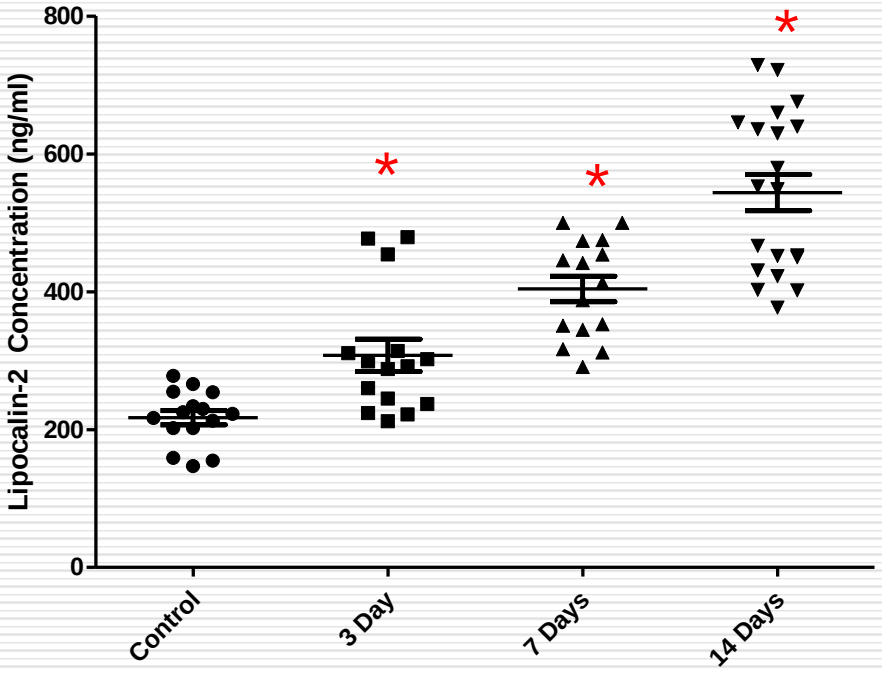
PharmOptima LLC

Value-Driven Research Services



14 day Rat Nephrotoxicity Testing with Kidney Injury Biomarker Panel

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Background

- The kidney is a major site of organ damage caused by drug toxicity
- Nephrotoxicity resulting from drug exposure has been estimated to contribute to 19–25% of all cases of acute kidney injury (AKI) in critically ill patients
- There is a paucity of biomarkers that reliably detect nephrotoxicity
- The Predictive Safety Testing Consortium (PSTC) determined several potential next generation biomarkers to predict kidney toxicity

Next-generation biomarkers for detecting kidney toxicity Nat Biotechnol. 2010 May ; 28(5): 436–440. doi:10.1038/nbt0510-436.

Biomarkers

- Meso Scale Discovery (MSD) Technology worked with PSTC to develop an electrochemiluminescence (ECL) based immunoassay to evaluate biomarker levels

- MSD developed a rat Kidney Injury Panel consisting of four biomarkers in rat urine that can be used to assess kidney toxicity
  - Lipocalin-2 also known as Neutrophil Gelatinase associated Lipocalin (NGAL)
  - Osteopontin (OPN)
  - Albumin
  - TIM-1/KIM-1 also known as Kidney Injury Molecule-1

