SUPPLEMENTARY MATERIAL for the manuscript entitled:
"A Standardized Frankincense Extract Reduces Disease Activity in Relapsing-Remitting Multiple Sclerosis in a Phase IIa Trial (The SABA proof-of-concept trial)" by Stürner et al.

I. Additional Methodological Details of the SABA Trial

II. Additional Outcomes

I. Additional Methodological Details of the SABA Trial

1. Patients: Full inclusion, eligibility and exclusion criteria.

- Inclusion criteria:
  - Aged 18 to 65 years
  - 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 MS according to published criteria (Polman *et al.*, 2011)
  - Subject is able to give informed consent
  - Signed informed consent
  - EDSS score between 0.0 and 5.5.
  - Disease is clinically stable, i.e. without relapse and patient not having received steroids within 30 days prior to inclusion
  - Patients have either failed to respond to standard treatment (interferon beta, glatiramer acetate) by clinical measures, or were not eligible for any of the available standard treatments, or chose not to start or 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.

- 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 four-month pre-treatment baseline period.
  - Subjects must not have had a relapse during the 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, treatment begin (day 1) is delayed at least until treatment starts not earlier than 30 days after the relapse and not earlier than 60 days in case i.v. corticosteroids had been given.

- Exclusion Criteria

  - The results of blood tests exceeding any of the limits defined below will necessitate exclusion from the study:

  - ALT (SGPT) or AST (SGOT) > three times the upper limit of normal
  - Total white blood cell count < 3,000mm^{-3}
  - Platelet count < 85,000mm^{-3}
  - Creatinine > 132.63μmol L^{-1}
  - Serological evidence of active hepatitis B or C infection, or other chronic liver disease
- Positive pregnancy test, or on-going breast-feeding
- Nausea and/or vomiting as a frequent complaint
- History or signs of immunodeficiency
- Similar clinical neurological symptoms caused by other diseases (e.g. syphilis, borreliosis, collagenosis or vasculitis)
- Concurrent, clinically significant (as determined by the investigator) cardiac, immunological, pulmonary, neurological, renal, and/or other major disorders

- Treatment history

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

- Definition of a confirmed relapse

A confirmed relapse was defined as the occurrence of new neurological symptoms or the worsening of pre-existing symptoms that lasted at least 24 h in a participant who had had a stable or improving neurological status in the previous 30 days, accompanied by an objective neurological change (worsening by 0.5 points on the EDSS or worsening by ≥1.0 points on the pyramidal, cerebellar, brainstem, or visual functional system scores) not due to fatigue alone, and not associated with fever or infection.

If a relapse occurred during the study, the patients were offered the option to discontinue the trial and revert to the standard treatment, and an informed re-consent was necessary if they chose to continue.

Supplementary Table 1: Restrictions on pre-treatments

Agent	Required time off the agent prior to trial drug administration
Glatiramer acetate (Copaxone™), Interferon beta (Betaferon™, Avonex™, Rebif™)	12 weeks
IV Ig, azathioprine (Imurek™), methotrexate, cyclophosphamide (Cytoxan™), mitoxantrone, plasma exchange, cyclosporine, oral myelin, cladribine, natalizumab, and other immunosuppressive treatments	24 weeks
Corticosteroids, ACTH	8 weeks

- History of alcohol or drug abuse within the five years prior to enrolment
- Female subjects who are not post-menopausal or surgically sterile, or who are not using a 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 presented prior to study begin
- 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 interfere with the subject's returning for follow-up visits on schedule
- Previous participation in this study
- Participation in other pharmaceutical trials during this study or during the three months before
- Patients hospitalised 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 in swallowing up to 12 capsules per day should not be recruited for screening.

