ONLINE SUPPLEMENTARY MATERIAL

Supplementary Table 1. Study inclusion and exclusion criteria.

Inclusion Criteria:

Eligible subjects must meet/provide **all** of the following:

1. Willing and able to give informed consent.
2. Willing and able to comply with the prescribed treatment protocol and evaluations, for the duration of the study.
3. Adult man or woman ≥18 and ≤65 years of age.
4. Women of childbearing potential (including those with an onset of menopause <2 years prior to screening, non-therapy-induced amenorrhea for <12 months prior to screening, or not surgically sterile [absence of ovaries and/or uterus]) must have negative serum/urine pregnancy tests during the Screening/MTX Run-in Period; subjects must agree to use 2 reliable forms of contraception during the study, one of which is recommended to be hormonal, such as an oral contraceptive. Hormonal contraception must be started ≥1 full cycle prior to Week -4 (start of MTX dosing) and continue for 30 days after the last dose of pegloticase or at least one ovulatory cycle after the last dose of MTX (whichever is the longest duration after the last dose of pegloticase or MTX). Highly effective contraceptive methods (with a failure rate <1% per year), when used consistently and correctly, include implants, injectables, combined oral contraceptives, some intrauterine devices, sexual abstinence, or vasectomized partner.
5. Men how are not vasectomized must agree to not impregnate their female partner during the study and for ≥3 months after the last MTX dose.
6. Hyperuricemia at the Screening, Week -4, or Week -2 Visit of the Screening or Run-in Period, as documented by sUA ≥6 mg/dL.
7. Uncontrolled gout, defined as meeting the following criteria:
    - sUA ≥6 mg/dL prior to the pegloticase + MTX Period (any laboratory tests during screening up to and including during the MTX Run-in Period) and at least one of the following:
        - inability to maintain sUA <6 mg/dL on other urate-lowering therapy
        - intolerable side effects associated with current urate-lowering therapy
        - functionally limiting tophaceous deposits (including those detected clinically or by DECT imaging)
8. Able to tolerate MTX 15 mg for 4 weeks during the MTX Run-in Period prior to the first dose of pegloticase.

Exclusion Criteria:

Subjects will be ineligible for study participation if they meet **any** of the following criteria:

1. Weight >160 kg (352 pounds).
2. Any serious active bacterial infection, unless treated and completely resolved with antibiotics, at least 2 weeks prior to the Week -4 visit of the MTX Run-in Period.
3. Severe chronic or recurrent bacterial infection, such as recurrent pneumonia, chronic bronchiectasis.
4. Current immunocompromised condition, including current or chronic treatment with systemic immunosuppressive agents, including prednisolone >10 mg/day or equivalent dose of other corticosteroid.
5. History of any transplant surgery requiring maintenance immunosuppressive therapy.
6. Known history of hepatitis B virus surface antigen positivity or hepatitis B DNA positivity.

7. Known history of hepatitis C virus RNA positivity.
8. Human Immunodeficiency Virus (HIV) positivity (tested at Screening Visit).
9. Glucose-6-phosphate dehydrogenase (G6PD) deficiency (tested at Screening Visit).
10. Severe chronic renal impairment (GFR <25 mL/min/1.73m^2) or currently on dialysis.
11. Non-compensated CHF or hospitalization for CHF within 3 months of the Screening Visit, uncontrolled arrhythmia, treatment for acute coronary syndrome (MI or unstable angina), or uncontrolled blood pressure (>160/100 mmHg) at the end of the Screening and MTX Run-in Period.
12. Pregnant, planning to become pregnant, breastfeeding, planning to impregnate female partner, or not on an effective form of birth control, as determined by the Investigator.
13. Prior treatment with pegloticase (KRYSTEXXA®), another recombinant uricase (rasburicase), or concomitant therapy with a PEG-conjugated drug.
14. Known allergy to PEGylated products or history of anaphylactic reaction to a recombinant protein or porcine product.
15. Contraindication to MTX treatment or MTX treatment considered inappropriate.
16. Known intolerance to MTX.
17. Receipt of an investigational drug within 4 weeks or 5 half-lives, whichever is longer, prior to MTX administration at Week -4 or plans to take an investigational drug during the study.
18. Current liver disease, as determined by ALT or AST levels >3 times upper limit of normal at the Screening Visit.
19. Currently receiving systemic or radiologic treatment for ongoing cancer, excluding non-melanoma skin cancer.
20. History of malignancy within 5 years other than non-melanoma skin cancer or in situ carcinoma of cervix.
21. Uncontrolled hyperglycemia with a plasma glucose value >240 mg/dL at screening that is not subsequently controlled by the end of the Screening/MTX Run-in Period.
22. Diagnosis of osteomyelitis.
23. Known history of hypoxanthine-guanine phosphoribosyl-transferase deficiency, such as Lesch-Nyhan and Kelley-Seegmiller syndrome.
24. Unsuitable candidate for the study, based on the opinion of the Investigator (e.g., cognitive impairment), such that participation might create undue risk to the subject or interfere with the subject's ability to comply with the protocol requirements or complete the study.
25. Alcohol use in excess of 3 alcoholic beverages per week.
26. Currently receiving allopurinol and unable to discontinue medication 7 days prior to MTX dosing at Week -4 and unable to discontinue treatment during the duration of the study.

