

Mechanism of Acrolein Toxicity



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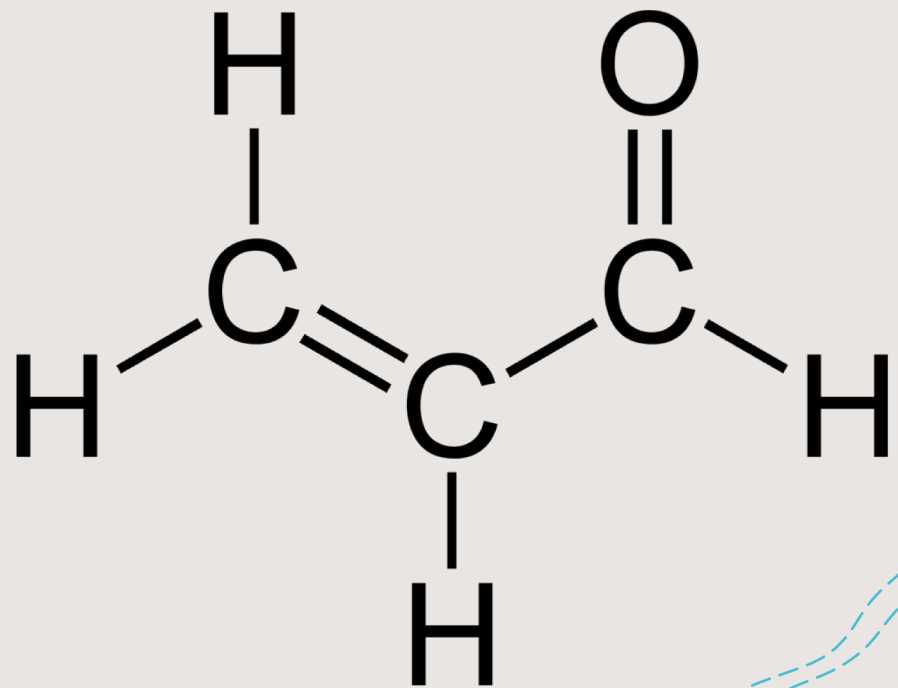
Effects of Acrolein on Health

- Burning of eyes, skin, nose, throat, etc.
- Routes of exposure:
 - Tobacco smoke
 - Automobile exhaust
 - Inhaling cooking oil and grease
- Common use of acrolein:
 - Biocide and herbicide
 - Production of amino acids



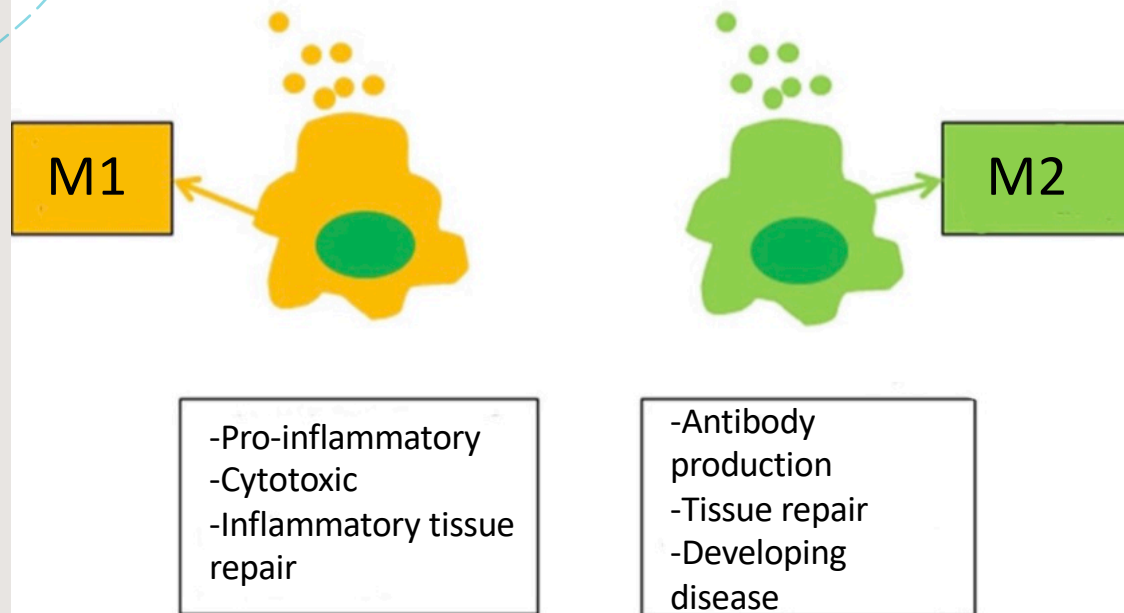
Chemistry of Acrolein

- Simplest unsaturated aldehyde
- Highly reactive
 - Electrophilic carbon
 - Carbonyl group
- Interacts with DNA, proteases, and proteins
 - Transient receptor potential cation channel (TRPA1)
 - Protein tyrosine phosphatase (PTP1B)