2. Dosing phase and dosing regimen

The standardised frankincense extract (SFE) was provided as capsules containing 400 mg. Patients were advised to swallow the capsules with a full glass of liquid before or during a meal.
SFE was titrated up to the maximum dose tolerated well by the patient, or to a maximum of 1600 mg t.i.d (whichever occurred first). Dose titration occurred over 28 days. After determining the maximum well-tolerated dose, each patient was continued on this dose for another 28 days to determine dose tolerability and for stabilization, followed by six months of treatment. Subsequent adjustments to the dose were made as necessary. A minimum tolerated dose of 2400 mg per day was mandatory to continue with the trial.
Definition of “not tolerated”: Moderate adverse events persisting at the next dosing time point, or whenever the investigator felt it necessary for the dose to be (temporarily) reduced or the dose to be omitted.
Definition of “well tolerated”: No or only mild adverse events which resolve before the next dose. Since the highest well-tolerated dose will in most cases be determined after moderate or severe adverse events have 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 following doses will be reduced until the “well-tolerated” dose is established.
“Dose” is defined as any daily dosing regimen that also allows for different numbers of capsules at the three dosing time points per day.

The titration rules for the dose finding period Stage 1 of the study were as follows:

The dose was increased only if the previous dose had been sufficiently well tolerated, i.e. no adverse event or only mild adverse event(s), which had resolved at the next dosing time point.
If an increase in the dose was not tolerated, the dose was temporarily reduced. No more than three temporary dose reductions were allowed during the dose escalation phase, i.e. after a third dose reduction the dose was fixed at that or a lower well-tolerated dose level.

<u>Dose Titration Scheme</u>

The dose unit for the following titration scheme was 400 mg/capsule. The doses were administered in the morning, at midday and in the evening with at least six hours between doses.

Supplementary Table 2: Titration scheme: The dose unit was 400 mg/capsule.

Days	Dosing schedule (capsules)	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	

Patients were requested to remain on each new dose for a minimum of three days before the next increase. Any dose increase after a dose reduction due to an adverse event required that either the treating physician or the study nurse be consulted by phone.
The patient had to report by phone any intolerance of a moderate or severe degree to either the treating physician or a study nurse.
The first dose was administered in the respective clinical centre. Each patient was contacted by the study nurse on days 1 (before morning dose), 4 (before morning dose), and on day 13 (before morning dose). The patients were seen at the centre on day 0 and day 28 and the morning dose was administered at the centre on those days.

3. Laboratory parameters

The specific laboratory parameters to be evaluated in this study were 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, albumin, lactate dehydrogenase, fibrinogen, CRP, CK.
- Hematology: complete blood count with differential and platelet counts, and coagulation studies (PT and PTT).

- Urinalysis: specific gravity, pH, protein, glucose, blood, ketones, nitrite, leucocytes and including a pregnancy test for women of child-bearing potential.
- Hepatitis serology: was either tested at screening, or prior test results will be accepted if the patient had already been tested for hepatitis during the past 12 months.

4. Further details on Magnetic resonance imaging

The section for multiple sclerosis imaging (SeMSi) at the Department of Neuroradiology of the UKE served as the central MRI reading and analysis center.
MRI scans were performed at months -3,-2, -1, 0, 1, 2, 3, 5, 6, 7, 8, 12, 24 and 36 months using a standardized protocol implemented at each site. The MRI protocol included a sagittal T2 Space Dark Fluid attenuated inversion recovery (FLAIR) (TR/TE = 4700ms/392 ms; TI=1800 ms; 192 slices, slice thickness = 0.9 mm, no gap; FOV = 240 mm; matrix = 320 x 320), an axial PD/T2 TSE transversal sequence (TR;TE=2800ms;18/95ms, 43 slices, slice thickness = 3 mm, no gap; FOV = 240 mm; matrix = 192 x 256), a sagittal magnetization prepared rapid acquisition gradient-echo (MPRAGE) T1 weighted sequence (TR/TE = 1900ms/2.46 ms; TI=900 ms; 192 slices, slice thickness = 0.9 mm, no gap; FOV = 240 mm; matrix = 256 x 256, flip-angle 9°, pre-/post Gadolinium, T1 / T1Gd). Contrast agent was applied with 0.1 mmol/kg using either MAGNOGRAF (Gadopentetat-Dimeglumin;MaRotrast GmbH Jena, Germany) or DOTAREM (Gadoteracid; Guerbet Sulzbach/Taunus, Germany).Minor changes were to protocol were allowed at both sites to accommodate different platforms and field strengths. All images had to pass quality control before and after image pre-processing to be included for analysis.
MRI data in Dicom format were pseudonymized prior to transfer and then uploaded to the central MRI reading center database. Prior to study onset, each site provided a dummy scan utilizing the standardized sequences for review by the central MRI reading center to verify scan quality and fidelity.
The scans underwent a standard review by a radiologist blinded to study details to assess for any new or unusual findings as a safety measure. Incoming imaging data was reviewed for completeness and fidelity to study pulse sequences by the imaging core investigators. Local radiologists and imaging core investigators were all blinded to the patient's timepoint in the study. The number of gadolinium-enhancing lesions and T2 lesions at each imaging timepoint was determined by an experienced neuroradiologist (SS). The number of CELs for the individual patient was the average value, while the CEL volume for the individual patient

