# Measuring pharmacodynamics in early clinical drug studies in multiple sclerosis

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# GSH-PEGYLATED LIPOSOMAL METHYLPREDNISOLONE IN COMPARISON TO FREE METHYLPREDNISOLONE: SLOW RELEASE CHARACTERISTICS AND PROLONGED LYMPHOCYTE DEPRESSION IN A FIRST-IN-HUMAN STUDY

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## ABSTRACT

**AIM** Intravenous high-dose free methylprednisolone hemisuccinate (MP) is the primary treatment for an acute relapse in relapsing-remitting (RR) multiple sclerosis (MS). However, it is inconvenient and its side effects are undesirable. Both dose and dosing frequency can be reduced by incorporating free MP in glutathione (GSH) PEGylated liposomes, creating a slow-release formulation with reduced toxicity and prolonged peripheral efficacy. This first-in-human study was designed to assess the safety, pharmacokinetics (PK) and pharmacodynamics (PD) of GSH-PEGylated liposomes containing MP (2B3-201).

**METHODS** The first part was a double-blind, 3-way cross over study in 18 healthy male subjects, receiving ascending doses of 2B3-201, active comparator (free MP) or placebo. Part 2 of the study was an open-label infusion of 2B3-201 (different doses), exploring pre-treatment with antihistamines and different infusion schedules in another 18 healthy male subjects, and a cross-over study in 6 healthy female subjects. MP plasma concentrations, lymphocyte counts, ACTH, osteocalcin and fasting glucose were determined. Safety and tolerability profiles were assessed based on adverse events, safety measurements and CNS tests.

**RESULTS** The most frequent recorded AE related to 2B3-201 was an infusion related reaction (89%). 2B3-201 was shown to have a plasma half-life between 24 and 37 hours and caused a prolonged decrease in the lymphocyte count, ACTH and osteocalcin, and a rise in fasting glucose.

**CONCLUSION** 2B3-201 is considered safe, with no clinically relevant changes in (CNS) safety parameters and no serious adverse events. In addition, 2B3-201 shows a long plasma half-life and prolonged immunosuppressive effects.




## INTRODUCTION

Multiple sclerosis (MS) is one of the most prevalent neuro-inflammatory diseases and the leading cause of chronic disability in young adults. In MS, central nervous system (CNS) infiltration of leukocytes leads to overt inflammation and demyelination and results in neuronal dysfunction[1]. High-dose methylprednisolone hemisuccinate (free MP), given 500-1000 mg daily for 3 to 5 consecutive days, is the primary treatment for an acute relapse in relapsing-remitting (RR) multiple sclerosis[2]. However, it is often given intravenously and causes undesirable short term and long-term side effects include insomnia, depression and agitation[3,4].

Both dose and dosing frequency of glucocorticoids may significantly be reduced by incorporating steroids in (PEGylated) liposomes, which is expected to result in reduced systemic toxicity while maintaining peripheral efficacy[5]. The additional conjugation of glutathione (GSH) to target active GSH transporters on the blood-brain barrier (BBB), has been shown to facilitate the delivery of the liposome-encapsulated drug into the brain[6,7].

2B3-201 is methylprednisolone hemisuccinate encapsulated in GSH-PEGylated liposomes and is developed with the aim to enhance the sustained delivery of MP into the brain, thereby potentially augmenting CNS activity. Preclinical studies in animal models showed that 2B3-201 at therapeutic levels in animal models had fewer behavioural side effects (unpublished) and a superior efficacy compared to methylprednisolone hemisuccinate[8-10]. Also, plasma circulation of 2B3-201 derived MP was significantly increased by encapsulation in GSH-PEGylated liposomes[11]. Based on these preclinical data we expected in human subjects a longer half-life and fewer side effects of 2B3-201 when compared to MP.

In this first-in-human study we aimed to assess the safety, pharmacokinetic and pharmacodynamic profile of 2B3-201 in healthy male and female subjects. Plasma concentrations of lymphocytes, osteocalcin, ACTH and fasting glucose were used as pharmacodynamic endpoints, as intravenous administration of prednisolone causes rapid inhibition of the hypothalamic-pituitary-adrenal (HPA) axis[12], glucose homeostasis disturbances, and depletion of osteocalcin[13,14] and lymphocytes[15]. CNS effects were measured with the NeuroCart[16].