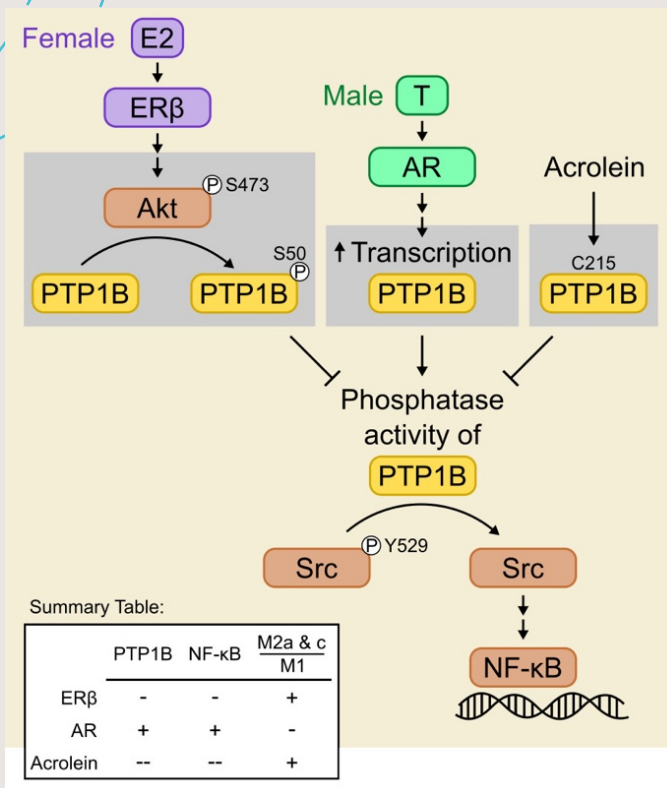
PTP1B Involvement with Macrophages

AM Phenotypes



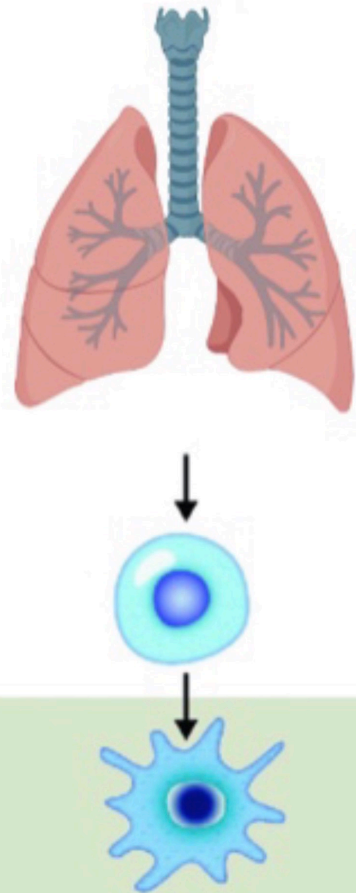
- PTP1B binds to Src kinase to activate cytokine production
- Src kinase activates cytokine production by signaling macrophages
- PTP1B inhibition increases M2 phenotype
- Acrolein alters PTP1B, not allowing it to activate to Src

Aims and Hypothesis

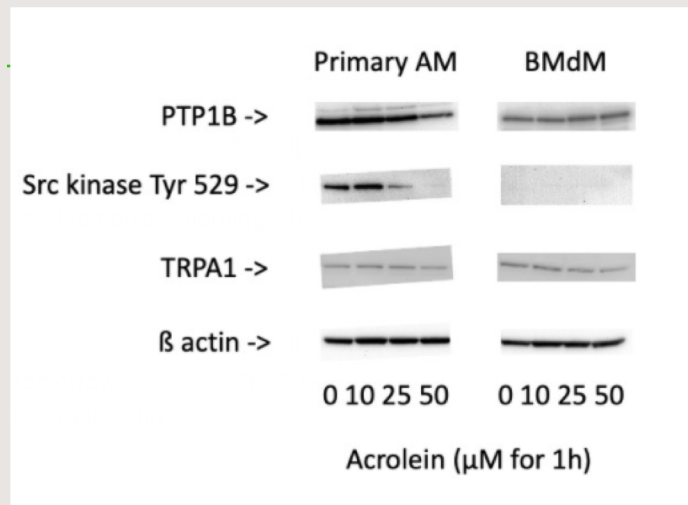


- Show that acrolein inhibits activity of PTP1B in alveolar macrophages in vitro
- Show that acrolein blocks PTP1B activity in lung macrophages in vivo
- Dose response

