Supplementary methods

# Clinical phase 1 study

## Study details

This phase 1 clinical study was performed by Quotient Sciences (Quotient), Ruddington, UK, a contract research organisation, on behalf of the sponsor, Norgine Ltd.

* **Quotient Study Number**: QSC204721
* **Sponsor Study Number:** NPJ5008-01/2020
* **EudraCT Number:** 2020-005719-35
* Quotient was responsible for the management of the study
* Clinical study site: Quotient Sciences, Mere Way, Ruddington Fields, Ruddington, Nottingham NG11 6JS, UK
* Clinical laboratory evaluation was performed by The Doctors Laboratory, London, UK on behalf of Quotient
* Evaluation of plasma concentrations of the investigational medicinal product (IMP) was the responsibility of Labcorp Drug Development, Harrogate, UK (formerly known as Covance Laboratories Limited) on behalf of the sponsor: ‘Validation of a Method for the Determination of Dantrolene and 5-Hydroxydantrolene (metabolite) in Human Plasma using Liquid Chromatography with Tandem Mass Spectrometric Detection, Labcorp study number 8407591, method ID DNTRHPP, Sponsor reference NPJ5008’
* Study monitoring was performed by Bionical EMAS, Hitchin, Hertfordshire, UK on behalf of Quotient
* Medical monitoring was performed by Norgine Ltd
* Pharmacovigilance was performed by Parexel International, Mohali, Punjab, India on behalf of the sponsor
* Data management, data reporting, pharmacokinetic (PK) parameter estimation, statistical analysis, and medical writing services were performed by Quotient
* Study dates: 2 April 2021 to 13 July 2021.

## Population selection

### Informed consent

Subjects were provided with a written explanation of the study at least the day before the screening visit. A physician or nurse explained to each subject the nature of the study, its purpose, the expected duration, and the benefits and risks involved in study participation. Subjects were informed that, for safety reasons, brief details of their involvement in the study may be revealed to other units and companies that carry out clinical studies nationally. Subjects were then given the opportunity to ask questions and were informed of their right to withdraw from the study without prejudice. After this explanation and before entering the study, the subject voluntarily signed an informed consent form (ICF). Until written consent had been obtained from the subject, no study-specific procedures or investigations were performed.

### Selection of study population

Subjects were selected from a panel of volunteers recruited by Quotient or by direct advertising to the public and were screened for inclusion in the study up to 28 days before dosing. Before subjects were admitted to the clinical unit, The Over Volunteering Prevention System was checked to ensure that each subject had not been dosed in a study within 90 days of the planned first dose of this study.

### Detailed exclusion criteria

Subjects were excluded from the study if one or more of the following statements were applicable:

1. Subjects who received any IMP in a clinical research study within the 90 days prior to first dose
2. Subjects who were immediate family members of a study site or sponsor employee
3. Subjects who had previously been administered IMP in this study
4. Subjects who had taken part in Part 1 were not permitted to take part in Part 2
5. Evidence of current severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection
6. Upper respiratory tract infection in the 14 days before first IMP administration or pneumonia in the 6 months prior to IMP administration
7. History of any drug or alcohol abuse in the past 2 years
8. Regular alcohol consumption in males >21 units per week and females >14 units per week (1 unit=½ pint beer, or a 25 ml shot of 40% spirit, 1.5–2 units=125 ml glass of wine, depending on type)
9. A confirmed positive alcohol breath test at screening or admission
10. Current smokers and those who had smoked within the past 12 months: a confirmed breath carbon monoxide (CO) reading of greater than 10 ppm at screening or admission
11. Current users of e-cigarettes and nicotine-replacement products and those who had used these products within the past 12 months
12. Females of childbearing potential including those who were pregnant or lactating (all female subjects must have had a negative highly sensitive urine pregnancy test at screening). A woman was considered of childbearing potential unless she was permanently sterile (hysterectomy, bilateral salpingectomy and bilateral oophorectomy) or was post-menopausal (had no menses for 12 months without an alternative medical cause and a serum follicle-stimulating hormone [FSH] concentration ≥40 IU l–1).
13. Subjects who did not have suitable veins for multiple venepunctures/cannulation and intravenous (i.v.) infusions as assessed by the investigator or delegate at screening
14. Clinically significant abnormal clinical chemistry, haematology or urinalysis as judged by the investigator
15. Subjects with Gilbert’s syndrome were not allowed
16. Subjects with aspartate aminotransferase (AST), alanine aminotransferase (ALT) or total bilirubin greater than the upper limit of normal
17. Positive hepatitis B surface antigen (HBsAg), hepatitis C virus antibody (HCV Ab) or human immunodeficiency virus (HIV) antibody results
18. History of clinically significant cardiovascular, renal, hepatic, dermatological, chronic respiratory or gastrointestinal disease, neurological disorder or psychiatric disorder, as judged by the investigator
19. Known allergy or adverse reaction to dantrolene or any of the formulation excipients (polyethylene glycol, 2-hydroxypropyl-beta-cyclodextrin, mannitol)
20. Presence or history of clinically significant allergy requiring treatment, as judged by the investigator. Hay fever was allowed unless it was active.
21. Donation of blood or plasma within the previous 3 months or loss of greater than 400 ml of blood
22. Subjects who were taking, or had taken, any prescribed or over-the-counter drug or herbal remedies (other than up to 4 g of paracetamol per day and hormone replacement therapy) in the 14 days before IMP administration. Exceptions may have applied on a case-by-case basis, if considered not to interfere with the objectives of the study, as determined by the investigator.
23. Evidence of significant airway restriction or obstruction as assessed by spirometry at screening, e.g. forced expiratory volume in 1 s (FEV1) <80% predicted or forced vital capacity (FVC) <80% predicted, FEV1/FVC <0.7 (70%) at screening.
24. Subjects with symptoms of or history of muscle disorder or scoliosis
25. Failure to satisfy the investigator of fitness to participate for any other reason.

### Additional restrictions

In addition to the inclusion and exclusion criteria, subjects also agreed to the following restrictions:

* No alcohol during the 24 h prior to screening and the 24 h prior to admission until discharge from the study at the follow-up call
* No food or drinks containing grapefruit, cranberry, Seville oranges, caffeine or other xanthines from 24 h prior to admission until discharge from the clinic
* No food containing poppy seeds for 48 h prior to screening and for 48 h prior to admission until discharge from the clinic
* No unaccustomed strenuous exercise from the 72 h before the screening visit and then from 72 h prior to admission until discharge from the study at the follow-up call
* No donation of blood or plasma (outside this study) from screening throughout the study duration, and for at least 90 days following the last dose of study medication
* No operating an automobile or engaging in other hazardous activity for 48 h post dose
* As dantrolene may possibly evoke a photosensitivity reaction, subjects were to minimise exposure to sunlight by spending a reduced amount of time outdoors/remaining inside the clinical unit during the residential periods of the study, wearing sunglasses and clothing that minimised exposure to sunlight and using sun cream on exposed areas when outdoors. No sunbed use was permitted. This restriction was advised from the first dose until 7 days post final dose.
* Male subjects additionally had to comply with the following restrictions:
	+ If sexually active with a partner of childbearing potential, must use, with their partner, a condom plus an approved method of effective contraception from the time of informed consent until 95 days after last IMP administration.