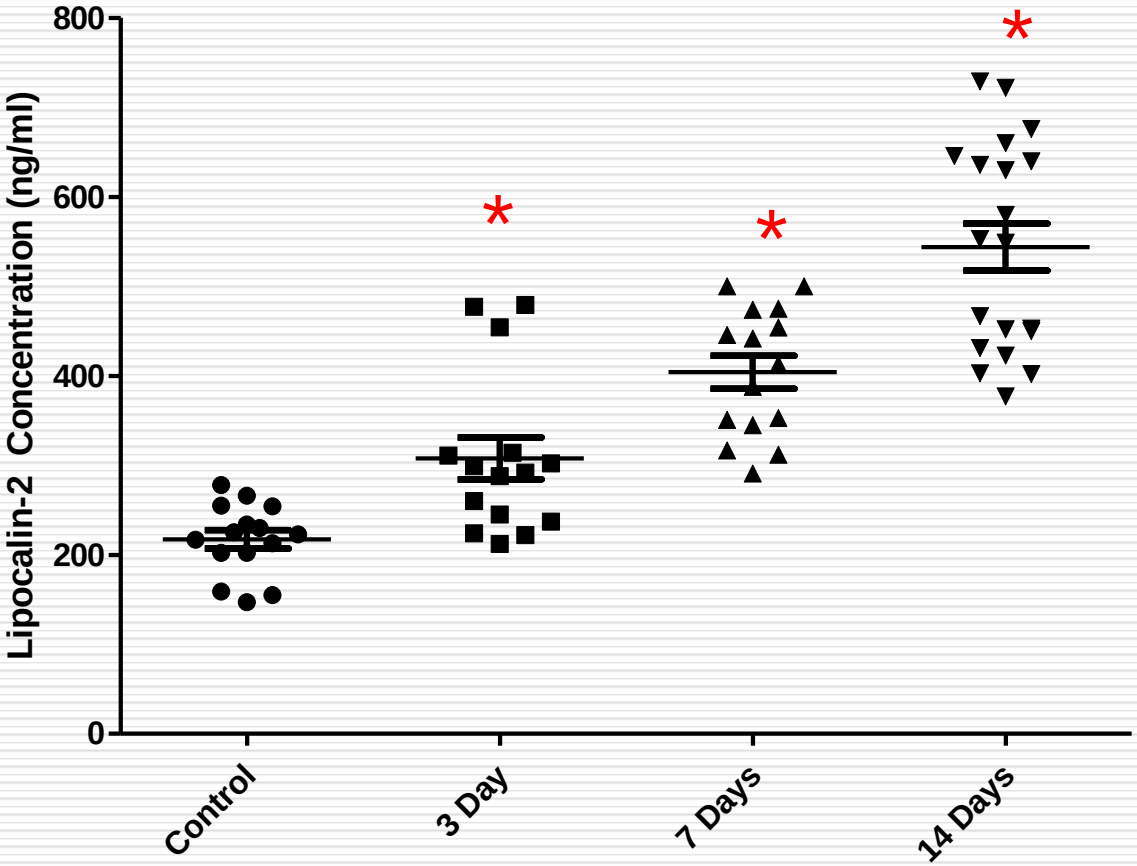
Testing NCE's in a 14 day Rat Nephrotoxicity Study with Kidney Injury Biomarkers at PharmOptima

- Protocol established and designed to test a new chemical entity (NCE) for kidney toxicity
- 14 day dosing of Wistar male rats 8-10 weeks of age 170-200 g.
- Urine from rats (n= 5 per group) collected for a period of 16 hours starting 8 hours after dosing on days 3, 7, and 14
- Collect blood for Serum Creatinine and BUN following 14 days of dosing
- Assay urine for biomarkers using ECL assay

Example 1: Test Gentamicin, a known kidney toxin

- Rats dosed daily with gentamicin (75 mg/kg bw) subcutaneously for 3 days, 7 days, and 14 days
- Previous study showed mild tubular degeneration and regeneration and a mononuclear cell infiltration (TOXICOLOGICAL SCIENCES 116(1), 8–22 (2010) doi:10.1093/toxsci/kfq029)
- Urine from rats collected on days 3, 7 and 14 of dosing and compared to urine samples from control (saline) treated rats using the MSD rat kidney toxicity biomarker panel
- Traditional clinical markers for kidney injury, Serum Creatinine and BUN (blood urea nitrogen), were evaluated after 14 days of dosing

