assessments

The specific laboratory parameters to be evaluated in this study are as follows:

- Blood chemistry: sodium, potassium, chloride, calcium, phosphate, creatinine, uric acid, urea, total bilirubin, GGT, ALT/GPT, AST/GOT, alkaline phosphatase, glucose, total protein, and albumin, lactate dehydrogenase, Fibrinogen, CRP, CK.
- Haematology: complete blood count with differential and platelet count, and coagulation studies (PT and PTT).

- Urinalysis: specific gravity, pH, protein, glucose, blood, Ketone, Nitrite, leucocytes and including a pregnancy test for women of child-bearing potential.
- Hepatitis Serology: will either be tested at screening or in case the patient has already been tested for Hepatitis during the last 12 months, these test results will be accepted.

12 Adverse Events

12.1 Adverse Events: Definition and management

The terms “relationship”, “serious” and “severity” used in this section, are defined in Table 3.

For the purposes of this study, adverse events are defined as any signs (including the clinical manifestations of abnormal laboratory results) or medical diagnoses noted by medical personnel, or symptoms reported by the patient, regardless of relationship to study drug, that:

- a) Have onset anytime after the first visit.

OR

- b) Have worsened since the event was previously reported (this includes worsening of signs, symptoms, or diagnoses that were present prior to dosing).

All adverse events reported by the patient or observed by study site personnel from the first visit until completion of a patient's participation in the study must be recorded in the patient's file and CRFs. Serious Adverse events are to be recorded regardless of relationship to the study protocol and reported to the Sponsor.

The information to be recorded in the patient file will include, but not be limited to, relationship of the event to study drug and severity of the event. Specific instructions for recording adverse events will be provided under separate cover and will be reviewed by the study site personnel.

Patients will be given the name and phone number of personnel at the study site that can be called in the event of an emergency or to report an adverse event that is of concern to the patient. The contact information will also be displayed in the patient consent form.

Any serious adverse event (SAE, for definition see table 3) occurring during any time of the study (baseline and treatment phase) will be reported by the investigator to the Sponsor (here his deputy: the PI) immediately, but no longer than 24 hours after it becomes known.

The Sponsor will document all Severe Adverse Events (SAEs) reported by the Investigator.

The Sponsor will report all SAEs and all adverse events to the DSMB every 3 months.

The Sponsor will report all Suspected Unexpected Serious Adverse Reactions (SUSARs) to the relevant Ethics Committees and the relevant Competent Authorities at the latest 15 days after it becomes known. He will also inform all Investigators involved in the trial and the DSMB.

In case of a fatal or life threatening SUSAR the Sponsor will report all information relevant for judging the event immediately, at the latest 7 days after the event becomes known to the relevant Ethics Committees and the relevant Competent Authorities, as well as to all Investigators involved in the trial and the DSMB.

The Sponsor will further report immediately, at the latest 15 days after it becomes known, all circumstances that require a revision of the risk-benefit analysis of the investigational product to the above mentioned authorities. This especially includes:

- Singular cases of expected severe adverse events with an unexpected outcome
- Increased incidence of expected severe adverse events that are judged as being clinically relevant
- SUSARs which occur after termination of the clinical trial (max. 2 weeks after termination or exclusion)
- Events related to study procedures or development of the study medication, which could affect a subject's safety

The Sponsor will once a year submit the annual safety report to the Competent Authoritie(s).

All person-related data will always be transmitted pseudonymised. Before reporting a SUSAR the subject will be unblinded.