### Physical examination

Subjects underwent a physical examination at screening, along with weight, body mass index (BMI) calculation and height measurements.

### COVID-19 test

COVID-19 tests (SARS-CoV-2 antibody and antigen tests) were performed at screening and pre-admission.

## Study conduct

### Data quality assurance

All study data were managed using a validated eCRF database system (TrialOne v4.3.8) and were subjected to data consistency and validation checks. Data queries were raised within the study eCRF database by data-management staff and resolved with the assistance of clinical staff. The clinical study report audit certificate is held by Norgine.

### Study stopping – termination criteria

#### Removal of participants

If a subject wished to leave the study at any time, they were permitted to do so. Subject completion was defined as the date of the last procedure conducted or last contact (e.g. phone call or unscheduled visit) for that subject. Subjects were to be withdrawn from the study drugs for the following reasons:

* Experiencing a serious adverse event (SAE) or severe adverse event (AE), including but not limited to:
	+ QT interval corrected with Fridericia’s formula (QTcF) of >500 ms or increase in QTcF of >60 ms from baseline (confirmed following a repeat electrocardiogram [ECG])
	+ ALT concentration >3× the upper limit of the reference range (confirmed following a repeat ALT blood test).
* Termination of the study
* Upon the subject’s request (withdrawal of consent)
* Pregnancy
* Significant deviation from the protocol
* Concurrent illness that would have adversely affected subject safety or data integrity or requirement for prohibited medication
* Evidence of current SARS-CoV-2 infection
* At the discretion of the investigator.

#### Study stopping

The study was to be halted, all dosing stopped and the risk to other subjects evaluated if any of the following criteria were met:

* A serious adverse reaction (i.e. an SAE considered at least possibly related to the IMP administration) in one subject
* Severe non-serious adverse reactions (i.e. severe non-serious AE considered at least possibly related to the IMP administration) in two subjects in the same cohort, independent of within or not within the same system organ class. Relatedness to IMP to be determined by the investigator.

If the study was halted, a temporary halt was to be submitted to the UK Medicines and Healthcare products Regulatory Agency (MHRA) and ethics committee (EC) in the form of a substantial amendment. The study could be resumed or terminated; however, it would not be resumed until a further substantial amendment to resume the study was submitted and approved by the MHRA and EC. If any of the stopping criteria were triggered, no further subjects could be dosed until the study was resumed.

#### Study termination

After the start of protocol activities but prior to the commencement of dosing, the study could be terminated by the sponsor and investigator, with notification of early termination provided to the MHRA and EC immediately and at the latest within 15 days after the study was terminated. Once exposure to dosing had begun, the study was to be completed as planned unless the following criteria were satisfied that required a temporary halt or early termination of the study:

* The occurrence of serious or severe AE(s), if considered to be related to the IMP
* New information regarding the safety of the IMP that indicated a change in the risk/benefit profile for the compound, such that the risk/benefit was no longer acceptable for subjects participating in the study
* Significant violation of Good Clinical Practice that compromised the ability to achieve the primary study objectives or subject safety.

If any of the above occurred, the study was to be terminated, with notification to the MHRA and EC, if careful review of the overall risk/benefit analysis demonstrated that the assumptions had changed and that the overall balance was no longer acceptable. Dosing was to be stopped immediately on safety grounds.

### Method of assigning subjects to groups

Subject numbers were allocated on the morning of dosing according to the code 101 to 116 (Part 1) and 201 to 205 (Part 2) using the lowest number available. For Part 1, subject numbers were allocated to treatment sequence test-reference (NPJ5008, DANTRIUM IV) or reference-test (DANTRIUM IV, NPJ5008) in a 1:1 ratio. The allocation was balanced, with eight subjects receiving each treatment sequence. For Part 1 Period 1, sentinel dosing was used; two subjects were dosed 24 h ahead of the remaining subjects, and the computerised randomisation schedule was constructed such that one of the sentinel subjects was randomised to test-reference and the other was randomised to reference-test. Part 2 of the study was non-randomised, but also included sentinel dosing of one subject ≥24 h ahead of the others.

### IMPs

Norgine Ltd was responsible for the manufacture of the IMPs (see Table 1).

**Table 1 Drug products used in NPJ5008-01/2020 (QSC204721)**

|  |  |
| --- | --- |
| **Regimen** | **Investigational medicinal producta** |
| A | NPJ5008 120 mg |
| B | DANTRIUM IV 20 mg |
| C | NPJ5008 120 mg |

a Supplied as the hydrated sodium salt form of dantrolene (dantrolene sodium hemiheptahydrate).

### Infusion time

In a previous clinical trial of i.v. dantrolene, severe AEs of muscle weakness, hypotension, dizziness, oxygen desaturation and respiratory muscle weakness were observed when doses of 1.75 mg kg–1 and 2 mg kg–1 were administered to healthy volunteers as 30 sec infusions. No severe AEs were reported when slower infusion times were used with doses up to 2.5 mg kg–1. Therefore, infusion times in this study were to have a minimum duration of 1 min (60 s). The rate of administration of DANTRIUM IV in a clinical trial setting is limited by the speed at which the volume can be delivered by available infusion pumps. While NPJ5008 could have been infused over a longer period to match DANTRIUM IV in this study, the infusion rate of both products was designed to be reflective of clinical practice as much as was technically feasible and safe in a phase 1 research unit environment.

## Pharmacokinetic and safety assessments

### Assessment schedule

A schedule of assessments, showing the PK and safety procedures, is shown in Table 2. When more than one procedure was scheduled for the same time point, the following applied to post-dose time points:

* PK samples took priority over other procedures scheduled at the same time point
* ECGs were taken prior to vital signs when both measurements were scheduled at the same time point
* Other assessments, e.g. physical examinations and spirometry measurements, were performed within the required time windows.