MTX, methotrexate; DECT, dual-energy computed tomography; GFR, glomerular filtration rate; CHF, congestive heart failure; MI, myocardial infarction; PEG, polyethylene glycol; ALT, alanine transaminase; AST, aspartate transaminase.

Supplementary Table 2. Schedule of assessments for Run-in and first 24 weeks of pegloticase + methotrexate treatment period

	Screening[1]/ MTX Run-in Period[2]			**Pegloticase + MTX Treatment[3]** (Day 1 through Week 24)														
	Screen Visit[4]	**-4 W** (±3d)	**-2 W** (±3d)	**D1**	**W1** (±1d)	**W2** (±3d)	**W4** (±3d)	**W6** (±3d)	**W7** (±1d)	**W8** (±3d)	**W10** (±3d)	**W12** (±3d)	**W14** (±3d)	**W16** (±3d)	**W18** (±3d)	**W20** (±3d)	**W22** (±3d)	**W24** (±3d)
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 history[5]	X	X																
Medication/substance use history[6]	X	X	X															
Physical examination[7]	X	X		X			X			X		X		X		X		X
Vital signs, height, weight[8]	X	X		X		X	X	X		X	X	X	X	X	X	X	X	X
Electrocardiogram[9]				X														
HIV antibody screening	X																	
AS/SAE assessment[10]	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
Document gout flares and intensity	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					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
DECT[11]				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 dispensed[12]		X	X	X		X	X	X		X	X	X	X	X	X	X	X	X
MTX dosing[13]		Once weekly from Week -4 to one week after the last pegloticase infusion																
Gout prophylaxis Rx filled[14]		Rxs filled as needed																
Fexofenadine Rx filled[15]		Rx filled as needed																

Folic acid Rx filled[16]		Rx filled as needed																
MTX compliance/reconciliation			X	X		X	X	X		X	X	X	X	X	X	X	X	X
	Screening[1]/ MTX Run-in Period[2]			**Pegloticase + MTX Treatment[3]** (Day 1 through Week 24)														
	Screen Visit[4]	**-4 W** (±3d)	**-2 W** (±3d)	**D1**	**W1** (±1d)	**W2** (±3d)	**W4** (±3d)	**W6** (±3d)	**W7** (±1d)	**W8** (±3d)	**W10** (±3d)	**W12** (±3d)	**W14** (±3d)	**W16** (±3d)	**W18** (±3d)	**W20** (±3d)	**W22** (±3d)	**W24** (±3d)
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**	
IR prophylaxis[17]				X		X	X	X		X	X	X	X	X	X	X	X	
IR prophylaxis compliance				X		X	X	X		X	X	X	X	X	X	X	X	X
FA/GF prophylaxis compliance			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	
Pre-infusion MTX polyGL sampling[18]				X			X			X							X	X
Pegloticase PK sampling[19]				X	X	X	X	X	X	X	X		X		X		X	X
sUA[20]	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 sample[21]				X	X	X	X	X	X	X	X		X		X		X	X
G6PD deficiency screening	X																	
Urine pregnancy test[22]	X	X	X	X		X	X	X		X	X	X	X	X	X	X	X	X
Partner pregnancy[23]																		
Investigator clinical status assessment[24]																		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; sUA, serum uric acid; G6PD, glucose-6-phosphate dehydrogenase.

