

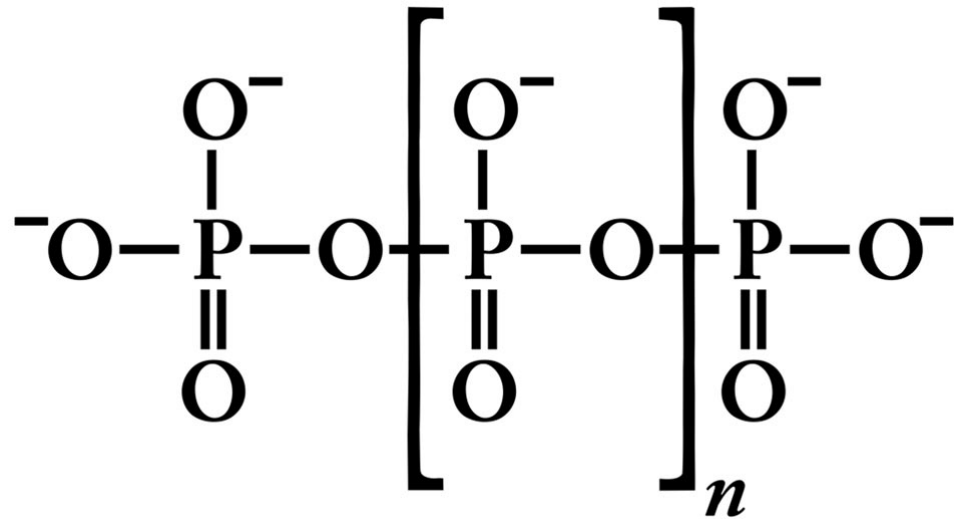
Monoclonal Antibody Against Inorganic Polyphosphate (PolyP)

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Polyphosphate (“PolyP”) are Highly Anionic Linear Polymers of Inorganic Phosphate



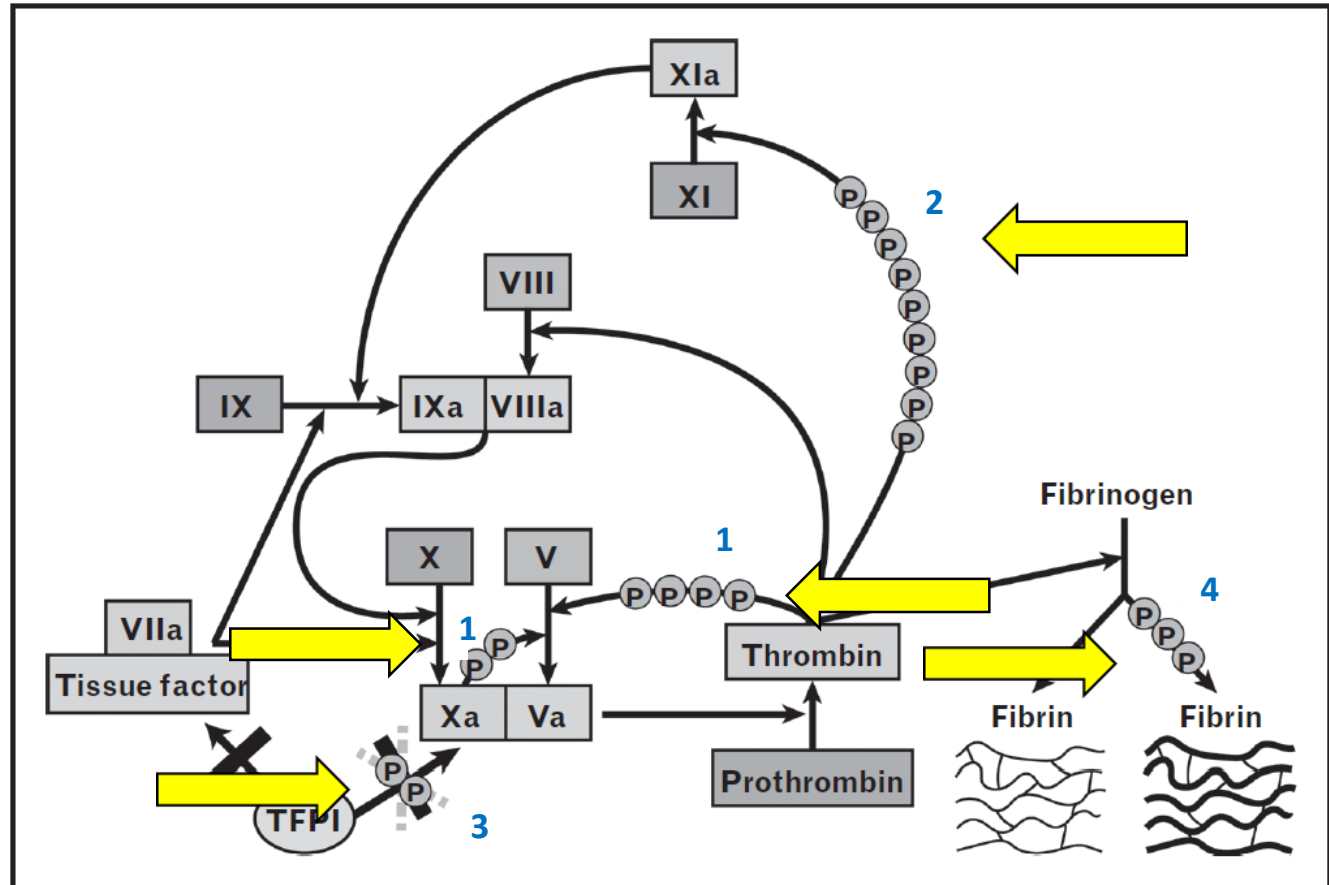
- Much of what is known of polyP is from prokaryotic & eukaryotic organisms. These microorganisms store long chain polyP (> 500-1000+ phosphates) as a source of energy and phosphate during nutrient deprivation. They also appear to use polyP to protect against metal toxicity.
- PolyP is found in lysosomes, secretory granules (platelet dense granules), mitochondria, & nuclei of mammalian cells