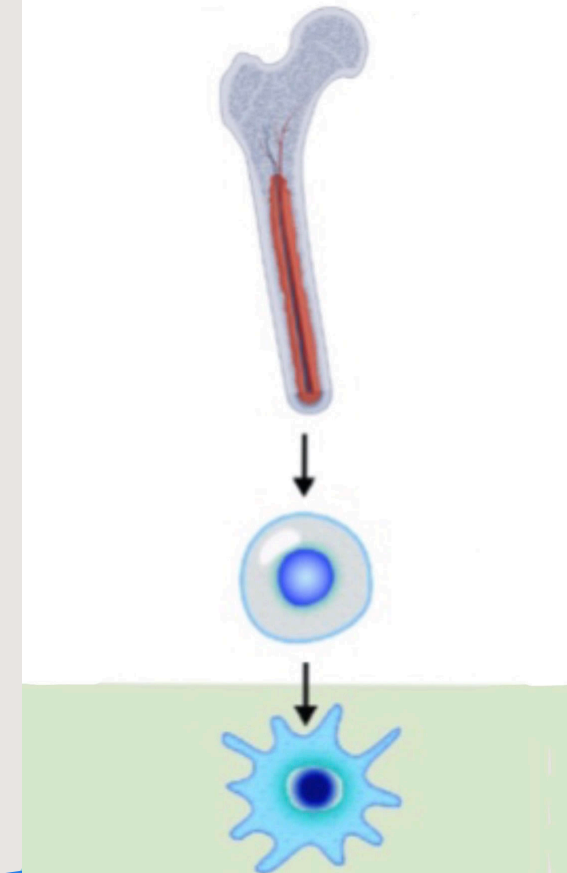
AM Model



- Alveolar macrophages (AM)- lung immune cell located in the airways and alveoli used for *in vivo* and *in vitro*
- Bone marrow derived macrophages (BMdM)- generated in lab and stimulated into macrophages for *in vitro* studies



BMdM Model

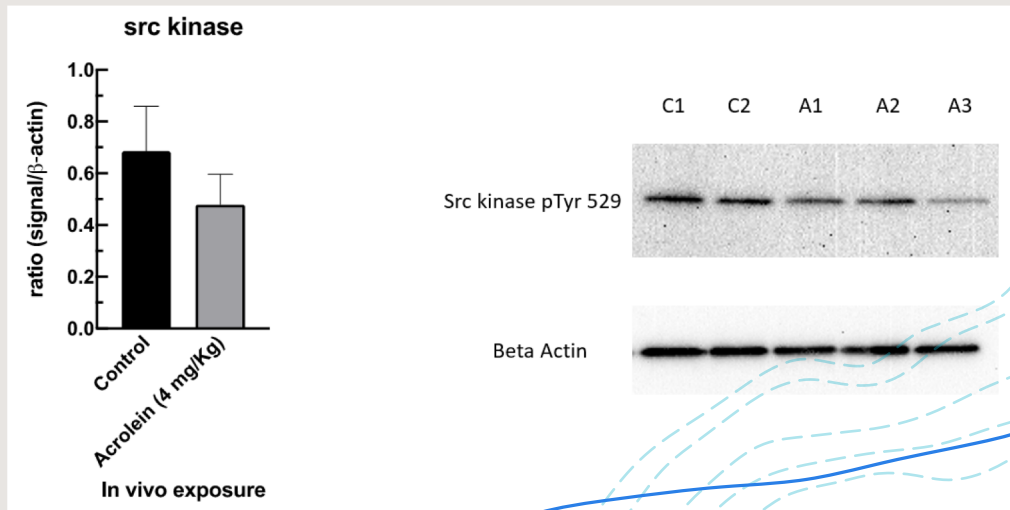
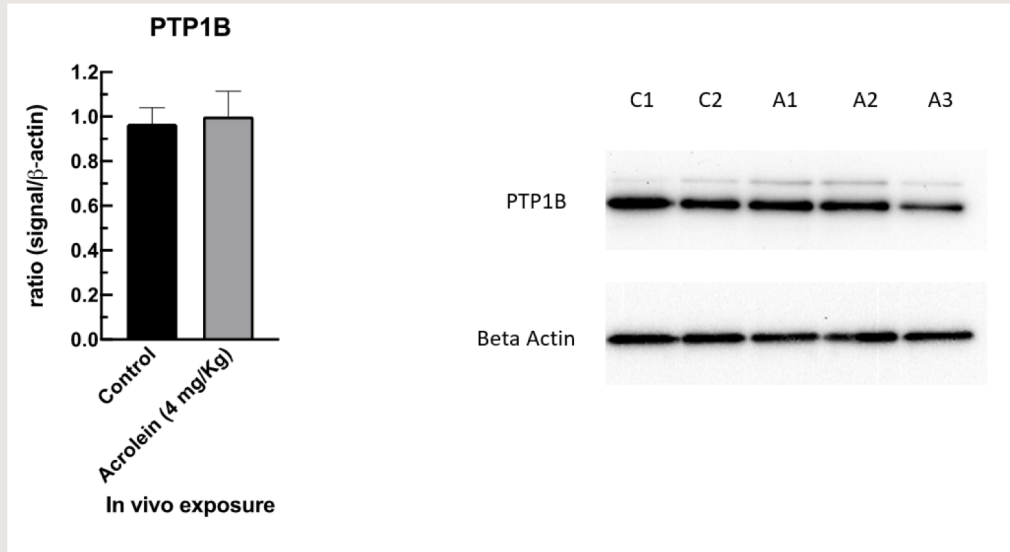