Results: Lipocalin 2 (NGAL) as a biomarker in PharmOptima gentamicin study



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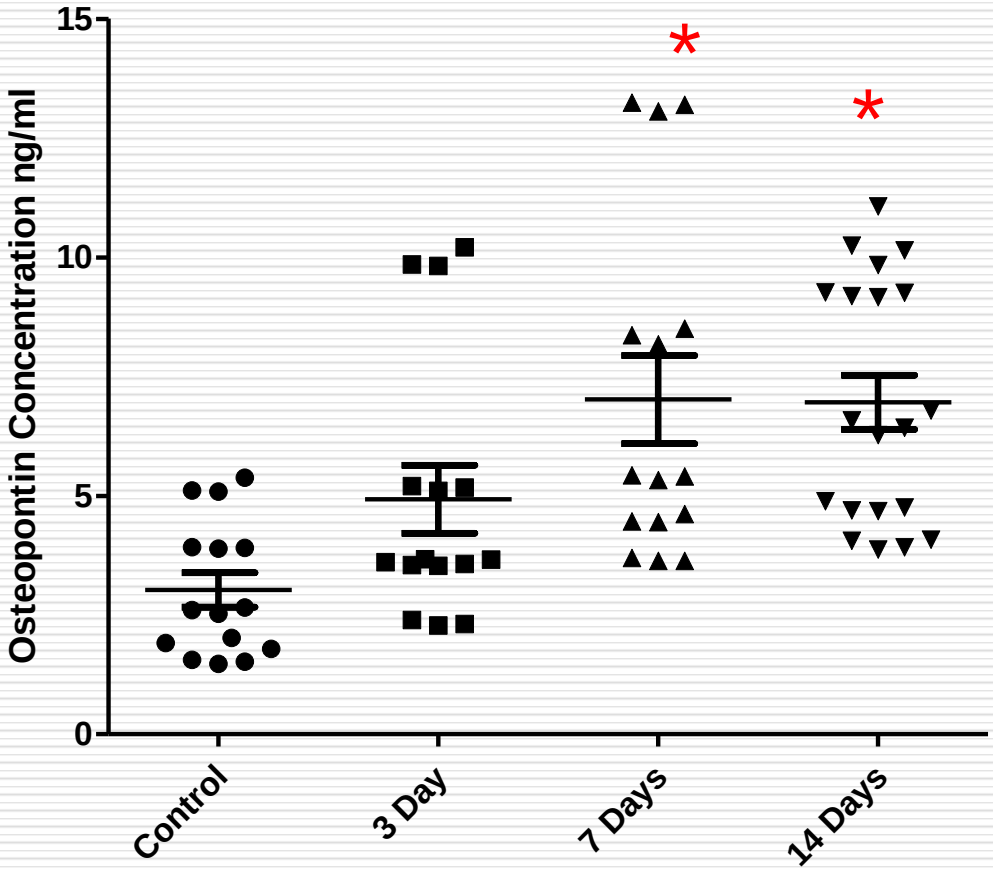
Groups that show significant increases compared to the saline treated control group as assessed by ANOVA are indicated by a red star ($p<0.05$)

Results: Osteopontin as a biomarker in PharmOptima gentamicin study



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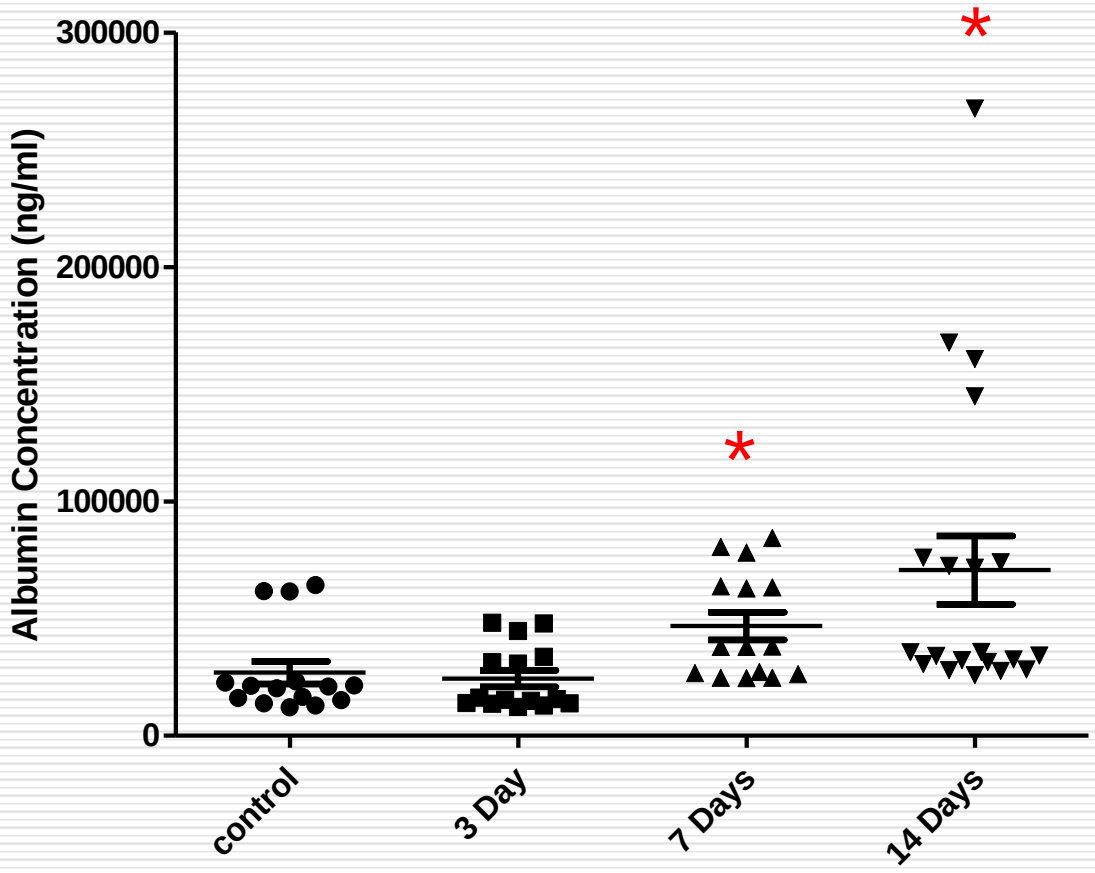
Results: Albumin as a biomarker in PharmOptima gentamicin study