Exception rules for the reporting:

In this clinical trial the following Serious Adverse Events are excluded from reporting:

- Severe or unexpected events which occur after screening or enrolment, but before first application of the investigational drug
- Hospital admissions for the purpose of diagnostic or therapeutic purposes which were planned before study inclusion
- Expected serious events, which could occur because of the administration of the investigational product and represent known effects of the investigational product.
- Hospital admissions for the purpose of treating a relapse

Table 3. Adverse Event Definitions

Serious Adverse Event	
Serious adverse events are defined as the following:	
<ul style="list-style-type: none"> ▪ Any death. ▪ Any life-threatening* event, i.e., an event that places the subject, in the view of the investigator, at immediate risk of death from the event as it occurred (does not include an event that, had it occurred in a more severe form, might have caused death). ▪ Any event that requires or prolongs in-patient hospitalization. ▪ Any event that results in persistent or significant disability/invalidity. ▪ Any congenital anomaly/birth defect diagnosed in a child of a subject who participated in this study and received study drug. 	
<p>* The term "life-threatening" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event, which hypothetically might have caused death if it were more severe.</p> <p>Important medical events that may not be immediately life-threatening or result in death, disability or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above should also usually be considered serious.</p>	
Relationship of Adverse Event to Study Drug	
Not related	Any reaction that does not follow a reasonable temporal sequence from administration of study drug <i>AND</i> that is likely to have been produced by the subject's clinical state or other modes of therapy administered to the subject.
Unlikely	Any reaction that does not follow a reasonable temporal sequence from administration of study drug <i>OR</i> that is likely to have been produced by the subject's clinical state or other modes of therapy administered to the subject.
Likely	A reaction that follows a reasonable temporal sequence from administration of study drug <i>OR</i> that follows a known response pattern to the suspected drug <i>AND</i> that could not be reasonably explained by the known characteristics of the subject's clinical state or other modes of therapy administered to the subject.
Definite	A reaction that follows a reasonable temporal sequence from administration of study drug <i>AND</i> that follows a known response pattern to the suspected drug <i>AND</i> that recurs with re-challenge, and/or is improved by stopping the drug or reducing the dose.
Severity of Adverse Event	
Mild	Symptom(s) barely noticeable to subject or does not make subject uncomfortable; does not influence performance or functioning; prescription drug not ordinarily needed for relief of symptom(s) but may be given because of personality of subject.
Moderate	Symptom(s) of a sufficient severity to make subject uncomfortable; performance of daily activity is influenced; subject is able to continue in study; treatment for symptom(s) may be needed.
Severe	Symptom(s) cause severe discomfort; symptoms cause incapacitation or significant impact on subject's daily life; severity may cause cessation of treatment with study drug; treatment for symptom(s) may be given and/or subject hospitalized.

12.2 Management

All adverse events reported by the subject or observed by the study site personnel from the start of the study until completion of a subject's participation

in the study (when all of the required post-dosing evaluations are completed) must be recorded in the subject's Clinical Center patient file.

Adverse events are to be recorded regardless of their relationship to the study drug.

All adverse events must be followed until the event resolves or until the subject's clinical course has stabilized, irrespectively of continuation of the study drug or the pre-specified observation period. This follow-up is also necessary if laboratory or clinical or MRI findings suggestive of a safety problem are noticed which do not fulfill the criteria of an adverse event.

12.3 Emergency procedures

In the event of a medical emergency (i.e., an event that requires immediate attention regarding the treatment of the subject, operation of the clinical study), the principle investigator will be contacted at

for Hamburg:

Institute of Neuroimmunology and Clinical MS Research, ZMHN, Tel.: +49 (0)40-74105-4076 or the inms clinical trial emergency cell phone Tel.: +49 (0)40- 74105- 8612 (24h hotline)

for Berlin:

NeuroCure Clinical Research Center, Charité Universitätsmedizin Berlin, Tel: +49 (0) 30 450 539 040 / 639 057 or the 24h-cell phone +49 (0)152-5466-2428

The investigator must ensure that all study site personnel responsible for the subject's medical care are familiar with the Medical Emergency Call number and its location and have access to it.

13 Statistical Statement and Analytical Plan

13.1 Sample Size Considerations

Based on analyses of a natural history cohort studied with monthly MRIs for a minimum of one year, a baseline to treatment designed trial incorporating 4 baseline MRIs and 4 treatment MRIs will require 12 patients to detect a 60% reduction in Gd-enhancing lesions with an alpha of 0.05 (one-sided). Regarding BA we expect an efficacy of 40% reduction of inflammatory lesions (Gd-enhancing) and estimate that 30 patients will be required to detect a 40% reduction with a power of 0.8 and an alpha of 0.05 (one-sided). Sample size estimation is based on published statistical simulations (64).