Chain Length of PolyP Determines Role in the Host

- Long chain polyP from microorganisms interact with the coagulation cascade at multiple points
- Dense granules in platelets of mammalian cells contain polyP of ~60-100 phosphate units in length.
- When platelets are activated in hyperinflammatory conditions, polyP is released in the circulation and play a role in coagulation/thrombosis.

PolyP has the Potential to be Pathologic if not Tightly Regulated

1. PolyP accelerates factor V activation at factor Xa & thrombin
2. Accelerates factor XI back-activation by thrombin
3. Inhibits tissue factor pathway inhibitor ability to inhibit factor Xa
4. Longer chain (>400 phosphates) enhance fibrin polymerization

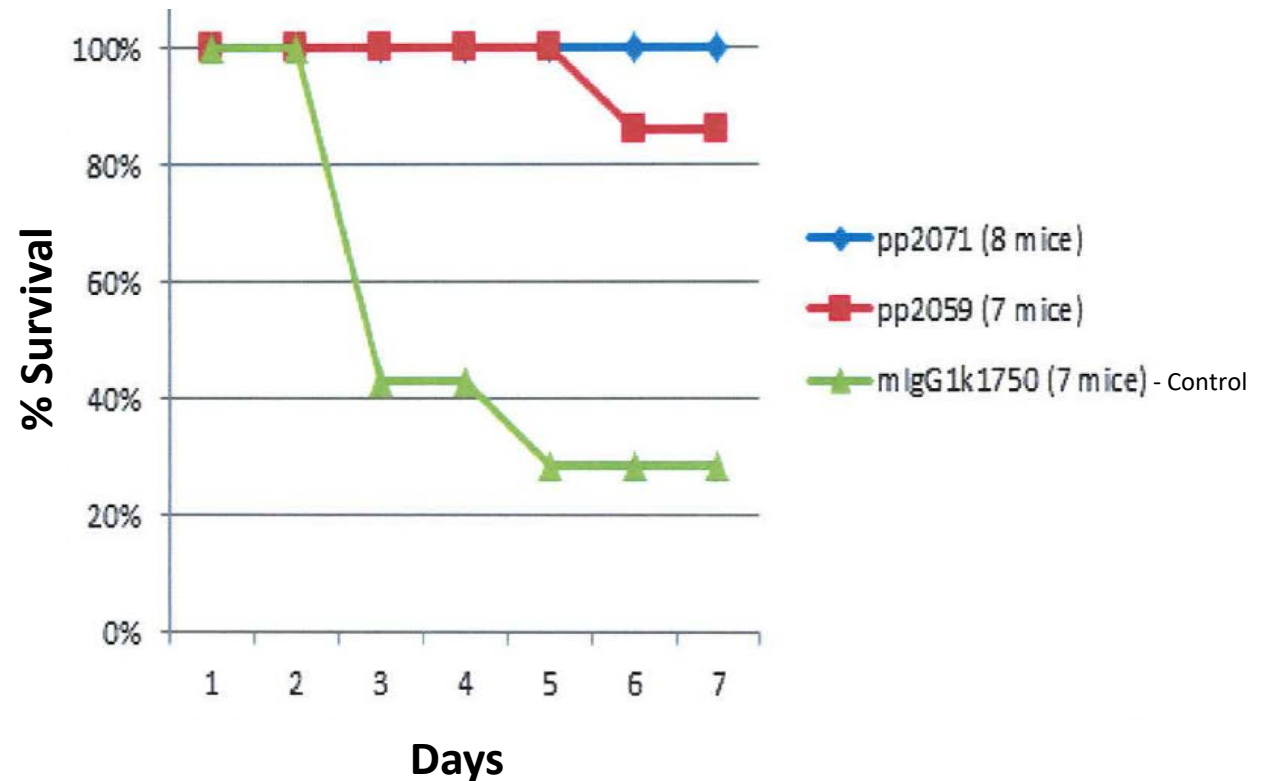


Adapted from Smith & Morrissey *Curr Opin Hematol* 2014, 21: 388-394

Anti-polyP Antibody Pre-Treatment Improves Survival in a Hyperinflammatory Challenge

Preliminary studies show that anti-polyP antibodies were protective in a hyperinflammatory LPS challenge model in mice.