was the sum value determined from the MRIs in the four-month baseline, resp. treatment period.

5. Immunological methods

5.1 Flow Cytometry

Multicolour flow cytometric analysis was performed longitudinally ex vivo in n = 26 patients at months -3, 0, 1, 3, 8 and 12, respectively. Measurements from whole blood samples of RRMS patients were performed using 200 µl EDTA blood. For surface marker analysis, cells were stained with monoclonal antibodies for 30 min at room temperature. Erythrocytes were lysed using lysis solution (BD Biosciences, USA) for 10 min at room temperature. The following antibodies were used: anti-CD3 (PE-Cy7, clone UCHT1, BioLegend, USA), anti-CD4 (PE-Cy7, clone RPA-T4, BioLegend or PacBlue, clone MT310, DakoCytomation, Denmark), anti-CD8 (AlexaFluor700, clone HIT8alpha, BioLegend or PerCPCy5.5, clone SK-1,BD Biosciences), anti-CD25 (APC, clone BC96, ebiosciences, USA), anti-CTLA-4 (PE, BD Biosciences), anti-CD127 (BV421, clone A019D5, BioLegend), anti-FoxP3 (PE, clone 3G3, Miltenyi Biotec, Germany), anti-CD45 (APC, clone HI30, BioLegend), anti-CD62L (APC, cloneDREG-56, BioLegend), anti-CD56 (PE, clone HCD56, BioLegend), anti-CD16 (Alexa Fluor® 700, clone 3G8, BioLegend), anti-CD14 (PacificBlue, clone M5E2, BioLegend), anti-CD19 (PacificBlue, clone HIB19, BioLegend), anti-CD27 (PE, clone M-T271, BioLegend), anti-IgD (FITC, clone IA6-2, BioLegend), anti-CD38 (APC, clone HIT2, BioLegend). Staining for FoxP3 was conducted according to the manufacturer's recommendations. Isotype controls were chosen according to manufacturer's recommendations.
For intracellular cytokine staining, PBMCs were separated by density gradient with Histopaque (PAA, Pasching, Austria) and rested overnight at 37°C. The cells were then stimulated with PMA (50 ng/ml, Sigma) and ionomycin (1 µg/ml, Sigma) in the presence of Brefeldin A (10 µg/ml, eBioscience) for 5 h. Next, they were stained with LIVE/DEAD® Fixable Dead Cell Stain Kit (AmCyan, Molecular Probes, Invitrogen), fixed and permeabilized with the corresponding buffers (eBioscience) and stained with anti-CD3 (PE, clone UCHT1, DakoCytomation), anti-CD8 (PacBlue, clone Clone DK25, DakoCytomation), anti-IFN-γ (FITC, clone 4S.B3, BDPharmingen), -IL-10 (PE-Cy7, clone JES3-9D7, eBioscience) and -IL-17A (Alexa Fluor®-647, clone eBio64DEC17, eBioscience) antibodies at room temperature. Sample acquisition was conducted using a LSRII (BD Biosciences) flow cytometer and data were analysed using the FlowJo software (Tree Star, Inc.). For data acquisitiona and analysis all involved researchers were blinded to the patient's timepoint in the study.