13.2 Background/Baseline Data

All appropriate background data will be summarized by presenting frequency distributions and/or basic summary statistics (mean, standard deviation, median, minimum, and maximum).

13.3 Evaluation of Safety

The safety population is defined as all subjects who received at least one dose of study drug and have at least one post-baseline assessment of the safety parameter available.

The primary objective of this study is to determine the safety and tolerability of BOSWELAN therapy in subjects with multiple sclerosis using clinical, MRI, and laboratory measures.

13.3.1 Adverse Events

The incidence of all adverse events reported by the investigator and/or the subject will be tabulated by severity and relationship to treatment. Adverse events resulting in treatment discontinuation or dose reduction will be identified. All adverse events will be documented according to the directions given in the respective CRFs.

13.3.2 Laboratory Data

Laboratory evaluations will be assessed to determine incidence of clinically notable abnormalities that emerge during the course of the study.

13.3.3 Clinical Safety Measures

The clinical measures are the changes in EDSS, SNRS, MSFC, and relapse rate. EDSS score will be obtained monthly as long as patients are treated with BOSWELAN either clinically or by telephone according to (62). The analyses will follow the same sequence of analyses as described for MRI. Comparison will always be made between baseline (week 0) and month 8 or earlier if treatment is stopped early.

14. Evaluation of Efficacy

Three efficacy populations will be analyzed for the primary outcome measures total Gd enhancing lesions and lesion volume:

The primary outcome in Stage 2 will first compare lesion (Gd-contrasting lesions) frequency (mean number of lesions) and area occurring during the 4 month baseline to lesion frequency and area occurring during months 5, 6, 7, 8 on treatment in patients treated with a dose of at least 800 mg t.i.d.

A secondary analysis will compare lesion frequency occurring over the 4 months of baseline to that occurring during over all treatment months.

An intention-to-treat analysis will be performed with all patients, all time on study.

Wilcoxon signed-rank test will be used for analysis of the primary endpoints.

15. POTENTIAL RISKS / DISCOMFORTS AND BENEFITS TO PATIENTS

Direct benefits for single patients

BOSWELAN is an orally available anti-inflammatory drug that has a very good safety profile as has been already shown pre-clinically and in clinical studies (see 2.4 and 2.5). For patients that opt not to start on one of the established treatments of multiple sclerosis due to the mode of application by injection or infusion and/or fear of the side effect profiles of these treatments, BOSWELAN offers an alternative treatment. The study design without placebo allows treatment for all included patients. The study duration of up to 12 months makes it highly unlikely that if BOSWELAN shows no effect patients will have a substantial disadvantage through the delay for initiation of a licensed drug. A highly well tolerated drug for patients with only moderate to low disease activity is still missing. BOSWELAN might meet this unmet need.

Indirect benefits for clinical trial development in MS

We expect that BOSWELAN will decrease the average number of monthly contrast-enhancing MRI lesions by 40% or greater (primary outcome). While in established treatments in MS monitoring for dose finding and responder profiling is an issue of recent research, well known biomarkers for the pharmacodynamics of BAs activity (i.e. Cat G and PGE₂) exist, that can be useful for dose finding and responder profiling. With this study a putative highly effective clinical screening design for investigational drugs will be further developed. We aim at paralleling primary endpoint MRI analysis with mechanistic studies and further development of more disease-prognosis associated MRI markers.

Scientific benefits

BOSWELAN offers a new therapeutic approach through a new mode of anti-inflammatory action by single- and dual inhibition of three enzymes (Cat G, 5-LO and mPGES) that has so far not been looked at in the context of multiple sclerosis in spite of evidence for a role of these enzymes in neuroinflammation (see 2.2). The study will clarify the relevance of these mechanisms in MS/CIS.