## MATERIALS

### Design

Initially a randomized, double-blind, placebo- and active comparator- controlled 3-way crossover study with three cohorts of 6 healthy males each, was performed. Subsequently the study was extended while applying a parallel open label design with four cohorts, each containing 6 healthy subjects.

In cohorts 1, 2 and 3, a single dose of 150 mg, 300 mg and 450 mg 2B3-201 respectively was tested and compared to free MP and placebo (table 1). The time interval between the occasions in the cross-over parts was 1 week. Cohorts 4, 5 and 6 had a single dose of 300 mg (cohort 5) and 450 mg (cohorts 4 and 6) 2B3-201 tested while applying altered infusion

schedules and pre-treatment with clemastine. Cohort 7 included females and compared 450 mg 2B3-201 to 1000 mg of free MP in a double-blind crossover design.

An interim analysis was conducted after completion of cohorts 1, 2 and 3 at which safety, pharmacokinetics and pharmacodynamics results were evaluated and a decision to continue to the next cohort was made.

The study was approved by the Medical Ethics Committee of the BEBO Foundation (Assen, The Netherlands). The study was conducted according to the Dutch Act on Medical Research Involving Human Subjects (WMO) and in compliance with Good Clinical Practice (ICH-GCP) and the Declaration of Helsinki.

## Subjects

Forty-six healthy subjects were recruited via the CHDR database and advertisements. All subjects gave written informed consent and were subsequently medically screened before entry into the study. Healthy subjects were not allowed to smoke more than ten cigarettes per day and had to refrain from smoking during the study days. In the 48 h prior to the study days they were asked not to drink alcohol and to avoid xanthine- containing drinks. The use of medication was not allowed during the study period (except occasional use of paracetamol, up to 1 g per day). Healthy subjects with a positive Mantoux test and or recent (less than 1 month prior to screening) or current significant infection, were not enrolled.

## Treatments

7 study cohorts with a total of 46 subjects received an infusion with 150 mg, 300 mg or 450 mg 2B3-201, 300 mg or 1000 mg free MP, or placebo. An overview of all cohorts can be found in table 1. Subjects in cohorts 1-3 had 3 study periods, during which they either received 2B3-201, free MP or placebo (5% dextrose). Cohorts 4 and 6 received open label infusions of 450 mg 2B3-201, and cohort 4 also assessed the pre-treatment effect of 2 mg clemastine on adverse events. Subjects in cohort 5 received 300 mg 2B3-201, and were also pre-treated with clemastine. In cohort 6 a longer infusion duration was assessed. In cohort 7 healthy female subjects received 450 mg 2B3-201 while being pre-treated with clemastine, and 1000 mg free MP in a double blind two-way cross-over fashion.

## Safety

Adverse events, electrocardiogram (ECG), lymphocyte count, fasting glucose, blood pressure, and heart rate measurements were collected throughout the study. Twelve-lead ECG recordings were made using Electrocardiograph Marquette 800/5500 or Dash 3000. Blood pressure and heart rate were assessed using a Nihon-Kohden BSM-1101K monitor or a Colin Pressmate BP 8800 or a Dash 4000. All ECG, blood pressure and heart rate measurements were performed after subjects had been resting in a supine position for at least 5 min.

## Pharmacokinetics

Whole blood samples were taken for assay of the active component methylprednisolone and the encapsulated pro-drug methylprednisolone hemisuccinate. Blood samples were taken 0.25 h pre-dose and 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 26, 48 and 72 hours post-dose for all cohorts, and up to 288 hours for cohorts 4-7. The blood was drawn in 2 mL NaF/K-oxalate tubes, directly placed on ice and then centrifuged (2000 g, 10 min, at 2-8 °C), transferred to 2 mL Sarsted tubes and stored at -80 °C within 30 min after sampling. The concentrations of methylprednisolone and methylprednisolone hemisuccinate (MPHS) in human sodium fluoride/potassium oxalate plasma were determined using a validated liquid chromatography with tandem mass spectrometry (LC-MS/MS) assays by Analytical Biochemical Lab (Assen, the Netherlands). The Lower Limit of Detection (LLOQ) was 1 ng/mL for methylprednisolone. Concentrations for methylprednisolone were calculated by interpolation from a calibration curve while applying a range of 1-1000 ng/mL.