As guidance, the preferred order of assessments was:

ECG → vital signs → PK blood sampling (nominal time) → other assessments, e.g. physical examinations, spirometry

**Table 2 Schedule of assessments**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Study day** | **–28 to –2** | **–1** | **1** | **2** | **3** | **4** | **6 to 8** |
| Time after dosing (end of infusion)(h) | S | Aa | P | 0 | EOI | 0.08 | 0.16 | 0.25 | 0.5 | 0.75 | 1 | 1.5 | 2 | 4 | 5 | 6 | 8 | 10 | 12 | 16 | 24 | 30 | 36 | 48 | 72b | Follow-up call(final doseonly) |
| Intravenous IMP administration |  | X |  |
| **Safety assessments** |
| Physical examination | X |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Targeted (symptom-driven) physicalexaminationc |  |  | X |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | X |  |  | X | X |  |
| Safety labsd | X |  | X |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | X |  |  |  |  |  |
| Urinalysis | X |  | X |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | X |  |  |  | Xe |  |
| 12-Lead ECG | X |  | X |  |  |  |  |  |  |  | X |  |  |  | X |  |  | X |  |  | X |  |  |  |  |  |
| Vital signsf | X | X | X |  |  |  |  |  | X |  | X |  | X |  | X |  | X | X |  | X | X |  |  | X | X |  |
| Continuous peripheral oxygen saturationmonitoringg |  |  |  | ---------------------------------------------------------------X--------------------------------------------------------- |  |  |  |  |  |  |  |  |  |
| Hand-grip testh | X |  | X |  |  |  |  |  |  |  | X |  | X |  | X |  |  | X |  | X | X |  |  |  |  |  |
| Spirometry | X |  | X |  |  |  |  |  |  |  |  | X |  |  |  |  | X |  |  |  | X |  |  |  |  |  |
| Adverse events | ---------------------------------------------------------------------------------------------------------------------X------------------------------------------------------------------------------------------------------------------------- |
| Prior and concomitant medication | ---------------------------------------------------------------------------------------------------------------------X------------------------------------------------------------------------------------------------------------------------- |
| **Pharmacokinetic assessments** |
| Plasma samples for dantrolene and5-hydroxydantrolene |  |  | X |  | Xi | X | X | X | X | X | X | X | X | X |  | X | X | X | X | X | X | X | X | X | X |  |

0.08=5 min; 0.16=10 min; 0.25=15 min; 0.5=30 min; 0.75=45 min.

A, admission; EOI, immediately after end of infusion; P, pre-dose; PA, pre-admission; S, screening.

a Part 1 Period 1 and Part 2 only. In Part 1, procedures on Day –2 (as required) and Day –1 (admission) took place in Period 1 only, with both Periods 1 and 2 completed within a single residency period; subjects were resident in the clinical unit from the evening of Day –1 in Period 1 until Day 4 in Period 2. There was a minimum 5-day washout between Day 1 of Part 1 Periods 1 and 2.

b Discharge from clinical unit. For Part 1, discharge was on Day 4 of Period 2 only (Periods 1 and 2 were completed as a single residency).

c Targeted (symptom driven) physical examination of the relevant body system(s) as clinically indicated, as per the investigator’s judgement.

d Haematology and clinical chemistry at each time point including virology and follicle-stimulating hormone (post-menopausal female subjects only) at screening.

e Part 1 Period 2 and Part 2 only.

f Blood pressure, heart rate, respiratory rate and peripheral oxygen saturation at each time point. Oral temperature at screening and pre-dose only.

g Continuous peripheral oxygen saturation monitoring took place from the start of infusion on Day 1 until 8 h post dose.

h There were three hand-grip measurements per time point, each separated by at least 30 s.

i The PK sample was to be taken immediately after the end of i.v. infusion ±2 min; all PK time points relative to the end of i.v. infusion.

### Plasma sample collection

Blood samples were collected by venepuncture or indwelling cannula, according to the time schedule in Table 2, into 2 ml K2EDTA tubes, which were inverted 8–10 times immediately after collection, stored at room temperature and processed within 30 min of collection by centrifugation at 2000 *g* for 10 min at room temperature. The resultant plasma was transferred into two appropriately labelled 3.5 ml polypropylene tubes. Plasma samples were stored at room temperature and protected from sunlight until frozen within 60 min of collection. The samples were stored at –70°C or below until they were shipped to Labcorp Drug Development for the analysis of dantrolene and 5-hydroxydantrolene.

### Bioanalytical method

Table 3 presents a summary of the method developed by Labcorp for this study to assess plasma concentrations of dantrolene and its major metabolite using liquid chromatography with tandem mass spectrometry detection.

**Table 3 Bioanalytical method summary**





Dantrolene is light-sensitive; therefore all dantrolene formulations were stored protected from light, and experiments and procedures were carried out in light-reduced conditions wherever possible.

### Statistics

#### Detailed sample size and power

In Part 1, the sample size estimate of intra-subject variability (CVw) of 11% for dantrolene was obtained using area under the curve AUC0–inf data reported from a 2 × 2 crossover study in male and female subjects of 2.5 mg kg–1 DANTRIUM IV and 2.5 mg kg–1 Ryanodex Suspension for Injection. Power calculations suggest that eight subjects with reliable PK parameter estimates on both test and reference treatments are expected to give at least 80% power to conclude equivalence. However, a minimum of 12 evaluable subjects were chosen based on regulatory guidance documents. Thus, a total of 16 subjects (an even number of subjects was required to ensure a balanced number of subjects randomised to each sequence) were recruited to allow for up to 20% drop-out. An evaluable subject for the bioequivalence assessment (Part 1) will have received both the test (NPJ5008) and reference (DANTRIUM IV) regimens, and have safety and PK data to 72 h post dose for both treatments.

For Part 2 of the study, no formal sample-size calculation was made; instead, numbers were based on previous experience. An evaluable subject for the safety arm (Part 2) will have received a 120 mg dose of NPJ5008 and have safety and PK data to 72 h post dose.

#### Detailed statistical analysis methods

The data were tested for normality and homogeneity of distribution and back-transformed on the logarithmic scale.

The CVw values were calculated across both regimens, as follows: CVw=100×[exp(mean square error)−1]1/2.

For the restricted maximum likelihood method of fitting the model, the denominator degrees of freedom for the fixed effects were calculated using Kenward and Roger’s method.

#### Part 1 assessment of bioequivalence

Only subjects who completed both the test (T) and reference (R) regimens within the relevant periods were included in the statistical analysis.

Comparisons between test and reference were made using the mixed effects model and two one-sided tests were performed to obtain the *P*-value for the test of the null hypothesis of non-equivalence. The one-sided tests tested the following hypotheses against the log-transformed lower and upper limits of the equivalence acceptance range.

* Test against the lower limit:
H01: µT–µR<ln(0.8)
H11: µT–µR≥ln(0.8)
* Test against the upper limit:
H02: µT–µR>ln(1.25)
H12: µT–µR≤ln(1.25)

where H01 and H02 were the null hypothesis (test and reference are not bioequivalent) for the lower and upper limits, respectively, and H11 and H12 were the alternative hypothesis (test and reference are bioequivalent) for the lower and upper limits respectively. µT–µR was the difference between the log-transformed mean values for NPJ5008 and DANTRIUM IV.