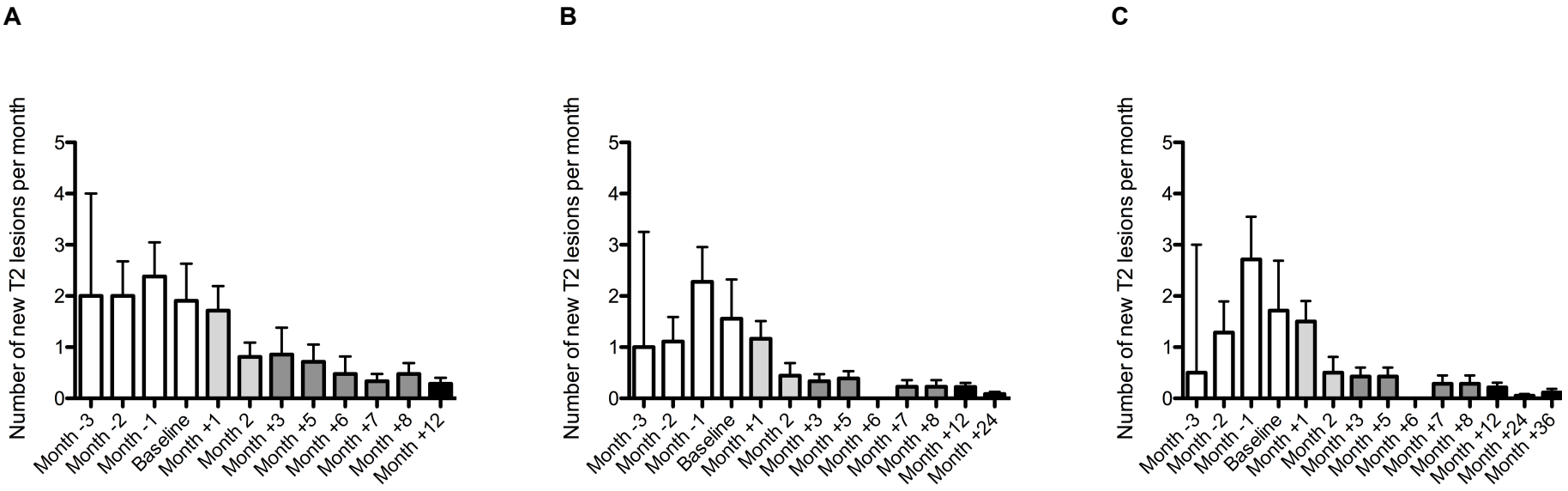
Values for CTLA-4 and CD62L surface expression were calculated as Median Fluorescence Intensity (MedFI) normalized to the Isotype control (MedFI/Isotype Control x 100).

5.2 Cytokine measurements

Serum cytokine levels were determined either by MesoScale Discovery (MSD) electrochemiluminescence technology (IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-17A, TNF-α, IFN-γ, TGF-β) or by Singulex SMC™ Erenna® Immunoassay technology (IL-17A). Serum concentrations of respective analytes were measured at months -2, 0, 1, 3, 8 and 12, respectively, in n = 28 samples for each time point after all patients had completed the study. IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, TNF-α were analysed with customized multiplex assays (MSD V-PLEX Human Proinflammatory Panel); IL-5, IL-17A, GM-CSF were analysed with customized multiplex assays (Mesoscale V-PLEX Human Cytokine Panel); TGF-β was analysed with single assays (Mesoscale Human TGF-β1 Kit, # K151IUC-1); IL-17A was analysed with Singulex SMC™ assays (Erenna # 03-0103-00; Lot No: 1509176). For data acquisition and analysis all involved researchers were blinded to the patient's time point in the study.