The following pharmacokinetic variables were calculated: Area under the plasma concentration-time curve (AUC) from time 0 to the time of the last quantifiable concentration ($AUC_{0\text{-}t}$) and from time 0 extrapolated to infinity ($AUC_{0\text{-}inf}$), maximal observed plasma drug concentration ($C_{max}$), time to maximum observed plasma drug concentration ($t_{max}$), half-life ($t_{1/2}$), volume of distribution (Vd) and clearance. For the non-compartmental analysis only MP concentrations up to 74 hours were used.

## Pharmacodynamics

**LYMPHOCYTE COUNT** Time points for measurement of lymphocytes were 2 hours pre-dose (cohorts 1-3 only), 15 minutes pre-dose (cohorts 4-7) and 1, 2, 4, 8, 12, 24, 48 and 72 hours post dose for all cohorts, and up to 288 hours post dose for cohorts 4-7. The 2 mL EDTA-sample was directly, without pre-processing, sent to a hospital haematology and chemistry lab for analysis. The normal range for lymphocyte count was 1.00-3.50 x10^9/L.

**OSTEOCALCIN** Serum osteocalcin was measured several times per occasion: pre-dose on day 0, 8, 24, 48 and 72 hours post dose for all cohorts, and up to 288 hours post dose for cohorts 4-7. Intact osteocalcin was measured in serum with ELISA [13], the normal range used was 0,4-4,0 nmol/L.

**ACTH** ACTH was measured 12 times per occasion. Samples were taken 0.25 h pre-dose and 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 26, 48 and 72 hours post-dose for all cohorts, and up to 288 hours post dose for cohorts 4-7. The ACTH samples (2 mL in an EDTA-tube) were put on ice directly, and centrifuged within 10 minutes. Normal range was <75 ng/L.

**FASTING GLUCOSE** As a pharmacodynamics and safety marker, measurement of fasting glucose levels was performed. Samples were taken pre-dose, 2, 6, 12, 24 and 72 hours post dose for all cohorts, and up to 288 hours for cohorts 4-7. 2 mL was collected in a NaF tube, the used normal range was 3.1-6.4 mmol/L.

**COMPLEMENT AND IGE** To confirm if the observed infusion related reactions in cohort 1 were complement mediated and not allergic reactions, we measured for cohorts 2-7 complement factors SC5b-9, C3a, C4d and Bb (4 mL blood EDTA tube) and IgE (2 mL blood, EDTA tube). These samples were taken pre-dose (depending on cohort at -20, -9 or -7 minutes) and 5, 30 and 120 minutes after start of the infusion.

**CNS TESTS** CNS tests performed with the NeuroCart included: pharmaco-EEG[17-19], maze learning[20], visual verbal learning test, Stroop test[21], adaptive tracking[22], VAS Bond & Lader[23] and VAS Bowdle[24] and saccadic and smooth pursuit eye movements[25].

### Statistics

To compare the pharmacodynamics and pharmacokinetics between treatments the mean and SD were calculated per time point by treatment. Cohorts with the same treatment are combined into one treatment group. For MP values below LLOQ are set to 0 ng/mL before dosing and set to half of LLOQ (0.5 ng/mL) after dosing. For ACTH all values below LLOQ were set to half of LLOQ (2.5 ng/L).

## RESULTS

### Demographics

A total of 46 subjects participated in the study of which 41 completed the study. 5 subjects retracted consent during the study, 4 of them were replaced. The subjects that participated in this study were all healthy young adults, subjects' characteristics are listed in table 2.

### Safety

No clinically relevant changes were observed in ECG, physical examination and vital signs (temperature, heart rate, systolic and diastolic blood pressure). Safety laboratory assessments for blood hematology, chemistry and urinalysis also showed no clinically meaningful abnormalities with the exception of a decrease in lymphocytes, which will be discussed in more detail in the pharmacodynamics section.