Each one-sided test was performed at a 5% significance level, i.e. a *P*-value <5% indicated there was evidence to reject the null hypothesis of non-equivalence.

#### Part 1 assessment of relative bioavailability

A formal statistical analysis to assess relative bioavailability was performed on the PK parameters Cmax, AUC0–6, and AUC0–72 (for both dantrolene and 5-hydroxydantrolene) and AUC0–last and AUC0-inf (for 5-hydroxydantrolene only). The null hypothesis was that there was no difference between test (60 mg NPJ5008) and reference (60 mg DANTRIUM IV). Only subjects who completed both the test and reference study periods within the relevant periods were included in the statistical analysis.

#### Part 1 and Part 2 assessment of relative bioavailability

Formal statistical analysis was performed on the Parts 1 and 2 NPJ5008 dose-corrected PK parameters Cmax/D, AUC0–last/D and AUC0–inf/D, calculated from the nominal dose for dantrolene only. The model included terms for regimen as a fixed effect and body weight at admission as a covariate. Adjusted geometric means ratios and 90% CI of the ratio were obtained, where the ratio was defined as 60 mg/120 mg for the following comparison:

* 60 mg NPJ5008 (Test; Regimen A) versus 120 mg NPJ5008 (Reference; Regimen C).

The statistical analysis was performed using the actual regimen received.

### Safety measurements

#### Adverse events

Defined in protocol as follows:

An AE is any untoward medical occurrence in a subject that occurs either before dosing (referred to as a pre-dose AE) or once a medicinal product has been administered, including occurrences that are not necessarily caused by or related to that product.

An adverse drug reaction is any AE where a causal relationship with the IMP is at least a reasonable possibility (possibly related or related). Adverse events will be monitored from the time the subject signs the ICF until after the final follow-up call. The severity of AEs should be assessed as follows:

* **Mild:** An AE that is easily tolerated by the subject, causes minimal discomfort and does not interfere with everyday activities
* **Moderate:** An AE that is sufficiently discomforting to interfere with normal everyday activities; intervention may be needed
* **Severe:** An AE that prevents normal everyday activities; treatment or other intervention usually needed.

Determination of causality achieved as follows:

Every effort should be made by the investigator to try to explain each AE and assess its relationship, if any, to the IMP. The temporal relationship of the event to IMP administration should be considered in the causality assessment (i.e. if the event starts soon after IMP administration and resolves when the IMP is stopped).

Causality should be assessed using the following categories:

* **Unrelated:** Clinical event with an incompatible time relationship to IMP administration and that could be explained by underlying disease or other drugs or chemicals, or is incontrovertibly not related to the IMP
* **Related:** Clinical event with a plausible time relationship to IMP administration and that cannot be explained by concurrent disease or other drugs or chemicals.

The degree of certainty with which an AE is attributed to IMP administration (or alternative causes, e.g. natural history of the underlying disease, concomitant therapy, etc.) will be determined by how well the experience can be understood in terms of one or more of the following:

* Known pharmacology of the IMP
* Reactions of a similar nature have been previously observed with the IMP or this class of drug
* The experience being related by time to IMP administration, terminating with IMP withdrawal, or recurring on re-challenge
* Alternative cause.

Any clinically significant abnormality in laboratory parameters, vital signs or ECG could be reported as an AE according to the judgement of the principal investigator, taking into account any associated clinical signs and symptoms, and pre-dose values. During each study visit, subjects were questioned directly regarding the occurrence of any adverse medical event according to the schedule in the source documents. AEs observed or volunteered at other times were also recorded. All AEs, whether ascribed to study procedures or not, were documented immediately in the source documents. This included the date and time of onset, a description of the AE, severity (mild, moderate, severe), duration, seriousness (yes/no), actions taken, outcome and an investigator’s current opinion on the relationship between the study drug and the event.

A diagnosis and final opinion on the relationship (related or unrelated) between the study drug and the event were provided at the end of the study by the investigator. Any subjects withdrawn from the study due to an AE were to be followed up until the outcome was determined, and written reports were provided by the investigator.

#### SAEs

An SAE was defined as any untoward medical occurrence that at any dose:

* Resulted in death
* Was life-threatening
* Required inpatient hospitalisation or prolongation of existing hospitalisation, unless hospitalisation was for:
	+ Routine treatment or monitoring of the studied indication, not associated with any deterioration in condition
	+ Elective or pre-planned treatment for a pre-existing condition that was unrelated to the indication under study and had not worsened since signing the ICF
	+ Treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of a SAE given above and not resulting in hospital admission
	+ Social reasons and respite care in the absence of any deterioration in the subject’s general condition.
* Resulted in persistent or significant disability or incapacity
* Consisted of a congenital anomaly or birth defect
* Was an important medical event, i.e. defined as an event that jeopardised the subject or may have required medical or surgical intervention to prevent one of the outcomes listed above.

Serious AEs were to be immediately reported to the sponsor and pharmacovigilance provider.

Suspected unexpected serious adverse reactions (SUSARs) were AEs that were believed to be related to an IMP and were both unexpected (i.e. the nature or severity was not expected from the information provided in the investigator’s brochure) and serious. SUSARs were subject to expedited reporting.

#### Laboratory parameters

The following laboratory assessments were performed on blood samples collected by venepuncture or indwelling cannula at the time points specified in Table 2:

* Haematology: Samples were collected into K3EDTA-coated tubes (2 ml). Haemoglobin, haematocrit, erythrocytes, erythrocyte mean corpuscular volume, erythrocyte mean corpuscular haemoglobin, erythrocyte mean corpuscular haemoglobin concentration, platelets, basophils, basophils/total cells, monocytes, lymphocytes, lymphocytes/total cells, leucocytes, neutrophils, neutrophils/total, monocytes/total cells, eosinophils and eosinophils/total cells were assessed.
* Clinical chemistry: Samples were collected into serum separator tubes (5 ml) containing clot activator and serum separator gel. Sodium, potassium, chloride, bicarbonate, urea, creatinine, bilirubin, alkaline phosphatase, AST, ALT, creatine kinase, gamma-glutamyl transferase, protein, albumin, calcium, phosphate and glucose were assessed.
* Urinalysis: The following analyses were performed on urine samples using Combi Screen® urine test strips: bilirubin, blood, glucose, ketones, leucocytes, nitrites, pH, protein, specific gravity and urobilinogen. Microbiology and/or urine microscopy were to be performed based on urinalysis results at the discretion of the investigator.
* Drug screen: Urine samples were tested for drugs, including drugs of abuse, at screening and admission
* Virology: Screening for HBsAg, HCV Ab and HIV was performed using the clinical chemistry blood sample
* Alcohol and CO breath tests: These were performed at screening and admission for each study period by Quotient. A positive result for CO (greater than 10 ppm) or alcohol excluded the subject from the study or from dosing during that admission, respectively.
* Pregnancy test: Pregnancy testing (urine β-human chorionic gonadotropin) was performed using the urinalysis sample for all female subjects.
* FSH test: Serum FSH tests were performed on an aliquot taken from the clinical chemistry sample if required for post-menopausal female subjects only.

