**Supplement Table 1a.** Schedule of assessments, screening thru Week 24.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Screen1** | **MTX Run-in2**  (4 weeks) | | **Pegloticase + MTX Treatment3**  (Day 1 through Week 24) | | | | | | | | | | | | | | |
|  |  | **-4 W** | **-2 W** | **D1** | **W1** | **W2** | **W4** | **W6** | **W7** | **W8** | **W10** | **W12** | **W14** | **W16** | **W18** | **W20** | **W22** | **W24** |
| **Study Procedure/Assessment** |  |  |  | **Inf 1** |  | **Inf 2** | **Inf 3** | **Inf 4** |  | **Inf 5** | **Inf 6** | **Inf 7** | **Inf 8** | **Inf 9** | **Inf 10** | **Inf 11** | **Inf 12** |  |
| Informed consent | X |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Enrollment |  |  |  | X |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Demographic data | X |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Inclusion/exclusion criteria | X | X | X | X |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Medical/surgical history4 | X | X |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Medication/substance use history5 | X | X | X |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Physical examination6 | X | X |  | X |  |  | X |  |  | X |  | X |  | X |  | X |  | X |
| Vital signs, height, weight7 | X | X |  | X |  | X | X | X |  | X | X | X | X | X | X | X | X | X |
| Electrocardiogram8 |  |  |  | X |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| HIV antibody screening | X |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| AS/SAE assessment9 | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Concomitant medications |  |  |  | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Gout flare assessment | X | X | X | X |  | X | X | X |  | X | X | X | X | X | X | X | X | X |
| Swollen and tender joint counts |  | X |  | X |  |  |  |  |  |  |  |  | X |  |  |  |  | X |
| HAQ | X | X |  | X |  |  |  |  |  |  |  |  | X |  |  |  |  | X |
| Patient global assessment | X | X |  | X |  |  |  |  |  |  |  |  | X |  |  |  |  | X |
| Physician global assessment | X | X |  | X |  |  |  |  |  |  |  |  | X |  |  |  |  | X |
| Joint pain assessment | X | X |  | X |  |  |  |  |  |  |  |  | X |  |  |  |  | X |
| DECT10 |  |  |  | X |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Tophi assessment | X |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | X |
| MTX dosing calendar |  | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| MTX dispensed11 |  | X | X | X |  | X | X | X |  | X | X | X | X | X | X | X | X | X |
| MTX compliance/reconciliation |  |  | X | X |  | X | X | X |  | X | X | X | X | X | X | X | X | X |
| IR prophylaxis compliance12 |  |  |  | X |  | X | X | X |  | X | X | X | X | X | X | X | X | X |
| FA/GF prophylaxis compliance13 |  |  | X | X |  | X | X | X |  | X | X | X | X | X | X | X | X | X |
| Pre-infusion MTX polyGL sampling14 |  |  |  | X |  |  | X |  |  | X |  |  |  |  |  |  | X | X |
| Pegloticase PK sampling15 |  |  |  | X | X | X | X | X | X | X | X |  | X |  | X |  | X | X |
|  | **Screen1** | **MTX Run-in2**  (4 weeks) | | **Pegloticase + MTX Treatment3**  (Day 1 through Week 24) | | | | | | | | | | | | | | |
|  |  | **W-4** | **W-2** | **D1** | **W1** | **W2** | **W4** | **W6** | **W7** | **W8** | **W10** | **W12** | **W14** | **W16** | **W18** | **W20** | **W22** | **W24** |
| **Study Procedure/Assessment** |  |  |  | **Inf 1** |  | **Inf 2** | **Inf 3** | **Inf 4** |  | **Inf 5** | **Inf 6** | **Inf 7** | **Inf 8** | **Inf 9** | **Inf 10** | **Inf 11** | **Inf 12** |  |
| SU16 | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Hematology | X | X | X | X |  | X |  | X |  |  |  |  | X |  |  |  | X | X |
| Clinical chemistry | X | X | X | X |  | X |  | X |  |  |  |  | X |  |  |  | X | X |
| Spot urine collection | X | X | X | X |  | X |  | X |  |  |  |  | X |  |  |  | X | X |
| Antibody sample17 |  |  |  | X | X | X | X | X | X | X | X |  | X |  | X |  | X | X |
| G6PD deficiency screening | X |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Urine pregnancy test18 | X | X | X | X |  | X | X | X |  | X | X | X | X | X | X | X | X | X |
| Partner pregnancy inquiry19 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Clinical status assessment20 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | X |

MTX, methotrexate; D, day; W, week; Inf, infusion; AE, adverse event; SAE, serious adverse event; HAQ, health assessment questionnaire; DECT, dual-energy computed tomography; IR, infusion reaction; FA/GF, Folic acid/gout flare; polyGL, poly glutamate; PK, pharmacokinetic; SU, serum urate.