The most frequently reported adverse events related to 2B3-201 were infusion related reactions, defined as any sign or symptom experienced by the subject within 4 hours after the start of the infusion[26,27]. Symptoms related to infusion that occurred within 4 hours after start of the infusion, such as chest discomfort, urticaria, angioedema and back pain, were clustered[28]. Infusion reaction related symptoms were reported by 41 of the 46 healthy subjects (89%). Other frequently reported adverse events were somnolence (15%), gastroesophageal reflux disease (8%), back pain (not assessed as an infusion related reaction) (8%), fatigue (8%) and dizziness (8%).

Pre-treatment with 2 mg clemastine (cohorts 4, 5 and 7) at 20 minutes before infusion did not result in fewer infusion related reactions: all subjects in these cohorts showed symptoms of an infusion related reaction (see table 3). Not all infusion related reactions resulted in (temporary) halt of the infusion and/or lowering of the infusion speed.

All adverse events were mild in severity, short lasting and self-limiting. One adverse event related to 1000 mg free MP was classified as moderate: a male subject (cohort 1) developed an acute tonsillitis with fever 3 days after the infusion. He was subsequently treated with feniticillin and fully recovered.

Complement and IgE measurements showed that 2B3-201 caused a parallel rise of C3a and Bb and no increase in C4d and IgE levels were observed (figure 1).

### Pharmacokinetics

A concentration-time graph for methylprednisolone plasma concentration at different dose levels of 2B3-201 derived MP and free MP is shown in figure 2. Pharmacokinetic parameters per cohort are listed in table 4. Plasma concentrations of 2B3-201 derived MP were measured up to 7 days (300 and 450 mg), for 300 mg and 1000 mg free MP concentrations were measurable until two days after infusion.

2B3-201 derived MP had a maximum plasma concentration of 545 mg/mL (450 mg 2B3-201), which contrasts the maximum plasma concentration of 7290 ng/mL for free MP (1000 mg). The $t_{max}$ was 5.9 hours for 2B3-201 derived MP while free MP had a $t_{max}$ of 2.16-4.2 hours. Plasma half-life for 2B3-201 derived MP was between 24 and 37 h. Free MP had a half-life of 2.2- 4 hr. A t-test showed a significant difference in AUC and $C_{max}$ (p-values of respectively 0.003 and 0.006) between males and females and in weight (p-value = 0.03), but not in BMI. Observed differences were tested for correlation with weight and BMI with a Spearman correlation. Correlation was found for weight, with values of -0.335 (weight and $C_{max}$, p-value=0.03) and -0.39 (weight and AUC, p-value=0.01), however not for BMI, with values of 0.11 (BMI and $C_{max}$) and 0.052 (BMI and AUC). Concentrations and pharmacokinetic parameters for MPHS are not reported (data on file).

### Pharmacodynamics

**LYMPHOCYTES** The effects of 2B3-201 derived MP, free MP and placebo on lymphocytes are shown in figure 3a. Administration of 2B3-201 and free MP resulted in a maximal decrease in lymphocyte count 6 to 12 hours after dosing. The decrease in lymphocyte count, persisted for 2 days after dosing after 150 mg 2B3-201 administration, for 3 days after dosing with 300 and 450 mg 2B3-201. Infusion of 300 mg and 1000 mg free MP resulted in a maximal decrease for 24 hours. 7 days after dosing lymphocytes values for all active groups had returned to baseline.

**ACTH** ACTH concentrations were below the lower limit of quantification for almost all subjects 3 hours after administration of active study medication. The decrease of ACTH was sustained for 3 days (150 mg) and 4 days (300 and 450 mg) in the 2B3-201 dosing groups, whereas for free MP ACTH plasma levels were no longer decreased after the first day after dosing, demonstrated a slight compensatory increase on days 2 and 3, and had returned to baseline values from day 4 onwards.

**OSTEOCALCIN** All the active treatment groups showed a decrease in osteocalcin concentrations in the first 24 hours after dosing. In the 1000 mg MP dosing group, osteocalcin concentrations started to rise again after 24 hours. For the 2B3-201 dosing groups, the decrease in osteocalcin concentrations persisted for at least 4 days.