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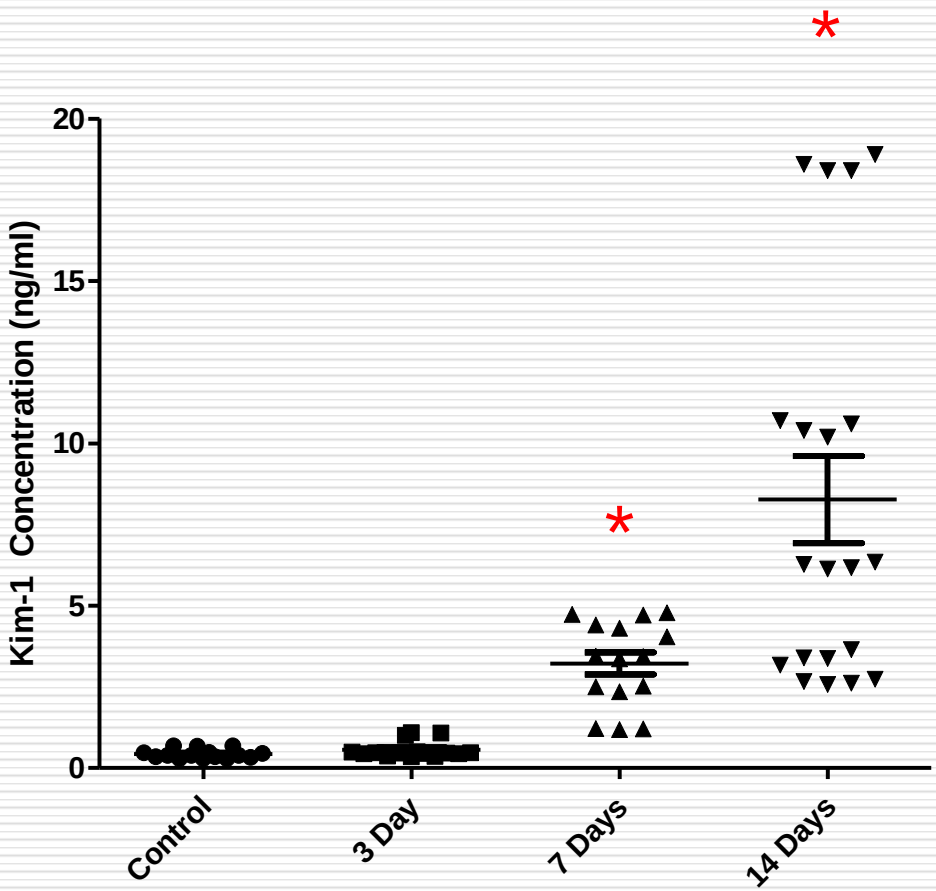
Results: KIM-1 as a biomarker in PharmOptima gentamicin study