1Screening included the MTX Run-in Period and the Screening visit occurred up to 2 weeks prior to the MTX Run-in period.

2Subjects took 15 mg oral MTX per week during the 4-week Run-in Period.

3Subjects took 15 mg oral MTX per week and received 8 mg intravenous pegloticase every 2 weeks (total of 12 infusions).

4The Investigator (or their designee) collected a complete gout history and other relevant medical/surgical history.

5Included gout medication history (from diagnosis through MTX Run-in Period), substance use history, and non-gout medications used within a year of Screening.

6Clinically significant findings were recorded as AEs. Assessment of tophi was conducted at Screening and Week 24.

7Heart rate and blood pressure was measured after subject had been sitting and calm/rested for ≥5 minutes. Recorded vital signs were not measured during pegloticase infusion. Weight was measured without shoes on Day 1 and at Weeks 8, 16, and 24. Height was measured only at the Screening visit.

8Electrocardiograms were recorded prior to the first pegloticase infusion.

9Investigators completed additional information for possible infusion reactions or anaphylaxis.

10At sites with DECT capabilities, DECT images were obtained at Day 1 and Week 24.

11MTX was dispensed and brought back to each visit to check compliance. If the subject required an MTX dose reduction, the Investigator changed the number of tablets to take weekly. The updated number of tablets, along with the date and time of each MTX dose, was recorded in the dosing calendar. MTX should have been taken 1 to 3 days prior to each pegloticase infusion, but was required ≥60 min prior to each infusion.

12Infusion prophylaxis consisted of oral fexofenadine (60 or 180 mg based on Investigator’s discretion) the day before and morning of infusion, oral acetaminophen (1000mg) the morning of infusion, and intravenous methylprednisolone (125 mg infused over 10-3- minutes) or hydrocortisone (200 mg) immediately prior to each infusion.

13 Subjects were required to complete at least one standard gout flare prophylaxis protocol (e.g., colchicine and/or non-steroidal anti=inflammatory drugs and/or low-dose prednisone ≤10 mg/day) at least 1 week before the first pegloticase dose and continue flare prophylaxis per American College of Rheumatology guidelines (the longer of 6 months or 3 months after first SU reading ≤6 mg/dL [6 months after SU reading ≤5 mg/dL for patients with tophaceous gout]). Subjects took 1 mg oral folic acid every day from Week -4 to the end of the study.

14Blood samples were collected prior to pegloticase infusion and after the end of infusion on Day 1 and at Weeks 4, 8, 22, and 24 to measure MTX polyglutamate levels.

15Blood samples were collected prior to pegloticase infusion and after the end of infusion on Day 1 and at Weeks 2, 4, 6, and 8 and prior to pegloticase infusion at Weeks 10, 14, 18, 22, and 24. Randomly selected subjects, who consented for frequent PK sampling, also had PK sampling at Weeks 1 and 7 (morning preferred).

16Serum samples for SU levels were collected at Screening (≤2 weeks of first MTX dose at Week -4), Week -4 (prior to first MTX dose), Week -2, and within 48 hours prior to and immediately after the end of each pegloticase infusion (except on Day 1). Randomly selected subjects who consented to more frequent sampling also had SU levels measured at Weeks 1 and 7. Two blood samples were collected for SU level measurement; one sample was tested by the site’s local laboratory and the other was sent to the central laboratory. A subject with an SU level ≥6 mg/dL at 2 consecutive visits, beginning at Week 2, was classified as a non-responder. The subjects continued in the study without further pegloticase/methotrexate therapy.

17Serum samples for evaluation of anti-PEG and anti-uricase IgG antibodies were collected prior to pegloticase infusion on Day 1 and at Weeks 2, 4, 6, 8, 10, 14, 18, 22, and 24. Visits for frequent sampling of a subset of subjects who consented for additional non-infusion visit PK sampling (random, morning preferred) also had antibody evaluation at Weeks 1 and 7. In the event of a suspected infusion reaction, a serum sample was collected at that time or at the subsequent visit for evaluation of pegloticase antibodies.