Commitment of time

Monthly visits require approximately 3 hours a month, at first drug administration 2 further hours have to be spent at the study center.

Risks of MRI

Gadolinium is as a contrast agent for use with MRI approved by the EMEA. No serious side effects have been associated with its use. Approximately 5-10 percent of patients develop transient headaches following administration, but it is not clear whether the headaches are associated with the drug. The effect of gadolinium on the developing fetus remains partly unknown. Animal studies have shown a delay in development but no developmental abnormalities. Consequently, women of childbearing potential will be entered into this study only if a highly effective method of birth control is in use. A pregnancy test will be done prior to beginning the study and monthly pregnancy tests will be done before MRIs are performed. The risk of nephrogenic systemic fibrosis in

increased in patients with profoundly impaired renal function (glomerular filtration rate <30mL/minute).

Risks of treatment

With respect to the clinical use of BAs, more than 500 patients (with different inflammatory, mainly autoimmune diseases) have been treated in clinical studies, and no serious adverse events have been reported from any of the clinical trials (49). The risk of therapy can therefore be considered reasonably low.

Hypersensitivity: In pre-clinical experiments the use of BOSWELAN did not show any risk of anaphylaxis. Cytokine response to the drug product has not been observed in animals, neither did any severe adverse events occur during treatment in clinical studies. Patients will be excluded if a hypersensitivity to frankincense is reported and will be monitored for two hours after the first drug administration at the study center which is equipped to provide immediate adequate medical measures in case anaphylaxis occurs.

Risk of hepatotoxicity: In animal toxicity studies indications for a liver toxic influence of Boswellic acids at very high doses (46) were observed (see 2.4). Although patients in this study receive a maximum dose of more than 50fold less than the doses that have been reported to show transient hepatotoxicity liver enzyme levels will be closely monitored and are one of the central safety outcome parameters.

Carcinogenicity: Carcinogenicity studies have previously not been considered necessary, as no reasons, e.g. chemical structure, previous demonstration of a carcinogenic potential in the product class that were considered relevant to man, or toxicological reasons including mutagenicity results, made such studies necessary (see 2.4). Moreover, frankincense extracts have already been used as antitumor agents in clinical cancer trials. The still ongoing discussion in regard to the genotoxic carcinogenicity and acceptable daily dose of estragole will be communicated to patients in a separate patient information as will be the need for further and more specific genotoxic carcinogenicity studies.

Reproductive and developmental toxicity: Reproductive and developmental toxicity were conducted in rats and rabbits (see 2.4). Testing of the effect on fertility, reproductive capacity and peri- and postnatal development was not conducted since there was no indication that the reproductive organs had been affected by the test substance.

No signs of embryotoxicity including malformations, variations and/or retardations at any of the tested dose levels, even at materno-toxic doses in rats. In rabbits the test substance possessed no teratogenic properties. A marginal embryotoxicity was noted only at the materno-toxic dose. The reproductive and developmental toxicity has therefore considered to be reasonably low. Nevertheless women of child-bearing potential have to sign an informed consent that they have to use acceptable methods of contraception.

16. Ethical Requirements

16.1 Subject Information and Consent

Prior to any testing under this protocol, including screening or pre-treatment tests and evaluations, written informed consent must be obtained from the subject in accordance with local practice and regulations. Study investigators will obtain informed consent.

Whenever possible, the primary physician will also be involved in this procedure. The background of the proposed study and the benefits and risks of the procedures and study will be explained to the subject. A copy of the informed consent document signed and dated by the subject and the investigator must be given to the subject. Confirmation of a subject's informed consent must also be documented in the subject's medical records prior to any testing under this protocol, including screening or pre-treatment tests and evaluations.

Information that is gained through this study will be available to the participating patients.

All patients will be informed about the transfer of their pseudonymic data according to the documentations- and reporting-duties of the Sponsor and investigators (see AMG § 12 und § 13). Patients not consenting to this transfer may not participate in the study (see patients' informed consent).