Researching PTP1B Inhibition *in Vivo*

- BCA assay to determine concentrations of proteins
- Western blot to show:
 - detection of proteins to determine acrolein is getting into the cells *in vivo*
 - reduction of PTP1B due to acrolein *in vivo*

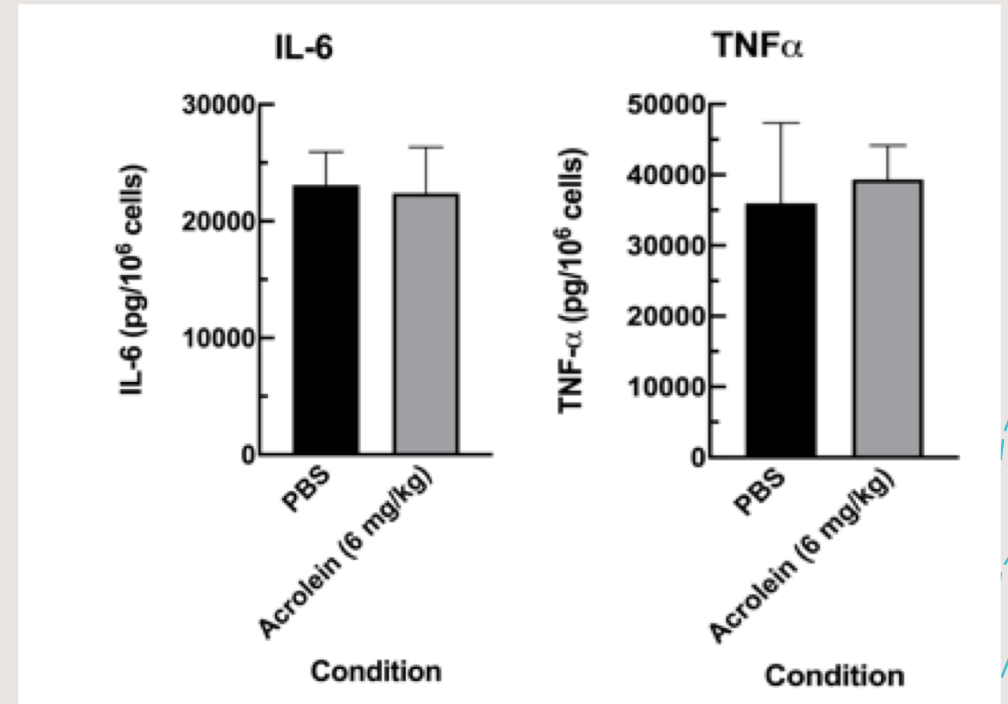


Results of *in Vivo* Exposure



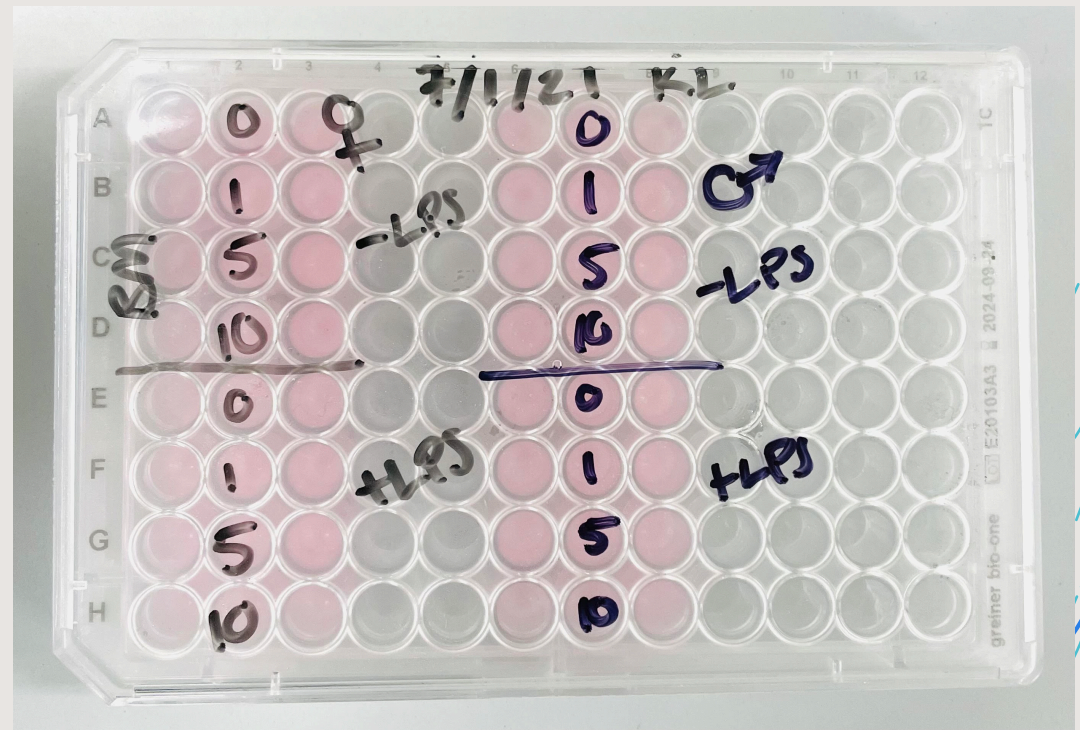
Results of *in Vivo* Exposure