18For women of childbearing potential, a serum pregnancy test was performed at Screening. A urine pregnancy test was performed at each visit (except Weeks 1 and 7 in high frequency PK sampling subjects) thereafter.

19Non-vasectomized males were asked about partner pregnancy.

20The Investigator reviewed the clinical status of the subject at Week 24.

**Supplement Table 1b.** Schedule of assessments, Weeks 26-52.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Pegloticase + MTX Treatment1**  (Week 26 through Week 50) | | | | | | | | | | | | | **End of infusions** | **EOS/ET** | **Safety Contact** | **MTX partner pregnancy follow-up** | **Follow-up** |
|  | **W26** | **W28** | **W30** | **W32** | **W34** | **W36** | **W38** | **W40** | **W42** | **W44** | **W46** | **W48** | **W50** | **≤2 wks of final inf** | **Wk 52** | **30 days after last inf** | **3 mo after last MTX** | **3 and 6 mo** |
| **Study Procedure/Assessment** | **Inf 14** | **Inf 15** | **Inf 16** | **Inf 17** | **Inf 18** | **Inf 19** | **Inf 20** | **Inf 21** | **Inf 22** | **Inf 23** | **Inf 24** | **Inf 25** | **Inf 26** |  |  |  |  |  |
| Physical examination2 |  |  |  |  |  | X |  |  |  |  |  |  |  | X | X |  |  | X |
| Vital signs, height, weight3 | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |  |  | X |
| AS/SAE assessment4 | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |  | X |
| Concomitant medications | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |  |  | X |
| Gout flare assessment | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |  |  | X |
| Swollen/tender joint counts |  |  |  |  |  | X |  |  |  |  |  |  |  | X | X |  |  | X |
| HAQ |  |  |  |  |  | X |  |  |  |  |  |  |  | X | X |  |  | X |
| Patient global assessment |  |  |  |  |  | X |  |  |  |  |  |  |  | X | X |  |  | X |
| Physician global assessment |  |  |  |  |  | X |  |  |  |  |  |  |  | X | X |  |  | X |
| Joint pain assessment |  |  |  |  |  | X |  |  |  |  |  |  |  | X | X |  |  | X |
| DECT5 |  |  |  |  |  | X |  |  |  |  |  |  |  | X | X |  |  | X |
| Tophi assessment |  |  |  |  |  | X |  |  |  |  |  |  |  | X | X |  |  | X |
| MTX dosing calendar | X | X | X | X | X | X | X | X | X | X | X | X | X |  |  |  |  |  |
| MTX dispensed6 | X | X | X | X | X | X | X | X | X | X | X | X | X |  |  |  |  |  |
| MTX compliance/reconciliation | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |  |  |  |
| IR prophylaxis compliance7 | X | X | X | X | X | X | X | X | X | X | X | X | X |  |  |  |  |  |
| FA/GF prophylaxis compliance8 | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |  |  |  |
| Pegloticase infusion | X | X | X | X | X | X | X | X | X | X | X | X | X |  |  |  |  |  |
| Pre-infusion MTX polyGL sampling9 |  |  |  |  |  | X |  |  |  |  |  |  |  |  |  |  |  |  |
| Pegloticase PK sampling10 |  |  |  |  |  | X |  |  |  |  |  |  |  | X | X |  |  |  |
| SU11 | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |  |  | X |
| Hematology |  |  |  |  |  | X |  |  |  |  |  |  |  | X | X |  |  | X |
| Clinical chemistry |  |  |  |  |  | X |  |  |  |  |  |  |  | X | X |  |  | X |
| Spot urine collection |  |  |  |  |  | X |  |  |  |  |  |  |  | X | X |  |  |  |
| Antibody sample12 |  |  |  |  |  | X |  |  |  |  |  |  |  | X | X |  |  | X |
| Urine pregnancy test13 | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |  |  |
| Partner pregnancy inquiry14 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | X |  |
| Clinical status assessment15 |  |  |  |  |  |  |  |  |  |  |  |  |  | X | X |  |  |  |

MTX, methotrexate; EOS/ET, end of study/early termination; D, day; W, week; mo, month; Inf, infusion; AE, adverse event; SAE, serious adverse event; HAQ, health assessment questionnaire; DECT, dual-energy computed tomography; IR, infusion reaction; FA/GF, Folic acid/gout flare; polyGL, poly glutamate; PK, pharmacokinetic; SU, serum urate.