LPS was premixed with anti-polyP antibodies (pp2071 & pp2059) or isotype control (mIgG1k1750) then delivered *i.v.* retroorbitally



Anti-polyP Antibodies as a Potential Therapy in Hyperinflammatory Conditions

- We have isolated multiple anti-polyP antibodies that bind to polyPs of varying lengths (U.S. Nat'l stage PCT priority date of 8/21/14; jointly owned rights of U. of IL & OMRF; OMRF is lead for prosecution & licensing)
- These antibodies have the potential to alleviate inflammatory & thrombotic complications due to platelet activation & polyP release
- There are multiple acute conditions where activated platelets and/or coagulopathies are implicated.
- Such conditions include Acute Respiratory Distress Syndrome (“ARDS”), Acute Pancreatitis, and Ischemia Reperfusion Injury (“IRI”)
- We used IRI as a proof of concept for our preliminary data

Anti-polyP Antibody Treatment Protects Against IRI (MRI)



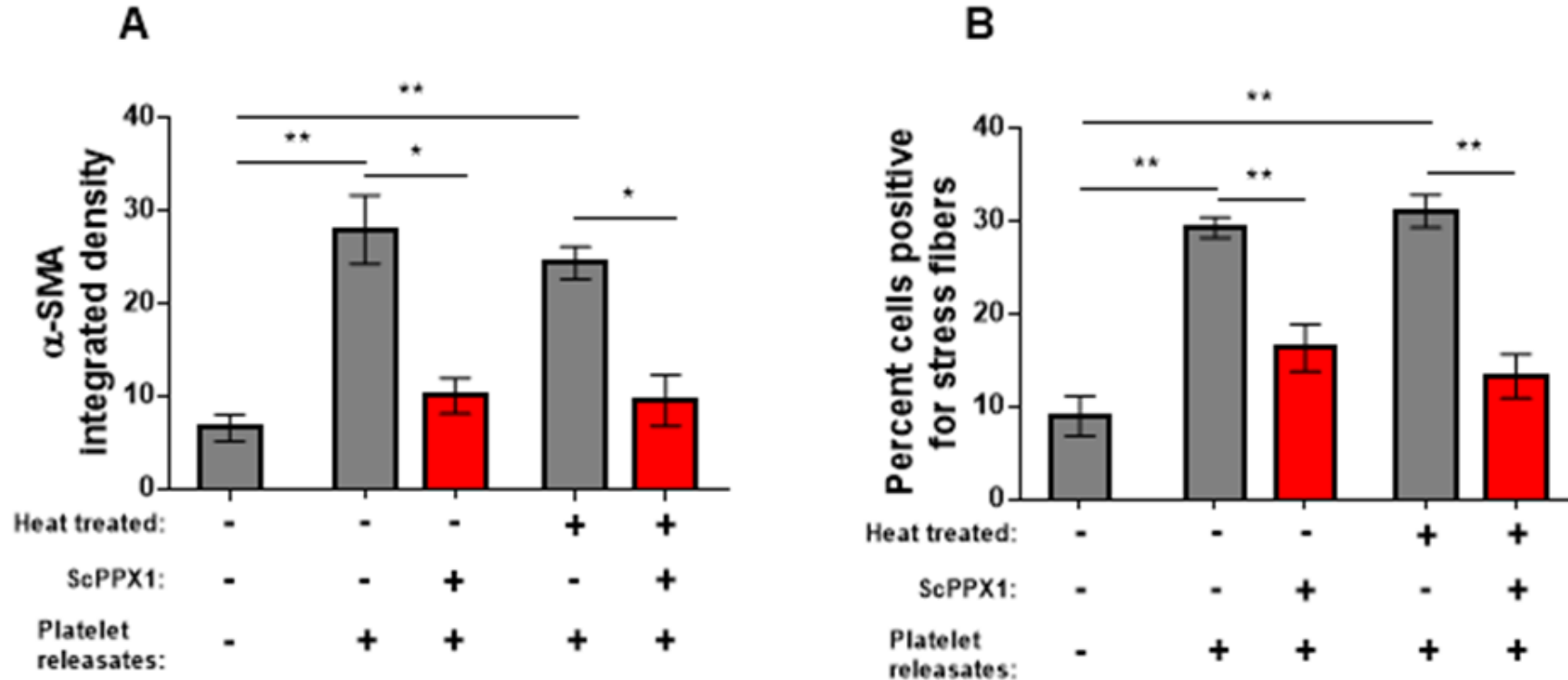
A rat model of IRI is used where the left kidney undergoes 30 minutes of ischemia followed by an hour of reperfusion before magnetic resonance imaging (“MRI”)