**FASTING GLUCOSE** An increase in fasting glucose was visible for all active treatment groups. Peak concentrations were measured 12 hours after dosing for free MP cohorts, and 15 hours after dosing for 2B3-201 cohorts. Fasting glucose concentrations were below 6 mmol/L (the upper limit of subjects in fasting condition) after 2 days for cohorts with free MP and 150 mg 2B3-201. For subjects who received 300 mg and 450 mg 2B3-201, fasting blood glucose had returned to levels below 6 mmol/L after 4 days.

**CNS TESTS** No relevant changes in the effects on CNS between 2B3-201 and free MP could be observed.

## DISCUSSION

This first-in-human study with 2B3-201, a formulation of methylprednisolone-encapsulated GSH-PEG liposomes, showed prolonged methylprednisolone concentrations in serum, and as a consequence a sustained decrease in the levels of lymphocytes, osteocalcin and ACTH and increased fasting glucose over a longer period of time.

Based on pharmacokinetic properties, 2B3-201 acts like a slow release product. The estimated terminal half-life of 2B3-201 derived MP is ten times longer than free MP. Also, the $C_{max}$ is lower for 2B3-201 (360-545 ng/mL) than for free MP (5120-7290 ng/mL). Based on graphical inspection, it is likely that the pharmacokinetics of 2B3-201 derived MP is characterized by first order kinetics. The observed pharmacokinetic profile of free MP corresponded with literature[29,30] and information in the Summary of Product Characteristics.

Pharmacokinetics of 450 mg 2B3-201 in women were different from 450 mg 2B3-201 in men: The $C_{max}$ and AUC were higher, and the half-life was longer (table 4). This can be explained by relative lower weight of women resulting in a higher concentration of MP in serum, and a longer residence time of 2B3-201 compared to men, as the clearance is comparable (men: 0.09-0.11 L/hr, women: 0.09 L/hr).

A limitation of the cross-over part of the study was the time interval between the cohorts. In cohort 3, dosing of 450 mg of 2B3-201 resulted for two subjects in low concentrations of MP study in pre-dose samples at the start of subsequent occasions. However, we believe that this did not influence the major outcome as pharmacodynamic parameters lymphocytes, ACTH and fasting glucose were back to baseline in less than 7 days after the infusion. Also, the other 4 subjects in cohort 3 did not have measurable pre-dose pharmacokinetic results.

As a consequence of prolonged plasma concentrations of 2B3-201 derived MP, a pronounced decrease in lymphocytes was observed for all dose levels for 3 days, an effect that lasted markedly longer than in the free MP groups (1 day). Similar prolonged pharmacodynamic effects were observed for the decreases in concentrations of osteocalcin and ACTH, as well as a rise in fasting blood glucose. All these effects were present over a longer period of time after dosing 2B3-201 in comparison to free MP. Even though we observed prolonged effects of 2B3-201, we could not observe significant differences in effects on CNS functioning between 2B3-201 and free MP.

Treatment with 2B3-201 led to the occurrence of mild infusion related reactions in 89% of all subjects. Increased levels of complement concentrations were found in all subjects after receiving 2B3-201 300 mg and 450 mg, although not all subjects reported symptoms related to an infusion reaction (table 3). From this study we can conclude that complement is activated due to administration of 2B3-201, resulting in a rise in C3a (figure 1). With a simultaneous rise of complement factor Bb (specific for the alternative pathway, figure 1), and a lack of rise in C4d (figure 1) concentration (specific for classical pathway), we can conclude that 2B3-201 activated the alternative complement activation pathway. IgE concentrations (figure 1) were not increased, indicating no anaphylactic reaction was initiated. These results correspond well with what is known as 'complement activation related pseudo allergy' (CARPA). The relationship between liposomal drug delivery and CARPA is well known, and the observed symptoms in this study match those previously described by others[31-33].

There were a couple of adjustments described that may decrease the development of a CARPA reaction. First adjustment is to start the infusion with a low infusion rate[31]. We lowered the infusion rate during cohort 1. Also, lowering the infusion speed when symptoms occur, and re-challenging subjects has also reported to be effective[28]. The same could be observed in our study: the infusions of only 3 subjects were eventually permanently halted as a result of an infusion related reaction. All other subjects received the complete infusion.