In cases where laboratory findings were outside the normal range and the investigator believed that the results could have been of clinical significance, repeat sampling was requested as clinically indicated. If the abnormal finding was clinically significant, appropriate actions were taken, e.g. the subject was not entered into the study or was withdrawn from the study. The subject was referred to their general practitioner or other appropriate provider (e.g. genitourinary medicine clinic) for further care. The same applied if the results of the HBsAg, HCV Ab or HIV test were positive, and the investigator ensured that adequate counselling was available if requested. Abnormal findings at follow-up assessments also required repeat testing if the investigator believed the results were of possible clinical significance.

Vital signsVital signs (blood pressure and heart rate) were recorded at the time points specified in Table 2 by an automated recorder after the subject had rested in the supine position for a minimum of 5 min. Temperature was measured at screening and pre dose only. Respiratory rate and peripheral oxygen saturation were measured on each occasion vital signs were checked. In addition, continuous peripheral oxygen saturation was monitored using a pulse oximeter from the start of i.v. dosing until at least 8 h post dose according to the time schedule presented in Table 2.

ElectrocardiogramSingle 12-lead ECGs were recorded after the subject had been in the supine position for a minimum of 5 min at the time points specified in Table 2.

Hand-grip testA hand-grip test was used to monitor the muscle-relaxant effects of the IMPs, according to the time schedule presented in Table 2. Grip strength was quantitated using a Jamar Hand Dynamometer, which measured grip force in kilograms. The same hand was used for each measurement. There were three hand-grip measurements per time point, each separated by at least 30 s.

SpirometryThe following lung function tests were performed using a standard calibrated spirometer: FEV1, FVC, peak expiratory flow rate and FEV1/FVC, according to the time schedule in Table 2. Predicted values were calculated according to GLI-2012 Quanjer and adjusted for age, sex and race. Throughout the study, age at the time of each spirometry assessment was used for assessment of predicted values.

# Pharmacy study

The preparation timings of NPJ5008 and DANTRIUM IV drug products (see Table 1) used in the NPJ5008-01/2020 (QSC204721) study were recorded in the pharmacy worksheets. The drug-product composition, quantity of diluent required and dosing information are presented in Table 4 (a) and (b).

**Table 4 (a) Composition of drug products with quantity of diluent required for reconstitution**

|  |  |
| --- | --- |
| **NPJ5008 120 mg** | **DANTRIUM IV 20 mg** |
| **Composition** | **Composition** |
| Dantrolene sodium | 120 mg | Dantrolene sodium | 20 mg |
| 2-hydroxypropyl-β-cyclodextrin | 3530 mg | Mannitol | 3030 mg |
| PEG3350 | 400 mg | Sodium hydroxide | 0.8–1.2 mg |
| **Diluent** | **Diluent** |
| Water for injection (WFI) | 20 ml | WFI | 60 ml |
| Final reconstituted volume | 22.6 ml | Final reconstituted volume | 62 ml |

**Table 4 (b) Drug dosing information**

|  |  |  |
| --- | --- | --- |
| **Regimen** | **Investigational medicinal product** | **Dose** |
| A | NPJ5008 120 mg powder for solution for injection (Test)Each vial reconstituted with 20 ml WFI | 60 mg as 11.3 ml of a 5.3 mg ml–1 solution |
| B | DANTRIUM IV 20 mg powder for solution for injection (Reference)Each vial reconstituted with 60 ml WFI | 60 mg as 186 ml of a0.32 mg ml–1 solution |
| C | NPJ5008 120 mg powder for solution for injection (Test)Each vial reconstituted with 20 ml WFI | 120 mg as 22.6 ml of a5.3 mg ml–1 solution |

The three different regimens (Regimens A, B and C) in the study used different pharmacy worksheets to prepare the correct drug product and amount for the subjects. Regimen A required a single vial of NPJ5008 to be prepared, whereas Regimens B and C required four and two vials to be prepared respectively. The differing number of vials used represented the total amount of drug product required for accurate delivery using a syringe driver and i.v. giving set. The total number of vials prepared in the study was dependent on the total number of subjects enrolled. The preparation steps for all three regimens are summarised in Table 5. All three regimens captured five time points during the preparation of a vial. These five discrete time points were captured in every regimen but occurred at different steps owing to the variation in the preparation of each regimen. The time points were captured in the format HH:MM:SS and can be summarised as:

1. Start time of water for injection (WFI) withdrawal
2. Time of WFI added to drug product vial
3. Time drug product fully reconstituted after vial vigorously shaken
4. Start time of drawing up of reconstituted drug product
5. End time of drawing up of reconstituted drug product

All times were entered into a pharmacy worksheet by the pharmacy operators, and the completed worksheets were provided to the Sponsor for analysis at the end of the study.

The raw data from all pharmacy worksheets were transcribed into an Excel spreadsheet for analysis and timing calculations, with the process quality controlled by two individuals.

# Laboratory simulation study

This study was to compare the preparation, reconstitution and administration times of both NPJ5008 and DANTRIUM IV vials. For this experiment, two types of cannula were used:

* 16G cannula to represent use in an adult
* 22G cannula to represent paediatric use

The procedures for each drug product are outlined in Table 5 (a) and Table 5 (b).