16.2 Alternative treatment

Currently four therapies are approved for the treatment of relapsing MS, interferon beta 1a (Avonex® or Rebif®), interferon beta 1b (Betaseron® or Extavia®), glatimer acetate (Copaxone®), and natalizumab (Tysabri®). Each of these treatments has been shown to reduce the frequency of relapses. While interferon beta-1a has been shown to delay progression in relapsing patients, no consistent effect on progression in patients with progressive MS has been demonstrated. In general, these therapies are approved for the treatment of patients, who have definite MS and who have had at least one relapse in the past year. Recent evidence indicates that treatment with interferon after an initial episode likely to be MS increases the time to a second relapse, which would confirm the diagnosis of definite MS. The risk of deferring conventional therapy would be that a relapse could occur that is not followed by complete recovery. In general, the risk would be higher in patients with a history of frequent relapses and with relapses that have not recovered completely. However, it needs to be noted that the approved therapies produce about a 30% reduction (60% for Tysabri) in relapse rate, so all relapses will not be prevented. There is no data, which address the potential risk of deferring initiation of approved therapy for short periods such as 1 year.

In addition to interferon beta and glatimer acetate, corticosteroids are used to treat acute episodes of worsening. Further, various forms of immunosuppressive, -modulatory therapy, i.e. mitoxantrone, natalizumab, cyclophosphamid and azathioprine are used by some physicians for patients having an aggressive course of disease.

Ethical issues relating to the entry of patients into a research study who are eligible for an approved therapy

Some patients recruited for the current study will be eligible for approved therapies. These will be patients who are still in the relapsing phase of the illness. Only those patients electing not to receive approved therapies independent of the current trial or those patients who have been found by their treating physician to have failed approved therapy will be considered eligible for entry. Consequently, the guidelines for entry of those patients will include the following:

1. Patients early in the course of MS who have not elected to receive approved therapy will have the indications for approved therapy and the benefits of that therapy discussed with the patient by the referring physician or by a physician at the MS outpatient unit at the UKE, Hamburg or the Charité Berlin not directly involved in the current study. This discussion will occur prior to discussion of the trial of BOSWELAN. Documentation of the patient's election to defer approved therapy will be documented in the medical record. Further, the informed consent will review the benefits of the approved therapies and the potential risk of deferring this therapy.
2. The patient together with the patient's treating physician outside the MS outpatient unit at the UKE (inims), Hamburg or the Charité, Berlin will make the determination that a patient has failed approved therapy. In general, treatment failure means that the patient has continued to have relapses at a rate similar to the frequency prior to treatment, that the patient is showing progression of disability or that the patients cannot tolerate the treatments. The reason for considering the patient a treatment failure will be documented in the medical record.

After discontinuation of treatment patients will be monitored for an additional four months (months 9-12) and be offered counselling regarding further treatment.

17. Optional Substudy on pharmacokinetic effects

To gain further insight into the dose-dependent pharmacokinetics of BOSWELAN and to see if there is a linear relationship between dose and plasma level, an optional pharmacokinetics substudy will be offered to a minimal of 5 and maximally 10 patients participating in the above mentioned study. These five to ten patients will be asked to donate blood samples during stage 2 of the study (dose titration) on the following days and at the described timepoints:

Days	Dosing schedule	Blood samples taken
Day 0	1-0-0	- pre-dose - 1/2 hrs. after intake - 1 hrs. after intake - 2 hrs. after intake - 3 hrs. after intake

		- 5 hrs. after intake - 8 hrs. after intake - 24 hrs. after intake
Day 9:	2-2-2	- pre-dose - 1 hrs. after intake - 2 hrs. after intake - 3 hrs. after intake
Day 18	3-3-3	- pre-dose - 1 hrs. after intake - 2 hrs. after intake - 3 hrs. after intake
Day 28	4-4-4	- pre-dose - 1 hrs. after intake - 2 hrs. after intake - 3 hrs. after intake
Month 6 (Steady State)	4-4-4	- pre-dose - 1 hrs. after intake - 2 hrs. after intake - 3 hrs. after intake

For elimination pharmacokinetics blood samples will be taken at the following timepoints after the patient took the last dose of BOSWELAN:

- immediately after last intake (0 hrs.)
- 1 hrs. after intake
- 5 hrs. after intake
- 8 hrs. after intake
- 24 hrs. after intake

Patients will be offered to receive remuneration of travel expenses for participating in the substudy on pharmacokinetic effects of BOSWELAN.