Another study reported that a low concentration of liposomes in the infusion fluid also led to fewer infusion related reactions[34], which was implemented in cohort 1. In cohorts 2-7 the concentrations of the liposomes were however still relatively high, as compared to the adjusted concentration in cohort 1 without the observed infusion related reactions. The effect of pre-treatment with clemastine has been discussed in literature[28,34], and although this had not been effective in all studies, it was decided to administer 2 mg clemastine 20 minutes before start of the infusion in cohorts 4, 5 and 7. In our study design, subjects received 2B3-201 once, so a reported decrease of infusion related reaction with multiple dosing[34] of the same compound was not addressed.

Our current actions did not lead to a decrease in the number of reported infusion related reactions, although we did observe a reduced need to change the infusion speed because of infusion related reactions. Reducing the concentration of the liposomes at the start of the infusion may offer a solution in the future.

Methylprednisolone is first choice in medication for acute relapses in MS[35,36]. In certain European countries usually 3 consecutive days of infusions are given[37]. Based on study results in 2015 which revealed that use of oral administration of MP was non-inferior to intravenous MP[38], the National Institute for health and Care Excellence (NICE, UK) adapted their guideline[39] accordingly. Nevertheless, use of intravenous MP remains part of clinical practice especially for patients who suffer from severe relapses and those who do not

respond to oral treatment. With these practices in mind, use of 2B3-201 as a one-day intravenous treatment may be a good alternative.

Moreover, to reduce the burden of 3-5 days of hospital visits, and healthcare costs related to the days of admissions in other countries, a single infusion of 2B3-201 could be beneficial for patients and reduce side effects caused by high doses of MP. In the current study, 2B3-201 derived MP was measurable and active for 7 days after infusion, resulting in a sustained decrease of lymphocyte count, ACTH and osteocalcin, and an increase in fasting glucose. Now, studies with single administrations in patients with RRMS and a relapse measuring clinical improvement and comparing single administrations of 2B3-201 to 3 day treatments with regular MP are warranted to demonstrate this further.

Despite the fact that the infusion related reactions were all mild and self-limiting, these reactions caused by 2B3-201 in the current setting were frequent and intense. MP treatment in MS reduces symptoms of the MS relapse on the short-term, but for most patients it does not influence the disease progression in the long-term[36]. It is important that the side effect profile is acceptable for the patient. The observed infusion related reactions, if not resolved, may therefore limit the future widespread use of 2B3-201 as a standard therapy for the treatment of relapses in patients with RRMS.



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39 NICE. NICE guideline on treatment of acute exacerbation in MS https://www.nice.org.uk/guidance/CG186/chapter/1-Recommendations#relapse-and-exacerbation

**TABLE 1 – Summary of study characteristics.**

| Cohort | 1 | 2 | 3 | 4 | 5 | 6* | 7 |
|---|---|---|---|---|---|---|---|
| **2B3-201 dose** | 150 mg | 300 mg | 450 mg | 450 mg | 300 mg | 450 mg | 450 mg |
| **Population** | Healthy males | Healthy males | Healthy males | Healthy males | Healthy males | Healthy males | Healthy females |
| **Design** | Randomized | Randomized | Randomized | | | | Randomized |
| | Crossover | Crossover | Crossover | | | | Crossover |
| | Placebo-controlled | Placebo-controlled | Placebo-controlled | | | | |
| | Active comparator: methyl-prednisolone | Active comparator: methyl-prednisolone** | Active comparator: methyl-prednisolone | | | | Active comparator: methyl-prednisolone |
| | Double blind | Double blind | Double blind | Open-label | Open-label | Open-label | Double blind |
| **Number of subjects** | 6 | 6 | 6 | 6 | 6 | 6 | 6 |

** Cohort 6 had an infusion that was twice as long, data from this cohort has not been used in our PK and PD analyses.*
*** Dose of methylprednisolone was an intravenous infusion of 300 (cohort 2 only) or 1000 mg.*