**Table 5 (a) Procedure for NPJ5008**

| **Step instructions** | **Timing recorded** |
| --- | --- |
| **Experiment 1: Five vials of NPJ5008 with a 16G cannula** |
| **Step 1 -** Remove the cap from the NPJ5008 vial. Remove the 20 ml syringe from its packaging. Remove a sterile needle from its packaging and add to the syringe. Open the bottle of WFI and withdraw 20 ml of WFI into the syringe.  | Record end-to-end time for all steps carried out without pausing | Record time taken to complete Step 1 |
| **Step 2 -** Reconstitute the vial with the filled syringe. Shake until the powder has been reconstituted.  | Record time taken to complete Step 2 |
| **Step 3 -** Using the same syringe and needle, collect all the solution from within the vial. Introduce 20 ml of air via the needle in order to equilibrate pressure within the vial. Attach the filled syringe to a 16G cannula via the Luer lock.  | Record time taken to complete Step 3 |
| **Step 4 -** Administer the NPJ5008 solution via the 16G cannula, into a labelled glass beaker.  | Record time taken to complete Step 4 |
| **Experiment 2: Five vials of NPJ5008 with a 22G cannula** |
| **Step 1 -** Remove the cap from the NPJ5008 vial. Remove the 20 ml syringe from its packaging. Remove a sterile needle from its packaging and add to the syringe. Open the bottle of WFI and withdraw 20 ml of WFI into the syringe.  | Record end-to-end time for all steps carried out without pausing | Record time taken to complete Step 1 |
| **Step 2 -** Reconstitute the vial with the filled syringe. Shake until the powder has been reconstituted.  | Record time taken to complete Step 2 |
| **Step 3 -** Using the same syringe and needle, collect all the solution from within the vial. Introduce 20 ml of air via the needle in order to equilibrate pressure within the vial. Attach the filled syringe to a 22G cannula via the Luer lock. | Record time taken to complete Step 3 |
| **Step 4 -** Administer the NPJ5008 solution via the 22G cannula, into a labelled glass beaker.  | Record time taken to complete Step 4 |

**Table 5 (b) Procedure for DANTRIUM IV**

| **Step instructions** | **Timing recorded** |
| --- | --- |
| **Experiment 1: Five vials of DANTRIUM IV with a 16G cannula** |
| **Step 1 -** Remove the cap from the DANTRIUM IV vial. Remove the 60 ml syringe from its packaging. Remove a sterile needle from its packaging and add to the syringe. Open the bottle of WFI and withdraw 60 ml of WFI into the syringe.  | Record end-to-end time for all steps carried out without pausing | Record time taken to complete Step 1 |
| **Step 2 -** Reconstitute the vial with the filled syringe. Shake until the powder has been reconstituted.  | Record time taken to complete Step 2 |
| **Step 3 -** Remove the filter needle from its packaging. Remove the safety cap from the filter needle. Insert spike into the bung of the reconstituted DANTRIUM IV vial. Discard the previously used needle. Connect the syringe and collect all the solution from within the vial. Introduce 60 ml of air via the needle in order to equilibrate pressure within the vial. Attach the filled syringe to a 16G cannula via the Luer lock.  | Record time taken to complete Step 3 |
| **Step 4 -** Administer the DANTRIUM IV solution via the 16G cannula into a labelled glass beaker.  | Record time taken to complete Step 4 |
| **Experiment 2: Five vials of DANTRIUM IV with a 22G cannula** |
| **Step 1 -** Remove the cap from the DANTRIUM IV vial. Remove the 60 ml syringe from its packaging. Remove a sterile needle from its packaging and add to the syringe. Open the bottle of WFI and withdraw 60 ml of WFI into the syringe.  | Record end-to-end time for all steps carried out without pausing | Record time taken to complete Step 1 |
| **Step 2 -** Reconstitute the vial with the filled syringe. Shake until the powder has been reconstituted.  | Record time taken to complete Step 2 |
| **Step 3 -** Remove the filter needle from its packaging. Remove the safety cap from the filter needle. Insert spike into the bung of the reconstituted DANTRIUM IV vial. Discard the previously used needle. Connect the syringe and collect all the solution from within the vial. Introduce 60 ml of air via the needle in order to equilibrate pressure within the vial. Attach the filled syringe to a 22G cannula via the Luer lock.  | Record time taken to complete Step 3 |
| **Step 4 -** Administer the DANTRIUM IV solution via the 22G cannula into a labelled glass beaker.  | Record time taken to complete Step 4 |

Supplementary results

# Clinical phase 1 study

## Participants

**Table 1 Summary of participant characteristics**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | **Part 1 – Treatment sequence** |  | **Part 2** |
|  |  | **NPJ5008 –****DANTRIUM IV** | **DANTRIUM IV – NPJ5008** | **Overall** | **NPJ5008 high dose** |
| Volunteers (n) |  | 8 | 8 | 16 | 5 |
| Age (years) | Mean (SD) | 44.3 (12.8)  | 34.6 (6.9) | 39.4 (11.1) | 43.6 (14.4) |
| Median (min–max) | 49.5 (23–55) | 35.5 (26–45) | 39.5 (23–55) | 46.0 (20–55) |
| Race/ethnicity | White non-Hispanic/Latino | 8 | 8 | 16 | 5 |
| Sex  | Male | 7 | 8 | 15 | 4 |
| Female | 1 | 0 | 1 | 1 |
| Height (cm) | Mean (SD) | 175.5 (6.1) | 182.9 (4.8) | 179.2 (6.5) | 177.0 (5.6) |
| Median (min–max) | 174.5 (164–182)  | 182.0 (174–189) | 181.5 (164–189) | 174 (172–184) |
| Weight (kg) | Mean (SD) | 79.83 (11.08)  | 82.05 (12.45) | 80.94 (11.44) | 86.88 (3.56) |
| Median (min–max) | 82.75 (61.5–96.3)  | 77.90 (69.8–105.5) | 82.05 (61.5–105.5) | 85.80 (83.2–92.4) |
| BMI (kg m–2) | Mean (SD) | 25.83 (2.49) | 24.58 (3.72) | 25.20 (3.13) | 27.78 (1.69) |
| Median (min–max) | 26.00 (22.0–29.4) | 23.40 (20.4–29.5) | 25.25 (20.4–29.5) | 27.90 (25.1–29.4) |
| Does subject smoke? | No | 8 | 7 | 15 | 3 |
| Previously | 0 | 1 | 1 | 2 |
| Alcohol consumption | None | 0 | 1 | 1 | 0 |
| Yes, not excessive | 8 | 7 | 15 | 5 |

BMI, body mass index; SD, standard deviation.

## Relative bioavailability

**Table 2Relative bioavailability of dantrolene in terms of Cmax and truncated area under the curve plasma pharmacokinetic parameters, following single intravenous infusion doses of 60 mg NPJ5008 (test) and 60 mg DANTRIUM IV (reference) in healthy male and female volunteers, including 90% CIs and statistical significance (*P*-values), and geometric coefficients of intra-subject variability measuring dispersion**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Parameter** | **Adjusted geometric mean****NPJ5008****(Test)** | **Adjusted geometric mean****DANTRIUM****(Ref)** | **Adjusted GMR****(Test:Ref)** | **90% CI** | ***P*-value** | **CVw** |
| Cmax (ng ml–1) | 1090 | 1170 | 92.76% | 78.27% to 109.93% | 0.45 | 27.80% |
| AUC0–6 (ng.h ml–1) | 4230 | 4790 | 88.35% | 84.95% to 91.89% | <0.001 | 6.32% |
| AUC0–72 (ng.h ml–1) | 12 200 | 13 500 | 90.27% | 85.89% to 94.88% | 0.003 | 8.01% |

P-value from two-sided test (null hypothesis of no difference)

AUC, area under the curve; Cmax, maximum observed drug concentration in plasma; CI, confidence interval; CVw, geometric coefficients of intra-subject variability; GMR, geometric mean ratio.