II. Additional Outcomes:

Supplementary Figure 1: Number of new T2-lesions per month during the extension (Stage 4) of the SABA trial



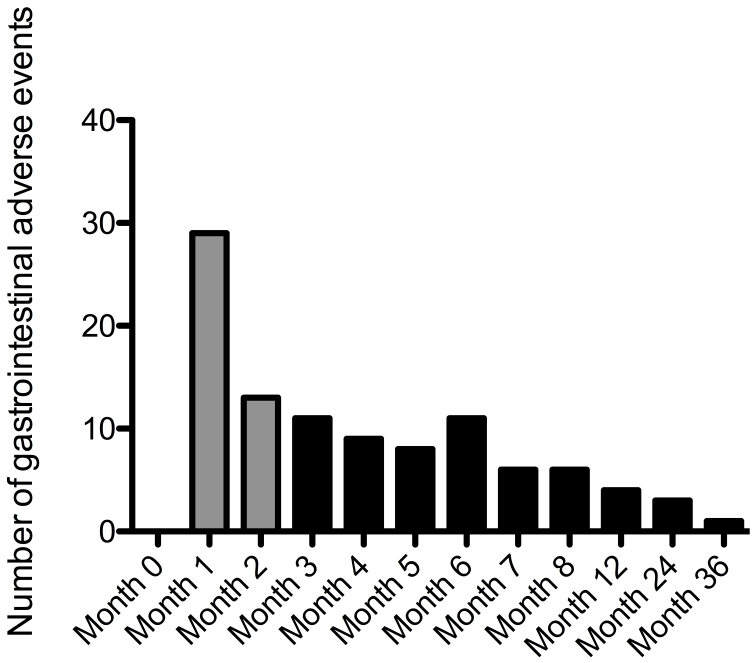
n= 25 until month 12

n=18 until month 24

n=12 until month 36

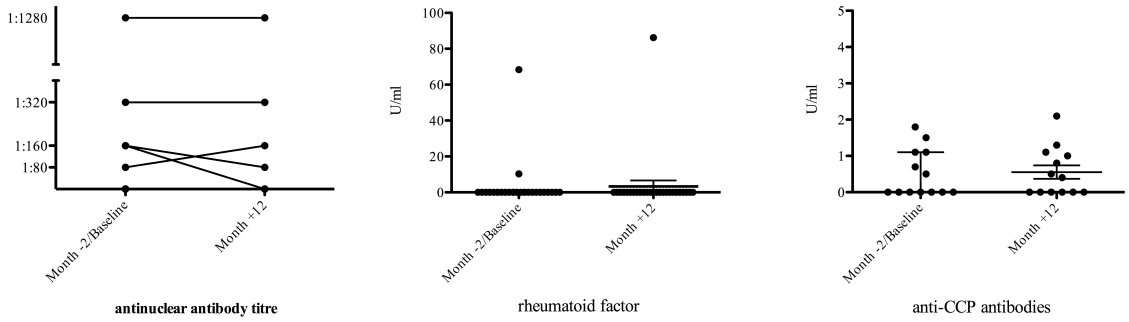
Number of new T2-lesions per month during **A** 12 months (n=25), **B** 24 months (n=18) and **C** 36 month (n=12) in the SABA trial.
White bars indicate the screening phase before start of treatment (months -3 to 0), light grey bars the dose-finding phase (months +1 and +2), Dark grey bars the treatment phase on a stabile individual dose and black bars the extension phase.

Supplementary Figure 2: Incidence of gastrointestinal adverse events in the SABA trial