A. MRI of control rat (untreated) showing damage in upper cortical & medullary regions (arrow) of ischemic kidney contralateral kidney serves as internal control

B. MRI of rat pretreated with anti-polyP antibody *i.v.* (tail vein) 30 minutes prior to ischemia.

Other qualitative data (not shown) from MRI reveal anti-polyP antibody treated kidneys maintain near normal levels of perfusion compared to ischemic controls. Also kidney metabolites that are elevated in IRI are lower in the anti-polyP treated kidneys

PolyP Antibodies & Treatment of Fibrosis



Releasates were generated by treating human platelets (at 1×10^9 /mL in Tyrode's buffer) with Thrombin Receptor-Activating Peptide (TRAP). Platelets were removed by centrifugation, and the supernatant was collected (termed "platelet releasate"). The protein concentration in releasates was determined using a NanoDrop spectrophotometer, and the releasates were subsequently diluted in Tyrode's buffer to a protein concentration of 300 μ g/ml. Some of the releasates were heated at 95 $^{\circ}$ C for 30 minutes to inactivate any protein activity, then cooled to room temperature (polyphosphate is heat-resistant, and many control experiments have shown that its activity is unaltered by this heat treatment). Some of the heated or non-heated releasates were subsequently treated with 40 μ g/ml of a recombinant polyphosphate-degrading enzyme, yeast exo-polyphosphatase (ScPPX1), for 1 hour at 37 $^{\circ}$ C. The variously treated releasates, or Tyrode's buffer control, were diluted twentyfold into DMEM containing 1.5% bovine calf serum and incubated with subconfluent NIH-3T3 fibroblasts for 48 hours. Cells were subsequently fixed, permeabilized and stained for α -SMA actin and imaged using a confocal microscope. Fluorescent integrated density was determined using ImageJ, and cells were scored for α -SMA localized to actin stress fibers. All values are mean \pm SEM, $n = 3$ with a minimum of 25 cells analyzed for each individual experiment and condition. * indicates $p < 0.05$, ** $p < 0.01$ compared to the no-polyphosphate control (unpaired t-tests).

Near Future (14-18 Months) Plan

- Determine a therapeutic window for anti-polyP antibody treatment i.e. treatment post injury
- Confirm that anti-polyP antibodies do not increase bleeding
- Follow up testing on in vivo inflammation & coagulation biomarkers
- Biodistribution & PK/Clearance Studies
- Antibody Characterization and Binding Assay
- Antibody Lead Selection & Humanization
- Animal Studies: ARDS/ALI, Acute Pancreatitis & Fibrosis (liver/lung)
- **Total Estimated Cost of Studies:** ~\$750,000 + Cost of mAb humanization