Groups that show significant increases compared to the saline treated control group as assessed by ANOVA are indicated by a red star ($p<0.05$)

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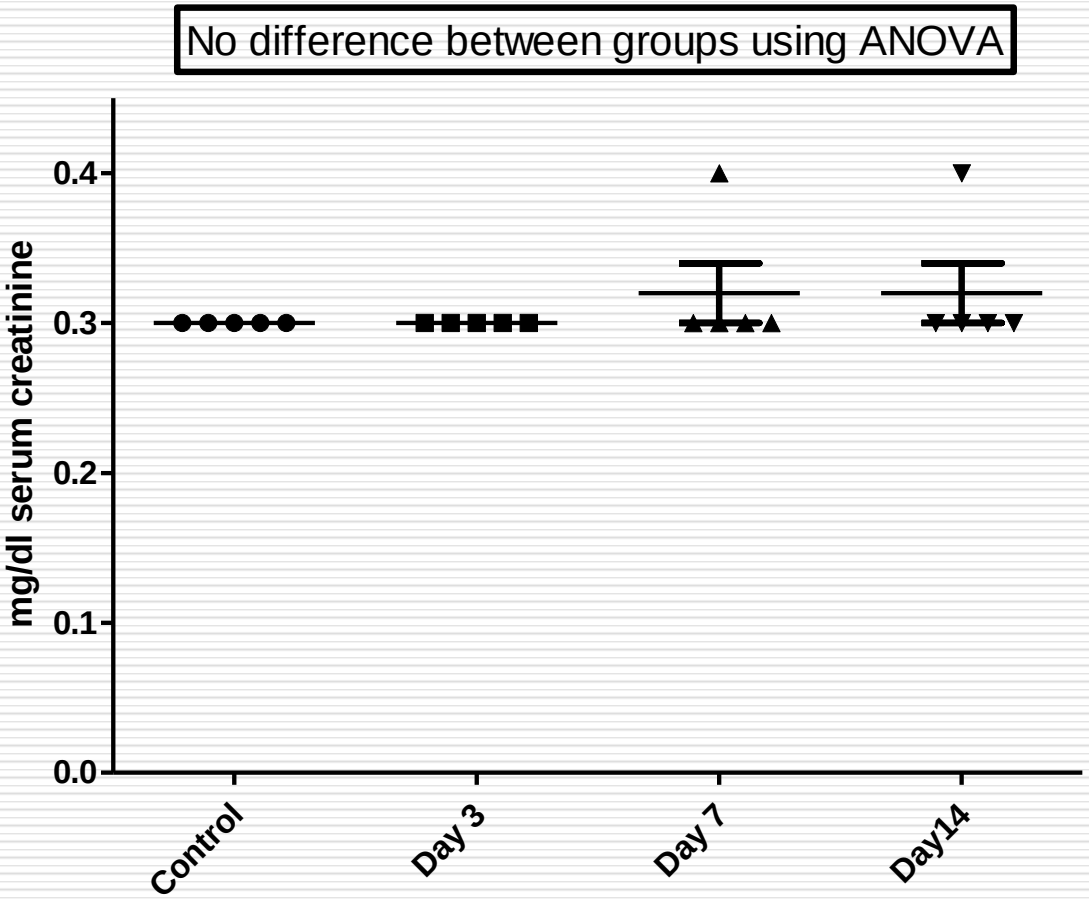
Results: Serum Creatinine as a clinical marker of kidney injury in PharmOptima gentamicin study