* Note no formal assessment of bioequivalence; however, results are very similar to those for AUC0–last and AUC0–inf.
* Ratios are close to 90%, i.e. mean exposure levels ~10% lower for NPJ5008 than for DANTRIUM IV.
* The peak exposure levels of dantrolene were not significantly different when comparing 60 mg NPJ5008 intravenous (i.v.) infusion and 60 mg DANTRIUM IV infusion. Note the CVw for Cmax is much greater than for AUCs.
* The overall exposure levels of dantrolene were significantly lower for 60 mg NPJ5008 i.v. infusion than for 60 mg DANTRIUM IV infusion. Exposure levels for NPJ5008 were 88.35% and 90.27% of those for the reference. However, the 90% CIs suggest that ratios of less than 84.95% or greater than 94.88% can be ruled out with some confidence.

**Table 3 Relative bioavailability of metabolite 5-hydroxydantrolene in terms of Cmax and AUC plasma pharmacokinetic parameters, following single intravenous infusion doses of 60 mg NPJ5008 (test) and 60 mg DANTRIUM IV (reference) in healthy male and female volunteers, including 90% CIs and statistical significance (*P*-values), and geometric coefficients of intra-subject variability measuring dispersion**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Parameter** | **Adjusted geometric****mean****NPJ5008****(Test)** | **Adjusted****geometric mean****DANTRIUM****(Ref)** | **GMR****(Test:Ref)** | **90% CI** | ***P*-value** | **CVw** |
| Cmax (ng ml–1) | 114 | 138 | 82.14% | 76.46% to 88.25% | <0.001 | 11.60% |
| AUC0–6 (ng.h ml–1) | 497 | 581 | 85.62% | 78.68% to 93.18% | 0.006 | 13.60% |
| AUC0–72 (ng.h ml–1) | 2890 | 3380 | 85.45% | 81.70% to 89.37% | <0.001 | 7.21% |
| AUC0–last (ng.h ml–1) | 2880 | 3370 | 85.26% | 81.48% to 89.22% | <0.001 | 7.30% |
| AUC0–inf (ng.h ml–1) | 2920 | 3420 | 85.58% | 81.90% to 89.43% | <0.001 | 7.07% |

P-value from two-sided test (null hypothesis of no difference)

AUC, area under the curve; Cmax, maximum observed drug concentration in plasma; CI, confidence interval; CVw, geometric coefficients of intra-subject variability; GMR, geometric mean ratio.

* On average, the peak exposure as measured by Cmax for 60 mg NPJ5008 i.v. infusion was 82.14% of that of 60 mg DANTRIUM IV infusion. This reduction was statistically significant. The 90% CI indicates the true measure is unlikely to be less than 76.46%.
* On average, overall exposure as measured by AUC0–6, AUC0–72, AUC0–last and AUC0–inf for 60 mg NPJ5008 i.v. infusion was on average between 85.26% and 85.62% of that for 60 mg DANTRIUM IV infusion. These reductions were statistically significant. The 90% CI suggests the true measure was unlikely to be less than 78.68% across all AUC parameters.

**Table 4 Metabolite-to-parent ratios (5-hydroxydantrolene:dantrolene)**

|  |  |  |
| --- | --- | --- |
|  |  | **Pharmacokinetic parameter** |
| **Regimen** | **Statistic** | **MPR Cmax**  | **MPR AUC0–last**  | **MPR AUC0–inf**  |
| 60 mg NPJ5008 i.v.(*N*=16) | *n* | 16 | 16 | 16 |
| Mean | 0.104 | 0.235 | 0.232 |
| SD | 0.030 | 0.052  | 0.051 |
| CV% | 28.5 | 22.0 | 21.8 |
| Median | 0.108 | 0.224 | 0.219 |
| Min | 0.05 | 0.14  | 0.14 |
| Max | 0.15 | 0.32  | 0.32 |
| Geometric mean | 0.099 | 0.230  | 0.227 |
| Geometric SD | 1.376 | 1.258 | 1.254 |
| Geometric CV% | 32.8 | 23.3 | 22.9 |
| 60 mg DANTRIUM IV(*N*=16) | *n* | 16 | 16 | 16 |
| Mean | 0.118 | 0.254  | 0.250 |
| SD | 0.034 | 0.080  | 0.078 |
| CV% | 28.5 | 31.5 | 31.2 |
| Median | 0.126 | 0.245 | 0.241 |
| Min | 0.04 | 0.16  | 0.16  |
| Max | 0.16 | 0.47 | 0.46 |
| Geometric mean | 0.112 | 0.243  | 0.240 |
| Geometric SD | 1.436 | 1.345 | 1.340 |
| Geometric CV% | 37.4 | 30.3 | 29.9 |

AUC, area under the curve; Cmax, maximum observed drug concentration in plasma; CV, geometric coefficient of variation; MPR, metabolite:parent ratio; SD, standard deviation.

**Table 5 Comparison of MPRs (5-hydroxydantrolene:dantrolene) and geometric mean (geometric coefficient of variation) plasma pharmacokinetic parameters following single doses of 60 mg NPJ5008 and 60 mg DANTRIUM IV in healthy participants (*n*=16)**

|  |  |  |
| --- | --- | --- |
|  | **NPJ5008 60 mg** | **DANTRIUM IV 60 mg** |
| MPR Cmax  | 0.099 (32.8) | 0.112 (37.4) |
| MPR AUC0–last  | 0.230 (23.3) | 0.243 (30.3) |
| MPR AUC0–nf  | 0.227 (22.9) | 0.240 (29.9) |

AUC, area under the curve; Cmax, maximum observed drug concentration in plasma; MPR, metabolite:parent ratio.

