# Oral treatment with PBI-1402 improves glomerular filtration rate by reducing tubulointerstitial fibrosis and sclerosis in 5/6-nephrectomized rats

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## Abstract

PBI-1402 is a novel orally active low molecular weight compound with erythropoiesis stimulating activity distinct from erythropoietin (EPO). Recently, we demonstrated that PBI-1402 could increase RBC and Hb in 5/6-nephrectomized (5/6-Nx) rats, a model of chronic kidney disease/end stage renal disease. The purpose of this study was to investigate the renoprotection of this compound.

Male Sprague Dawley rats (6 week-old) were subjected to 5/6-Nx or sham operations. Treatment started at day 21 with water or PBI-1402 (oral, 200 mg/kg once a day), ended at day 56 and rats were sacrificed on day 63. Serum creatinine and GFR were assessed on day 21 (before treatment) and day 42.

Rats treated with PBI-1402 demonstrated a significant ($p<0.001$) lower serum creatinine level than control. In fact, remission, as defined by a significant improvement (>20 µmol/L) of serum creatinine at day 42 relative to day 21, was 8.3% for the control versus 58.3% for PBI-1402-treated rats. A GFR improvement was observed in 33% of control rats versus 61.5% of PBI-1402-treated rats ($p<0.01$). This improvement was 13% in the control, versus 55% for PBI-1402-treated rats. Histological lesion scores of kidney were also significantly ($p<0.05$) reduced in PBI-1402-treated rats (2.8±1.0) compared to control (4.2±0.9), as determined by HPE, PAS and Masson's trichrome staining. Tubulo-interstitial fibrosis and sclerosis were significantly ($p<0.05$) reduced by treatment with PBI-1402.

These results suggest that PBI-1402 offers the potential for a novel therapy by prevention and/or reduction of fibrosis and sclerosis.

## Background

PBI-1402 increases the production of immature progenitor stem cells (CFU-GEMM) from bone marrow and promotes the maturation of BFU-E and CFU-E. These effects result in increased production of red blood cells (RBC) in three animal models: immunosuppressed (chemo- and radio-therapy) mice and 5/6-Nx rats.

Furthermore, in a phase I clinical trial, PBI-1402 increased significantly both the relative and absolute numbers of reticulocytes in healthy volunteers. A phase Ib/II clinical trial confirmed that PBI-1402 increased RBC counts and hemoglobin (Hb) levels in patients with chemotherapy-induced anemia (CIA).

## Methods

5/6-NEPHRECTOMIZED RAT MODEL

Male Sprague Dawley rats (6 week-old) were subjected to 5/6 nephrectomy or sham operations.

Experimental Design:


**Day 0:** Partial Nx (2/3 left kidney)
**Day 7:** Total Nx (right kidney). Follow-up for 14 days (BW measurement and food intake).
**Day 21:** Measure GFR (creatinine clearance). Serum creatinine must be >125 mmol/L to be enrolled. This is equivalent to ~500 in humans or stage 5 ESRD. Start treatment (PBI-1402; oral, 200 mg/kg once a day, EPO; sc, 25U diw).
**Day 42:** Measure GFR (creatinine clearance)
**Day 56:** Stop treatment
**Day 63:** Sacrifice

Histopathological scoring:

Histological changes were evaluated by quantitative measurement of glomerular, tubular and interstitial injuries. Tubular injury was assessed by grading tubular dilatation, epithelial desquamation, hyalin deposition and atrophy in 10-20 randomly chosen, non-overlapping fields (×100 magnification). Glomerular injury was assessed by grading protein deposition, mesangial and endothelial cell proliferation or sclerosis, epithelial cell (crescent) proliferation. Interstitial injury was assessed by grading inflammation and fibrosis (collagen formation). The lesions were graded on a scale from 0 to 3: 0=normal; 0 to 1=0-25%; 1 to 2=25-75% and 2 to 3=>75% damaged tissue. The tubular and interstitial injury was averaged and this resulting score was added to the glomerulus injury score for a total grading scale of 6.