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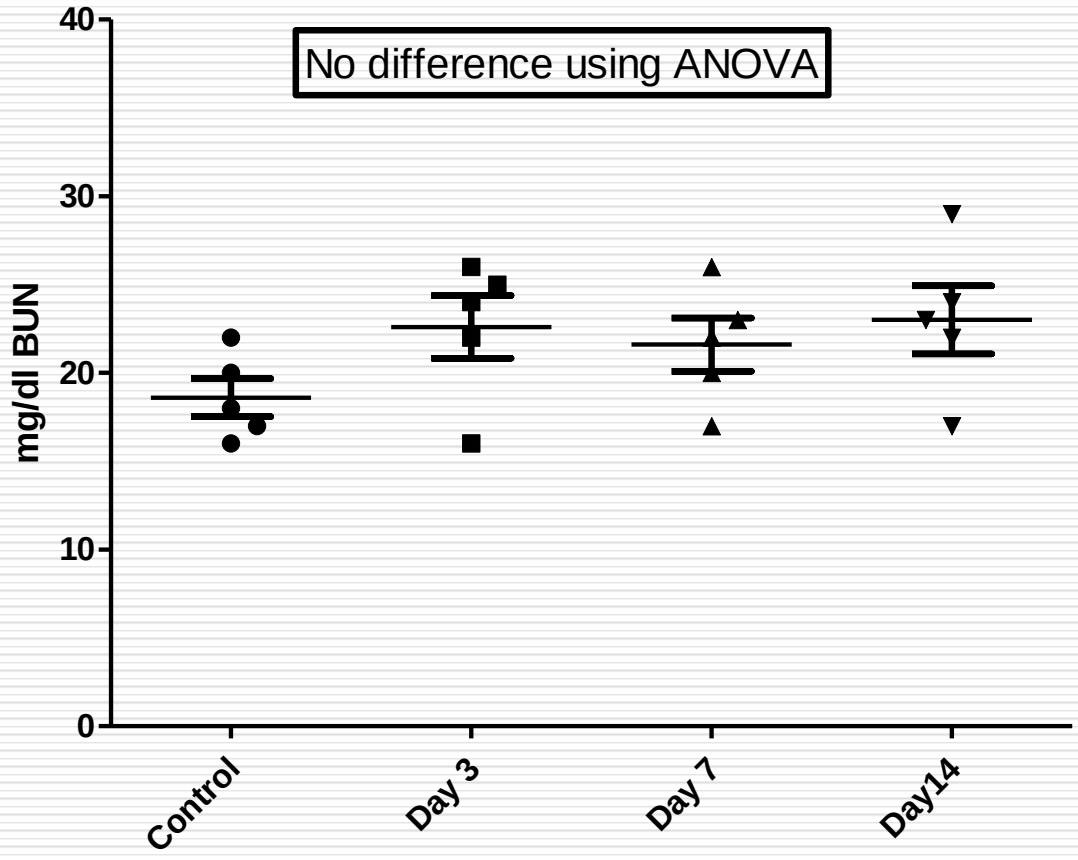
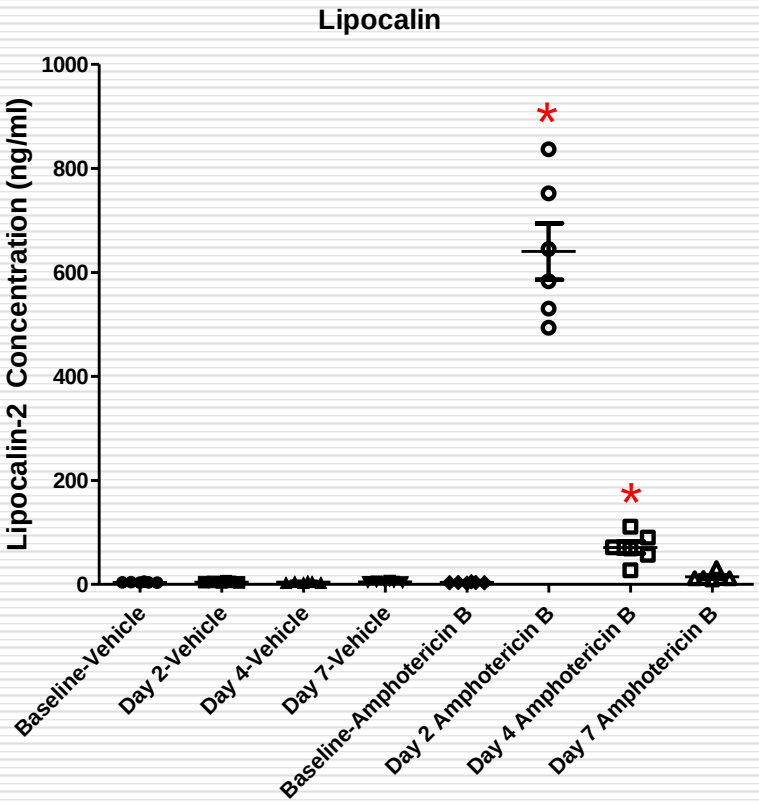
Results: BUN as a clinical marker of kidney injury in PharmOptima gentamicin study