**TABLE 2 – Subject characteristics.**

| Cohort | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| **2B3-201 dose** | 150 mg | 300 mg | 450 mg | 450 mg | 300 mg | 450 mg | 450 mg |
| **Population** | Healthy males | Healthy males | Healthy males | Healthy males | Healthy males | Healthy males | Healthy females |
| **Age (years) mean (range)** | 25 (20-30) | 24.9 (19-36) | 25.3 (20-45) | 21.3 (19-25) | 25.5 (20-35) | 19.8 (18-23) | 23 (20-30) |
| **Weight (kg) mean (range)** | 77.6 (66-95) | 71.3 (66-77) | 75.5 (68-88) | 81.1 (68.9-116.6) | 71.0 (59.8-85.6) | 72.1 (65.9-77.8) | 66.8 (54.4-81.2) |
| **Height (cm) mean (range)** | 183.9 (177-191) | 179.9 (159-189) | 183.4 (165-194) | 184.9 (178.4-199.6) | 177.3 (165.0-188.2) | 184.2 (179.2-191.5) | 171.5 (162.7-179.0) |
| **BMI (kg/m²) mean (range)** | 23.0 (20-28) | 22.1 (20-26) | 22.6 (18-26) | 23.5 (21.5-29.3) | 22.7 (18.4-25.2) | 21.2 (20.2-23.1) | 22.8 (19.1-26.9) |
| **Number of subjects** | 6 | 6 | 6 (1 dropout) | 6 | 6 | 5 (1 dropout) | 6 (2 dropouts) |

**TABLE 3 – Infusion related reactions (IRRS) per cohort.**

| Cohort | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| 2B3-201 dose | 150 mg | 300 mg | 450 mg | 450 mg | 300 mg | 450 mg | 450 mg |
| No. of IRRS | 4 | 5 | 5 | 6 | 6 | 6 | 6 |
| Infusion (temporary) stopped due to IRR symptoms | 2 | 3 | 5 | 2 | 4 | 6 | 3 |

**TABLE 4 – Pharmacokinetic parameters of methylprednisolone, calculated from 0-72 hours.**

| Dose (mg) | Cohort | Compound | $C_{max}$ (ng mL-1) (SD) | $t_{max}$ (h) | AUCK-inf (ng h mL-1) (SD) | $K_a$ (h-1) (SD) | $T_{½}$ (h) (SD) |
|---|---|---|---|---|---|---|---|
| 150 | 1 (n=6) | 2B3-201 | 138 (37.1) | 4.56 (1.3) | 4370 (1000) | 0.029 (0.0045) | 24.4 (3.9) |
| 300 | 2 (n=6) | 2B3-201 | 375 (96.6) | 4.48 (1.4) | 13400 (3220) | 0.028 (0.0032) | 25 (2.5) |
| 300 | 5 (n=6) | 2B3-201 | 282 (35) | 4.19 (0.9) | 14800 (4060) | 0.025 (0.0086) | 31 (9.5) |
| 450 | 3 (n=6) | 2B3-201 | 501 (144) | 4.85 (0.75) | 17900 (2690) | 0.024 (0.0029) | 29.2 (3.5) |
| 450 | 4 (n=6) | 2B3-201 | 360 (57.5) | 5.12 (1.4) | 17400 (4020) | 0.024 (0.0034) | 28.9 (4.3) |
| 450 | 7 (n=6) | 2B3-201 | 545 (99.7) | 5.90 (2.9) | 31800 (15500) | 0.0250 (0.010) | 37.0 (29) |
| 300 | 2 (n=6) | Free MP | 2140 (371) | 2.67 (0.20) | 11900 (2490) | 0.258 (0.039) | 2.74 (0.44) |
| 1000 | 1 (n=6) | Free MP | 5930 (580) | 2.16 (0.37) | 27800 (7370) | 0.286 (0.041) | 2.47 (0.38) |
| 1000 | 3 (n=6) | Free MP | 5120 (1120) | 3.95 (0.029) | 28800 (10800) | 0.286 (0.032) | 2.45 (0.30) |
| 1000 | 7 (n=6) | Free MP | 7290 (1740) | 4.20 (0.26) | 42900 (13400) | 0.208 (0.082) | 3.81(1.6) |

