Supplementary Online Content


eMethods. Pegloticase antibody assays

eFigure 1. Standardized serial digital photographs of tophus size reduction over time of biweekly pegloticase treatment: patient 314-001

eFigure 2. Standardized serial digital photographs of tophus size reduction over time of biweekly pegloticase treatment: patient 111-006

This supplementary material has been provided by the authors to give readers additional information about their work.
eMethods

Pegloticase antibody assays
Antibody responses to pegloticase were measured in serum samples collected at baseline and at selected
time points after administration of study medication. Specific assays were designed to detect: 1) IgG
and IgM antibody to pegloticase and 2) neutralizing antibody to pegloticase.

1) IgG and IgM antibody to pegloticase. Antibody to pegloticase was assessed using pegloticase
as the capture antigen in microtiter ELISA plates. Serum samples were applied followed by
administration of peroxidase-conjugated secondary antibody recognizing human IgG, IgM, or all
human antibody immunoglobulin heavy chain isotypes. Specific antibody levels were detected by
addition of substrate and quantitated by spectrophotometry. A human serum pegloticase-antibody
positive control was used. Study serum samples were initially diluted 1/30 in assay buffer (0.05%
Tween 20 in blocker casein in phosphate buffered saline) for screening purposes. Samples with
results exceeding a statistically defined negative cut-off were defined as screen-positive for antibody
to pegloticase. The specificity of antibody responses was determined by competition against soluble
pegloticase, and antibody titers and immunoglobulin heavy chain isotypes (IgG and IgM) were
determined.

2) Neutralizing anti-pegloticase antibody. The neutralization assay was a coupled
enzymatic/fluorometric assay in which the capacity of anti-pegloticase antibody to inhibit the
enzymatic activity of pegloticase in vitro was determined.
eFigure 1. Standardized serial digital photographs of tophus size reduction over time of biweekly pegloticase treatment: patient 314-001
eFigure 2. Standardized serial digital photographs of tophus size reduction over time of biweekly pegloticase treatment: patient 111-006