Example 2: Test Amphotericin B, a known kidney toxin in a 7-day study

- Amphotericin B has been shown to be a potent nephrotoxin (Gilbert Deray (2002) Amphotericin B nephrotoxicity. Journal of Antimicrobial Chemotherapy 49: Suppl. S1 37-41)
- In this study, rats were dosed daily with Amphotericin B 1.56 mg/ml, 4 ml/kg, 25 mg/kg/day SC QID on days 1-7
- Urine from rats was collected on days 2, 4 and 7 of dosing and compared to urine samples from control (saline) treated rats using the MSD rat kidney toxicity biomarker panel
- Traditional clinical markers for kidney injury, Serum Creatinine and BUN (blood urea nitrogen), were evaluated after 7 days of dosing

Results:Lipocalin-2 (NGAL) as a biomarker in PharmOptima Amphotericin B study



Note: groups showing a significant increase compared to the saline treated control group as assessed by ANOVA are indicated by a red star ($P < 0.05$)

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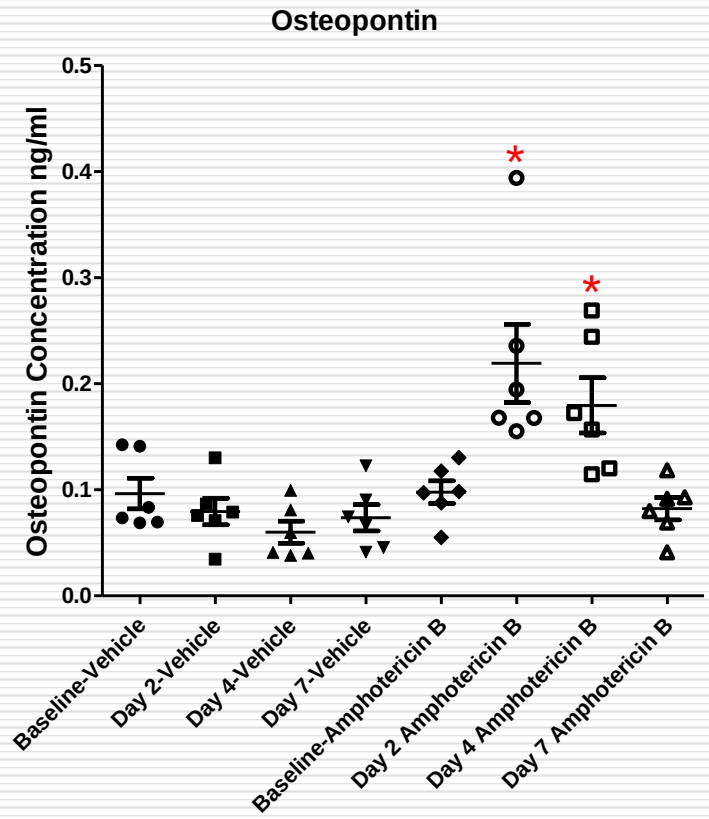
Results: Osteopontin as a biomarker in PharmOptima Amphotericin B study