1Subjects took 15 mg oral MTX per week and received 8 mg intravenous pegloticase every 2 weeks (total of 12 infusions Weeks ).

2Clinically significant findings were recorded as AEs. Assessment of tophi was conducted at Week 36, the End of Pegloticase Treatment, Week 52 (or End of Study/Early Termination), and Post Treatment 3 and 6 month Follow-up visits.

3Heart rate and blood pressure were measured after subject had been sitting and calm/rested for ≥5 minutes. Recorded vital signs were not measured during pegloticase infusion. Weight was measured without shoes at Week 36, the End of Pegloticase Treatment, Week 52 (or End of Study/Early Termination), and Post Treatment 3 and 6 month Follow-up visits. Height was measured only at the Screening visit.

4Investigators completed additional information for possible infusion reactions or anaphylaxis.

5At sites with DECT capabilities, DECT images were obtained at Week 36, the End of Pegloticase Treatment, Week 52 (or End of Study/Early Termination), and Post Treatment 3 and 6 month Follow-up visits.

6MTX was dispensed and brought back to each visit to check compliance. If the subject required an MTX dose reduction, the Investigator changed the number of tablets to take weekly. The updated number of tablets, along with the date and time of each MTX dose, was recorded in the dosing calendar. MTX should have been taken 1 to 3 days prior to each pegloticase infusion, but was required ≥60 min prior to each infusion.

7Infusion prophylaxis consisted of oral fexofenadine (60 or 180 mg based on Investigator’s discretion) the day before and morning of infusion, oral acetaminophen (1000mg) the morning of infusion, and intravenous methylprednisolone (125 mg infused over 10-3- minutes) or hydrocortisone (200 mg) immediately prior to each infusion.

8Subjects were required to complete at least one standard gout flare prophylaxis protocol (e.g., colchicine and/or non-steroidal anti=inflammatory drugs and/or low-dose prednisone ≤10 mg/day) at least 1 week before the first pegloticase dose and continue flare prophylaxis per American College of Rheumatology guidelines (the longer of 6 months or 3 months after first SU reading ≤6 mg/dL [6 months after SU reading ≤5 mg/dL for patients with tophaceous gout]). Subjects took 1 mg oral folic acid every day from Week -4 to the end of the study.

9Blood samples were collected prior to pegloticase infusion and after the end of infusion at Week 36 to measure MTX polyglutamate levels.

10Blood samples were collected prior to pegloticase infusion and after the end of infusion on Day 1 and at Weeks 2, 4, 6, and 8 and prior to pegloticase infusion at Week 36, the End of Pegloticase Treatment, and Week 52 (or End of Study/Early Termination).

11Serum samples for SU levels were collected at within 48 hours prior to and immediately after the end of each pegloticase infusion, at the End of Pegloticase Treatment, at Week 52 (or End of Study/Early Termination), and Post Treatment 3 and 6 month Follow-up visits. Two blood samples were collected for SU level measurement; one sample was tested by the site’s local laboratory and the other was sent to the central laboratory. A subject with an SU level ≥6 mg/dL at 2 consecutive visits, beginning at Week 2, was classified as a non-responder. The subjects continued in the study without further pegloticase/methotrexate therapy.

12Serum samples for evaluation of anti-PEG and anti-uricase IgG antibodies were collected prior to pegloticase infusion at Week 36 and the End of Pegloticase Treatment. Samples were also collected at Week 52 (or End of Study/Early Termination) and Post Treatment 3 and 6 month Follow-up visits. In the event of a suspected infusion reaction, a serum sample was collected at that time or at the subsequent visit for evaluation of pegloticase antibodies.

13For women of childbearing potential, a urine pregnancy test was performed at each visit.

14 Subjects who were non-vasectomized males were asked 3 months after MTX discontinuation regarding partner pregnancy. This occurred at a regulatory scheduled visit or by a separate phone/email/site visit.

15The Investigator reviewed the clinical status of the subject at End of Pegloticase Treatment and Week 52 (or End of Study/Early Termination).