1. The Screening Period is inclusive of the MTX Run-in Period.
2. During the MTX Run-in Period, subjects took oral MTX 15 mg weekly, which began 4 weeks prior to the first dose of pegloticase. Subjects unable to tolerate 15 mg of MTX during the Run-in period were considered screen failures.
3. It was recommended that before a subject began the Pegloticase + MTX Period, he or she had been taking the per protocol standard gout flare prophylaxis regimen for ≥1 week prior to pegloticase infusion. During the Pegloticase + MTX Period subjects continued taking MTX weekly until 1 week after the last pegloticase infusion. Subjects received pegloticase infusions every 2 weeks Day 1 through Week 22.
4. The Week -6 Visit was designated the Screening Visit and occurred any time within the 2 weeks prior to the first dose of MTX at Week -4.
5. The Investigator or designee collected a complete gout history and other relevant medical/surgical history.
6. Medication history (i.e., prior medications) included gout medications, starting at the time of diagnosis and up to (but not including) the Day 1 Visit; substance use history; History of all prior gout medications was collected. History of non-gout medication use in the year prior to Screening was collected.

7. A complete physical examination was performed at the Screening Visit and included assessments for presence of tophi, as well as gout history and symptom severity. A targeted physical examination (included heart, lungs, and abdominal exam and exam for joint and skin evaluation and assessment of AEs) was conducted based on potential risk for or occurrence of AEs at Week -4, Day 1, and prior to administration of pegloticase at Weeks 4, 8, 12, 16, and 20. Clinically significant findings from the targeted physical examinations were recorded as AEs. At Week 24, an assessment for presence of tophi was conducted during the targeted physical examination.
8. Heart rate and blood pressure measurements were taken after the subject had been in a sitting position and in a rested and calm state for at least 5 minutes and, for study visits during the Pegloticase + MTX Period, before the pegloticase infusion and any time after the end of the infusion, but prior to subject's discharge/release from the site. Weight was measured in kilograms or pounds without shoes and recorded at Screening Visit; prior to pegloticase infusions on Day 1 and at the Week 8, 16, and 24 follow-up visits. Height was collected at the Screening Visit only.
9. Electrocardiogram was completed prior to the pegloticase infusion at Day 1 Visit.
10. AEs/SAEs were collected from signature of the ICF. Serious AEs were captured/monitored at the Safety Follow-up Phone/Email/Site Visit 30 days after the last dose of MTX. At the Safety Follow-up Phone/Email/Site Visit, females of childbearing potential were asked to confirm if ovulation has occurred since the last dose of MTX. If the subject had not ovulated, a urine pregnancy test was required. For each AE, Investigators recorded if the event was possibly an infusion reaction or anaphylaxis and if so, were prompted to complete additional CRFs.
11. For sites with DECT capability, DECT was obtained at Day 1 and 24. The DECT was completed within +/- 5 days of the scheduled timepoint.
12. MTX was dispensed and brought back at each visit to check compliance. If subjects required an MTX dose reduction, the Investigator prescribed the subject 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.
13. MTX was taken 1 to 3 days prior to pegloticase infusion; however, if a subject did not do so, MTX was taken ≥60 minutes prior to pegloticase infusion.
14. For gout prophylaxis, subjects were required to take at least one protocol standard gout flare prophylaxis regimen (i.e. colchicine and/or non-steroidal anti-inflammatory drugs and/or low-dose prednisone ≤10 mg/day) for ≥1 week before the first dose of pegloticase and continue flare prophylaxis per American College of Rheumatology guidelines for the greater of 1) 6 months, 2) 3 months after achieving target serum urate (sUA < 6 mg/dL) for patients with no tophi detected on physical exam, or 3) 6 months after achieving target serum urate (sUA < 5 mg/dL) for patients with one or more tophi detected on initial physical exam that have since resolved. For IR prophylaxis, fexofenadine (60 mg or 180 mg orally based on the Principal Investigator's discretion) was taken the day before each infusion; fexofenadine (60 mg or 180 mg orally based on the Principal Investigator's discretion) and acetaminophen (1000 mg orally) were taken the morning of each infusion; and methylprednisolone (125 mg IV) given over the infusion duration 10-30 minutes (recommended) or hydrocortisone (200 mg IV) was administered immediately prior to each infusion given over the infusion duration 10-30 minutes was administered immediately prior to each infusion.
15. For IR prophylaxis, fexofenadine (60 mg or 180 mg orally based on the Principal Investigator's discretion) was taken the day before each infusion.
16. Subjects took folic acid 1 mg orally every day beginning at Week -4 (the start of MTX) through Week 24 during the Pegloticase + MTX Period.
17. Infusion reaction prophylaxis included fexofenadine (60 mg or 180 mg orally based on the Principal Investigator's discretion) administered the day before each infusion; fexofenadine (60 mg or 180 mg orally based on the Principal Investigator's discretion) and acetaminophen (1000 mg orally) administered on the morning of each infusion; and methylprednisolone (125 mg IV) given over the infusion duration 10-30 minutes (recommended) or hydrocortisone (200 mg IV) was administered immediately prior to each infusion, administered immediately prior to each infusion.
18. Blood samples were collected prior to pegloticase infusion on Day 1 and at Weeks 4, 8, 22, and 24 during the Pegloticase + MTX Period for MTX Polyglutamate levels.