**Table 6 Results for dantrolene (free acid) based on Pharmacokinetics Analysis Set Part 1 (*n*=16) and Part 2 (*n*=5) relative bioavailability including weight at admission as covariate (exploratory analysis)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameter****(dose-corrected)** | **Adjusted geometric mean****NPJ5008****60 mg****(Test)** | **Adjusted geometric mean****NPJ5008****120 mg****(Ref)** | **Adjusted****GMR****(Test:Ref)** | **90% CI** | ***P*-value** |
| Cmax/D (ng ml–1 mg–1) | 22.8 | 23.0 | 99.26% | 77.51% to 127.11% | 0.96 |
| AUC0–last/D (ng.h ml–1 mg–1) | 250 | 332 | 75.21% | 61.40% to 92.13% | 0.026 |
| AUC0–inf/D (ng.h ml–1 mg–1) | 257 | 338 | 76.15% | 62.06% to 93.45% | 0.033 |

* The peak exposure of dantrolene corrected for nominal dose received (Cmax/D) was not significantly different between 60 mg and 120 mg NPJ5008 i.v. infusion dose levels, i.e. there was no statistical evidence that the peak exposure per milligram (following salt correction) was different between the two dose levels
* The overall exposure of dantrolene corrected for nominal dose received (AUC0–last/D and AUC0–inf/D) was significantly lower for the 60 mg than for the 120 mg NPJ5008 i.v. infusion, i.e. the overall exposure per milligram (following salt correction) for the 60 mg dose level was approximately 76% of that for the 120 mg dose level.

## Safety assessments

### Haematology and blood chemistry

There were no clinically important changes in mean haematology and clinical chemistry values from baseline (Day 1, pre dose) to 24 h post dose for any treatment group; the majority of values fell within the normal reference range *t*. However, a small proportion of individual values shifted from within to outside the reference range after dosing. Such shifts were recorded for no more than two subjects, with the following exceptions:

* Erythrocyte mean corpuscular haemoglobin concentration: four subjects each for 60 mg NPJ5008 and 60 mg DANTRIUM and one subject for 120 mg NPJ5008 shifted to above the reference range at the 24 h assessment after administration.
* Protein: five subjects shifted to below the reference range at the 24 h assessment after administration of 60 mg NPJ5008, as did one subject after receiving 120 mg NPJ5008.
* Calcium: five subjects shifted to below the reference range at the 24 h assessment after administration of 60 mg NPJ5008.

The majority of these values were marginally outside the reference range.

### Hand-grip strength

**Table 7 Hand-grip strength decrease at different time points after administration of 60 mg DANTRIUM IV and 60 and 120 mg NPJ5008**

|  |  |  |
| --- | --- | --- |
|  |  | **Percentage change from baseline** |
| **Regimen** | **Time point** | **Mean** | **SD** | **Median** | **Minimum** | **Maximum** |
| 60 mg NPJ5008 i.v. (*N*=16) | Baseline |  |  |  |  |  |
|  | 1 h | −9.351 | 7.780 | −8.178 | −27.59 | 4.62 |
|  | 2 h | −10.442 | 10.566 | −8.010 | −27.32 | 3.78 |
|  | 5 h | −7.172 | 8.516 | −5.488 | −21.07 | 5.11 |
|  | 10 h | −8.492 | 9.833 | −6.450 | −25.60 | 7.33 |
|  | 16 h | −14.957 | 7.910 | −14.369 | −31.72 | −0.67 |
|  | 24 h | −6.573 | 6.894 | −5.578 | −19.11 | 5.00 |
| 60 mg DANTRIUM IV (*N*=16) | Baseline |  |  |  |  |  |
|  | 1 h | −7.483 | 6.385 | −7.322 | −20.37 | 1.20 |
|  | 2 h | −7.342 | 7.541 | −8.252 | −21.61 | 10.05 |
|  | 5 h | −4.272 | 7.929 | −2.940 | −24.56 | 6.11 |
|  | 10 h | −4.171 | 8.825 | −5.761 | −22.61 | 8.88 |
|  | 16 h | −11.701 | 13.272 | −12.855 | −31.56 | 28.72 |
|  | 24 h | −3.174 | 8.887 | −4.020 | −21.28 | 14.14 |
|  |  | **Percentage change from baseline** |
| **Regimen** | **Time point** | **Mean** | **SD** | **Median** | **Minimum** | **Maximum** |
| 120 mg NPJ5008 i.v. (*N*=5) | Baseline |  |  |  |  |  |
|  | 1 h | −24.942 | 14.311 | −19.149 | −48.30 | −11.40 |
|  | 2 h | −23.348 | 17.555 | −19.400 | −43.19 | −6.88 |
|  | 5 h | −20.151  | 21.871  | −16.000  | −46.17  | 7.02 |
|  | 10 h | −14.371  | 20.238  | −9.400  | −38.94  | 6.88 |
|  | 16 h | −21.003  | 15.905 | −17.660 | −39.79  | 1.72 |
|  | 24 h | −7.911 | 10.156 | −10.600 | −17.34 | 8.17 |

i.v., intravenous; SD, standard deviation.

## Pharmacy record analysis

These data were collected in a phase 1 clinical research unit under Good Clinical Practice conditions with quality control measures to provide safe preparation of investigational medicinal products for healthy volunteers, where speed of preparation was not an objective. Notwithstanding this, the results can offer insight into the preparation times in a clinical setting and the difference between the two products.

Data from 91 vials from three treatment regimens (27×NPJ5008 vials and 64×DANTRIUM IV vials) were available for analysis. The timings showed that the preparation of a single vial of drug product in this setting was not completed in a continuous end-to-end process by the pharmacy operators. Therefore, the full end-to-end timing could not be evaluated, but interval timings could be calculated to evaluate several preparation steps within the process. For the evaluation of an interval timing, a discrete start time and end time were required for the interval. The procedure during the interval also needed to be identical between regimens, to allow comparison between products. The captured time points from the pharmacy worksheets were annotated with descriptors to indicate whether this was a START or END time:

1. Start time of water for injection withdrawal (START)
2. Time of water for injection added to drug product vial (START)
3. Time drug product fully reconstituted after vial vigorously shaken (END)
4. Start time of drawing up of reconstituted drug product (START)
5. End time of drawing up of reconstituted drug product (END).

For valid further analysis, only interval timings with a discrete ‘start’ and ‘end’ time that had
an identical process for all three regimens were included. The only interval timing to meet the set criteria was ‘Reconstitution Time’. This timing had discrete start and end times, and is standardised across all three regimens. The reasons for exclusion of all other interval timings are presented in Table 8. Four of the 91 vials used to calculate reconstitution time were deemed invalid (most common reason: entry error on pharmacy worksheet) and were excluded from the final analysis. The valid results from 25×NPJ5008 and 62×DANTRIUM IV vials were included.

**Table 8 Interval timing review and reasons for exclusion**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Interval timing name** | **Interval timing** | **Start and end time** | **Standardised (A, B, C)** | **Included or excluded** | **Comment for exclusion** |
| Water for injection draw-up time | 1 → 2 | No | Yes | Excluded | No end time recorded for water draw up |
| Reconstitution time | 2 → 3 | Yes | Yes | Included |  |
| Draw up time | 4 → 5 | Yes | No | Excluded | Regimen C draw up not a continuous process and not a representative timing (30 ml obtained from two vials) |
| End-to-end time | 1 → 5 | Yes | No | Excluded | Not a continuous process and not a representative timing |