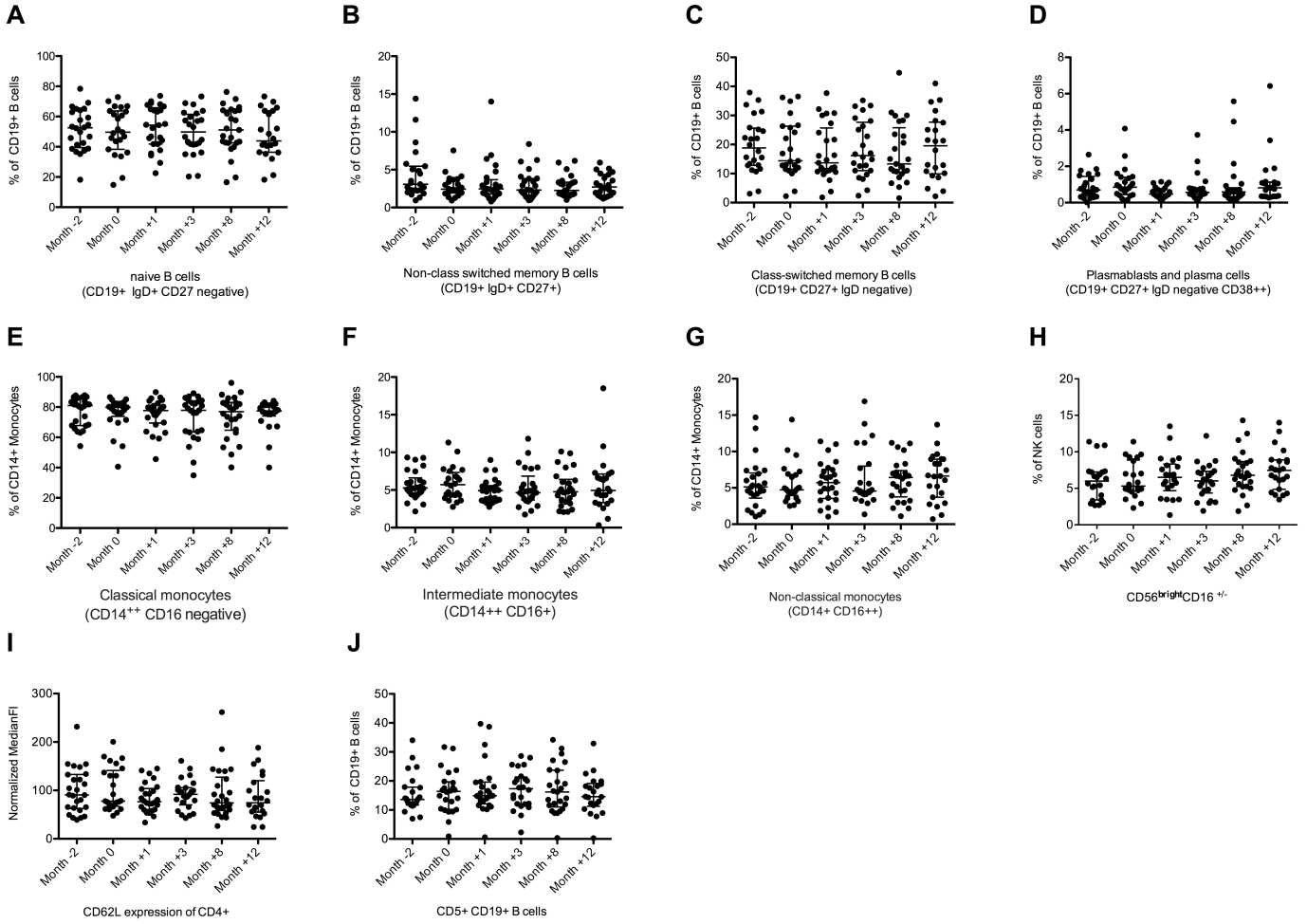
Grey bars depict the dose-finding phase (Stage 2), black bars the treatment phase (Stage 3).

Supplementary Figure 3: Results from analyses of serum samples from the total SABA patient cohort



A antinuclear antibody titre, **B** rheumatoid factor, **C** anti-cyclic citrullinated peptide (anti-CCP) antibodies. Rheumatoid factor and anti-CCP antibodies are presented as median with IQR.

Supplementary Figure 4: Further results from flow-cytometric immune-phenotyping of the SABA patient cohort.



A Percentage of naïve B cells (CD19+ IgD+ CD27 negative), n = 25 patients. No significant changes ($p > 0.05$).

B Percentage of non-class-switched memory B cells (CD19+ IgD+ CD27+), n = 25 patients. No significant changes ($p > 0.05$).

C Percentage of class-switched memory B cells (CD19+ CD27+ IgD negative), n = 25 patients. No significant changes ($p > 0.05$).