19. For all subjects, serum samples for PK analysis were collected prior to pegloticase infusion and after the end of infusion (prior to discharge) on Day 1 and at the Weeks 2, 4, 6, and 8 visits and prior to pegloticase infusion only at the Weeks 10, 14, 18, and 22. Visits for frequent sampling of a subset of subjects who consented for additional non-infusion visit PK sampling (random, morning preferred) occurred at Week 1 and Week 7.
20. Serum samples for measurement of sUA levels were collected at the Screening Visit (within 2 weeks prior to the first dose of MTX at Week -4), the Week -4 Visit (prior to the first dose of MTX), and the Week -2 Visit; within 48 hours prior to each pegloticase infusion (except on Day 1 when only 1 pre-infusion sample is required and will be drawn at the site just prior to the infusion); and after the end of each pegloticase infusion prior to discharge, from the Pegloticase + MTX Period through week 24. Visits for frequent sampling of a subset of subjects who consented for additional non-infusion visit PK sampling (random, morning preferred) occurred at Week 1 and Week 7. Two separate samples/tubes of blood were collected within 48 hours prior to the pegloticase infusion (except on Day1 when only 1 pre-infusion sample was required for the central laboratory). One sample/tube was assessed by the site's local laboratory to be used for on-study subject management; pre-infusion sUA results must be reported by the local or central laboratory prior to each pegloticase infusion. If a local laboratory sample was drawn (within 48 hours prior to the pegloticase infusion), a sample for the central laboratory was drawn prior to the pegloticase infusion on the day of the visit. The second sample/tube was sent to the central laboratory for analysis and recording in the database. A subject with sUA level >6 mg/dL (based on the local or central laboratory) at 2 consecutive study visits, beginning with the Week 2 Visit, was classified as a non-responder
21. Serum samples for evaluation of anti-PEG and anti-uricase IgG antibodies were collected prior to the pegloticase infusion on Day 1 and at the 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) occurred at Week 1 and Week 7. In the event of an AE suspected to be an infusion reaction, a serum sample was collected at that time or at the subsequent visit for evaluation of pegloticase antibodies.
22. For women of childbearing potential, a serum pregnancy test was performed at the Screening Visit. A urine pregnancy test was performed at each visit until 30 days after the last MTX dose if the subject had not ovulated and at the 30 day follow up phone/e-mail/site visit it is determined that the subject had not ovulated since the last dose of MTX; a urine pregnancy test was performed at all other indicated visits.
23. 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.
24. The Investigator reviewed the clinical status of the subject at the Week 24 Visitor the Early Termination Visit.