**FIGURE 1 – Mean values of Bb, C4d, C3a en IgE concentrations for 2B3-201, methylprednisolone and placebo.**

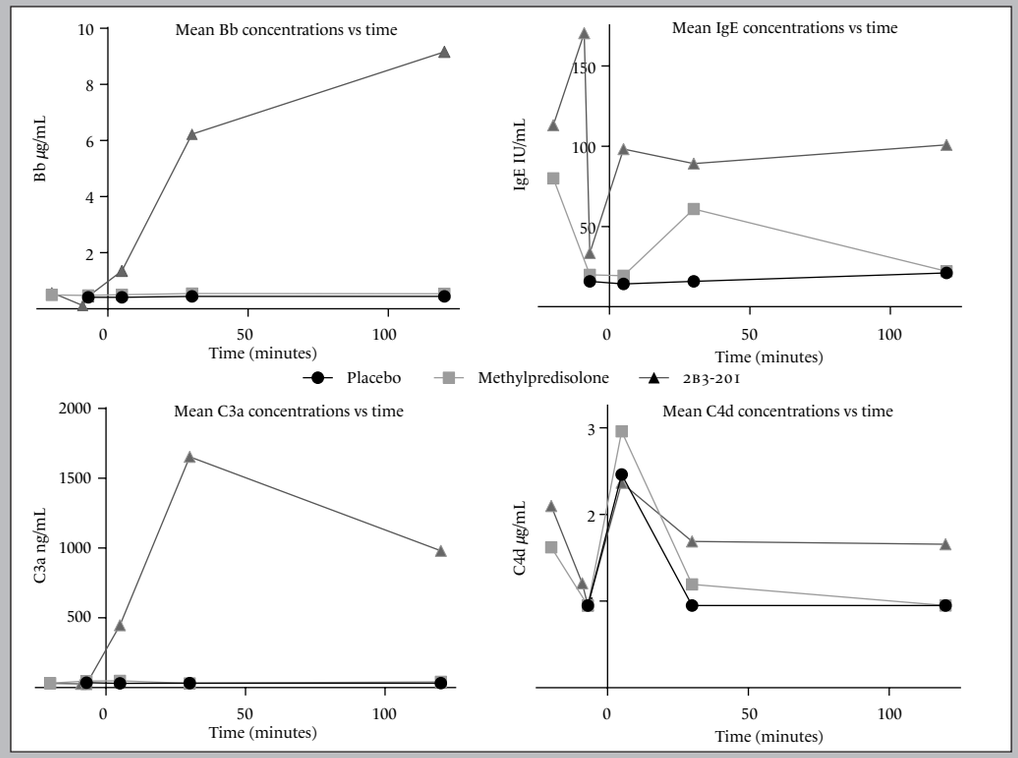

**FIGURE 2 – Serum methylprednisolone (MP) concentrations for 150, 300 and 450 mg 2B3-201, and 300 and 1000 mg MP. The concentrations of 150 mg 2B3-201 have only been measured for 50 h.**

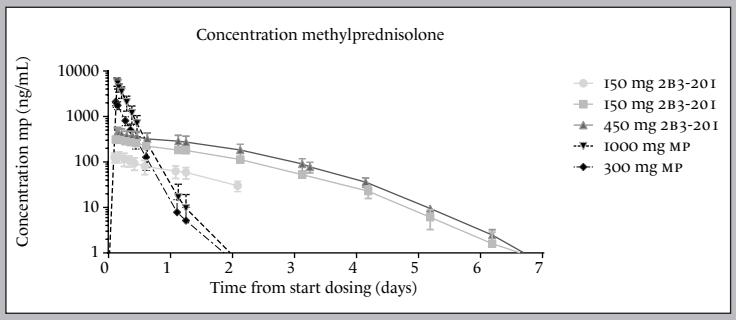


**FIGURE 3 – Pharmacodynamic measurements: graphs of lymphocyte count (A), ACTH concentrations (B), Osteocalcin concentrations (C) and fasting glucose concentrations (D) for different 2B3-201 doses, 300 and 1000 mg free methylprednisolone, and placebo.**