D Percentage of plasma blasts and plasma cells (CD19+ CD27+ IgD negative CD38++), n = 25 patients. No significant changes ($p > 0.05$).

E Percentage of classical monocytes (CD14++ CD16 negative), n = 25 patients. No significant changes ($p > 0.05$).

F Percentage of intermediate monocytes (CD14++ CD16+), n = 25 patients. No significant changes ($p > 0.05$).

G Percentage of non-classical monocytes (CD14+ CD16++), n = 25 patients. No significant changes ($p > 0.05$).

H Percentage of Natural Killer bright cells (CD56 ^{bright} CD16+/- CD3 negative), n = 25 patients. No significant changes ($p > 0.05$).

I CD62L expression on CD4+ T cells, n = 25 patients. No significant changes ($p > 0.05$).

J Percentage of CD5+ B cells, n = 25 patients. No significant changes ($p > 0.05$).

All data are presented as median with IQR.

Supplementary Table 3: Results from cytokine serum level measurements in the SABA patient cohort (n=28)

Cytokine	Time period	Minimum	Median	Maximum	Change from baseline: p-value
GM-CSF	Month -2 – 0 **Month 1-3** Month 8-12	0.002 0.008 0.011	0.105 **0.056** 0.077	0.404 0.163 0.382	 **p=0.0196** p=0.2280
IFN-γ	Month -2 – 0 Month 1-3 **Month 8-12**	2.254 1.293 1.547	5.649 4.263 **3.857**	286.65 138.44 32.145	 p=0.4078 **p=0.0088**
IL-10	Month -2 – 0 Month 1-3 Month 8-12	0.035 0.090 0.044	0.314 0.234 0.212	3.689 1.717 3.079	 p=0.6662 p=0.1978
IL-12p70	Month -2 – 0 Month 1-3 Month 8-12	0.000 0.022 0.021	0.146 0.135 0.131	1.201 0.458 0.401	 p=0.3229 p=0.0823
IL-13	Month -2 – 0 Month 1-3 Month 8-12	0.000 0.148 0.259	1.979 1.516 1.591	7.515 3.282 3.031	 p=0.1705 p=0.1383
IL-17A	Month -2 – 0 **Month 1-3** Month 8-12	0.084 0.065 0.058	0.318 **0.216** 0.201	3.717 1.374 1.270	 **p=0.0496** p=0.1263
IL-1β	Month -2 – 0 Month 1-3 Month 8-12	0.000 0.000 0.000	0.000 0.000 0.000	0.167 0.112 0.025	 p=0.3125 p=0.1250
IL-2	Month -2 – 0 Month 1-3 **Month 8-12**	0.000 0.000 0.000	0.121 0.084 **0.065**	2.300 0.571 0.543	 p=0.0682 **p=0.0032**
IL-4	Month -2 – 0 **Month 1-3** **Month 8-12**	0.006 0.003 0.007	0.061 **0.031** **0.031**	0.234 0.101 0.088	 **p=0.0292** **p=0.0088**
IL-5	Month -2 – 0 **Month 1-3** **Month 8-12**	0.058 0.065 0.020	0.163 **0.137** **0.126**	0.399 0.253 0.311	 **p=0.0039** **p=0.0028**
IL-6	Month -2 – 0 Month 1-3 Month 8-12	0.092 0.130 0..,082	0.633 0.450 0.419	2.733 1.271 2.502	 p=0.1383 p=0.1620
IL-8	Month -2 – 0 Month 1-3 Month 8-12	6.350 6.621 5.876	10.189 10.635 11.391	176.60 44.681 111.01	 p=0.2497 p=0.1240
TGF-β	Month -2 – 0 **Month 1-3** **Month 8-12**	9160.0 8558.2 7100.9	17057 **14524** **13220**	26454 21474 19681	 **p=0.0005** **p<0.0001**
TNF-α	Month -2 – 0 Month 1-3 Month 8-12	1.613 1.892 1.663	2.438 2.553 2.667	4.702 4.551 6.467	 p=0.1793 p=0.2075

Statistically significant results are highlighted in bold print.