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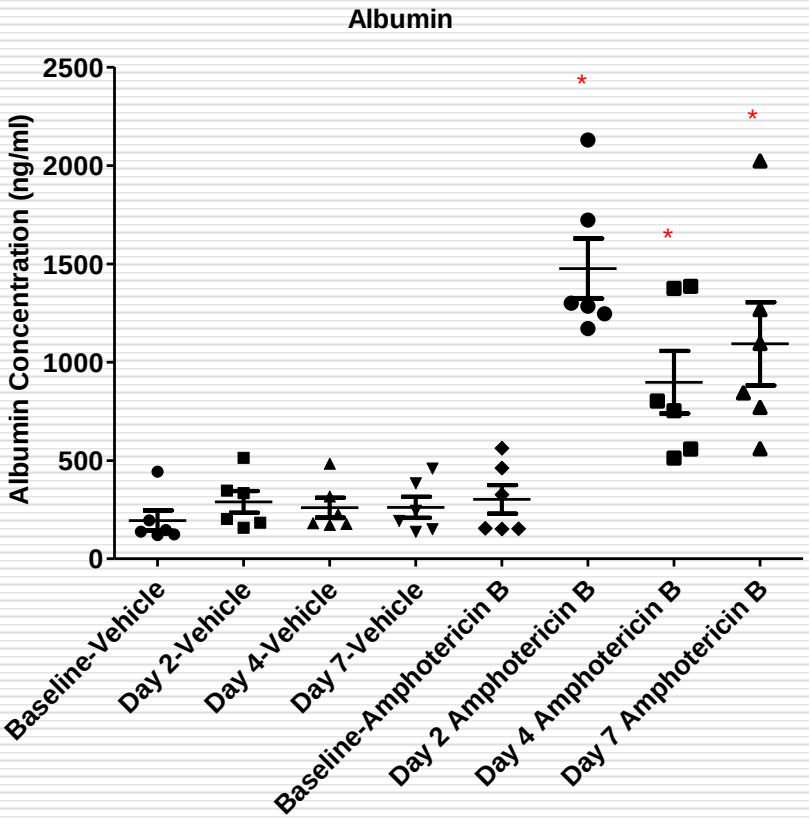
Results: Albumin as a biomarker in PharmOptima Amphotericin B study



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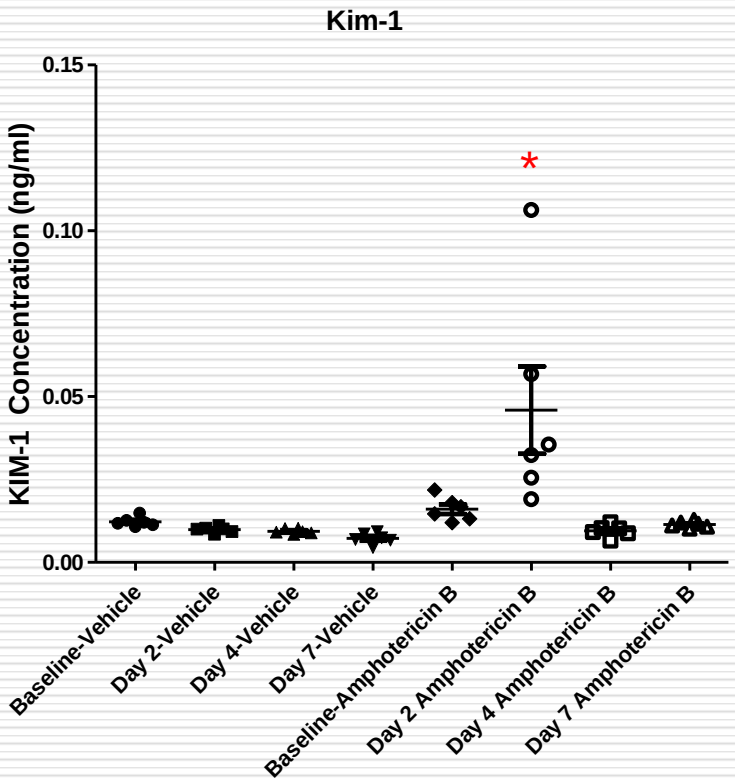
Results:Kim-1 as a biomarker in PharmOptima Amphotericin B study



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Conclusions: PharmOptima gentamicin and amphotericin B studies

- **MSD biomarker panel identified gentamicin and amphotericin B as potential nephrotoxins**
- Lipocalin-2 identified gentamicin and Amphotericin B as a nephrotoxin, at the doses tested, after only 3 and 2 days of administration, respectively
  - Osteopontin, Albumin, and Kim-1 identified gentamicin and Amphotericin B as a potential nephrotoxins, during the 14 day or 7 day studies, respectively
  - The use of the MSD biomarker panel for assessing nephrotoxicity shows differences in the response for an individual biomarker and may reflect differences in the mechanism of toxicity for a given nephrotoxic agent
- Traditional clinical markers of kidney toxicity, Serum Creatinine and BUN, **would not** have identified gentamicin or Amphotericin B as a nephrotoxins
- **PharmOptima ECL Biomarker Nephrotoxicity Testing**
  - Cost effective method
  - Faster than traditional histopathology method
  - More sensitive than traditional clinical markers

Contact Us

To have your New Chemical Entity tested for nephrotoxicity using PharmOptima's MSD ECL technology contact:

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