18. Optional study extension

Upon completion of the extended 12 month trial duration patients will be offered to continue in an extension study up to further 24 months or until primary efficacy measures have been analysed. Study visits will be performed at month 0, 3, 6, 9, 12, 15, 18, 21, 24. Visit Month 0 of the extension study may be Visit Month 12 from the core study.

On month 3, 9, 15, 21 the following procedures will be obtained:

- Monitoring of adverse events

On month 0, 6, 12, 18, 24 the following procedures will be obtained:

- Monitoring of adverse events.
- A complete physical examination.
- Vital signs and body weight.

- Haematology: see 11.3..
- Blood chemistries: see 11.3..
- Urinalysis: see 11.3..
- Urine pregnancy test for women of child-bearing potential
- Biomarker Assessment.
- EDSS, SNRS, MSFC.
- HAQUAMS, MSIS29, HADS, Fatigue Severity Scale
- Recording of concomitant medication.

On month 12 and 24 cranial MRI will be performed.

Data will recorded in additional eCRF pages. The same safety, SAE and SUSAR as in the core study will be applied.

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Appendix A: Study Flow Sheet

	SCREENING				STAGE 1 DOSE TITRATION				STAGE 2 STABILI-ZATION	STAGE 3 TREATMENT			STAGE 4 FOLLOW-UP											
	Month -3 (Screening)	Month -2 (+7 days)	Month -1 (+7 days)	Day 0 (+7 days)	Day 1 (± 1 days)	Day 4 (± 1 days)	Day 7	Day 10	Day 13 (± 1 days)	Day 15	Day 18	Day 22	Day 25	Day 28	Month 2 (+7 days)	Month 4 (+7 days)	Month 5 (+7 days)	Month 6 (+7 days)	Month 7 (+7 days)	Month 8 (+7 days)	Month 9** (+7 days)	Month 10** (+7 days)	Month 11 ** (+7 days)	Month 12 /Early termination (+7 days)
Medical history	•																							
MS history	•																							
Physical examination	•			•												•	•	•	•	•	•		•	
AE documentation		•	•	•												•	•	•	•	•	•		•	
Inclusion/Exclusion criteria	•			•																				
Vital signs	•	•	•	•												•	•	•	•	•	•		•	
Body weight	•	•	•	•												•	•	•	•	•	•		•	
Blood chemistries	•			•												•	•	•	•	•	•		•	
Haematology	•			•												•	•	•	•	•	•		•	
Urinalysis	•			•												•	•	•	•	•	•		•	
Hepatitis status	•																							
Immunology		•		•																			•	
Biomarker Assessment				•																			•	
Pregnancy test	•	•	•	•												•	•	•	•	•	•		•	
First study drug dose				•																				
Telephone Call (AEs)					•	•		•									•				•	•	•	
Dose escalation					•	•	•	•	•	•	•	•	•	•										
Clinic observation						•																		
EDSS (* by telephone)	•	•	•	•												•	•	•	•	•	•	•*	•*	•
SNRS	•	•	•	•												•	•	•	•	•	•			•
MSFC	•		•	•												•	•	•	•	•	•			•
MRI	•	•	•	•												•	•	•	•	•	•			•
Concomitant medication	•	•	•	•												•	•	•	•	•	•			•
HAQUAMS, MSIS29, HADS, FSS	•			•												•	•	•	•	•	•			•
HARAS	At Month -3 for each Relapse within the last 12 Months and afterwards in case of Relapse/ Relapse follow-up																							

** for patients deciding to continue on study medication after month 8