## Results

**Percentage of 5/6-Nx rats showing improvement in serum creatinine and GFR (creatinine clearance) from day 21 to day 42 treated with PBI-1402, EPO and EPO + PBI-1402.**

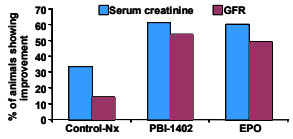


Figure 1

**Effect of PBI-1402, EPO and EPO + PBI-1402 on GFR (creatinine clearance) in 5/6-Nx rats that showed improvement.**

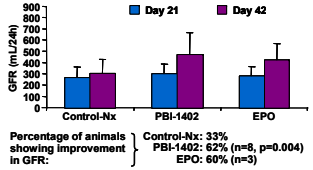


Figure 2

**Effect of PBI-1402, EPO and EPO + PBI-1402 on serum creatinine in 5/6-Nx rats.**

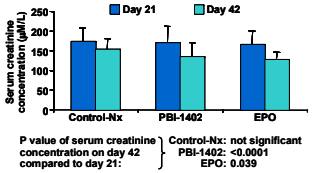


Figure 3

**Effect of PBI-1402, EPO and EPO + PBI-1402 on urine creatinine in 5/6-Nx rats.**

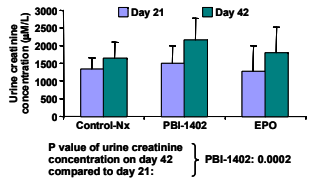


Figure 4

**Figures 5** and **6** are representative renal cortical sections in 5/6-Nx rats treated with PBI-1402 and 5/6-Nx rats treated with EPO. 5/6-Nx rats exhibited severe lesions, fibrosis and necrosis in both glomerular and tubular compartments. These lesions were mostly abrogated by treatment with PBI-1402.

**Histological micrographs of renal tissues from vehicle (Control) and PBI-1402-treated 5/6-Nx rats at day 63. Hematoxylin-Eosin staining (x40, x100 and x400).**

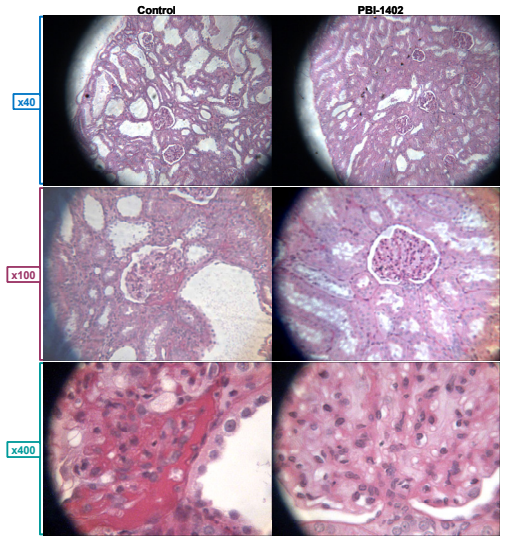


Figure 5

**Histological micrographs of renal tissues from vehicle (Nx) and PBI-1402-treated and EPO-treated rats at day 63. Masson's trichrome staining (x40, x100 and x400).**

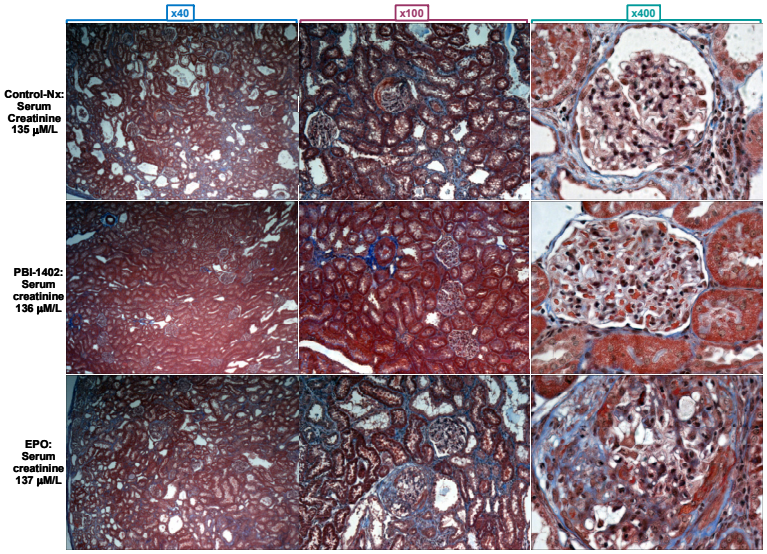


Figure 6

## Conclusion

These results suggest that PBI-1402 offers the potential for a novel therapy by prevention and/or reduction of fibrosis and sclerosis. PBI-1402 preserves renal function as evidenced by:

- ↑ GFR (creatinine clearance)
- ↓ Histological damage
- ↓ Fibrosis and sclerosis