- Exposed mice to acrolein
- Treated lavaged AM's with LPS
- Ran ELISA to determine if cytokine signaling pathway was being inhibited
 - TNF alpha
 - IL-6
- Results were inconclusive that acrolein was inhibiting PTP1B or the signaling pathway

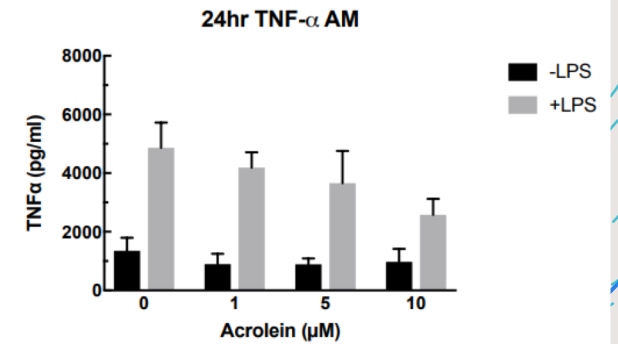
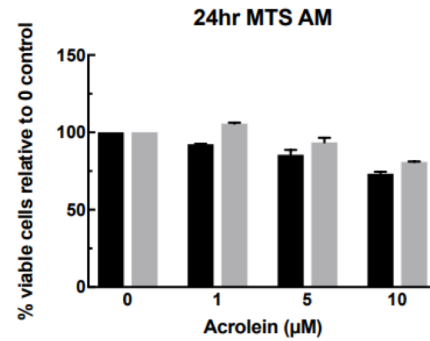
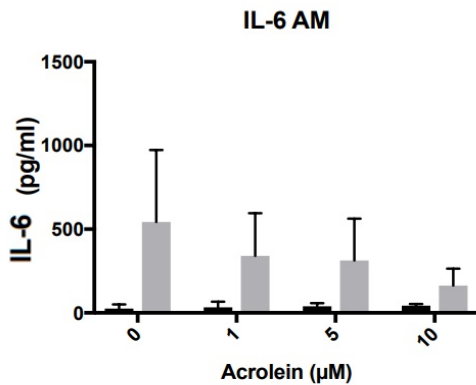
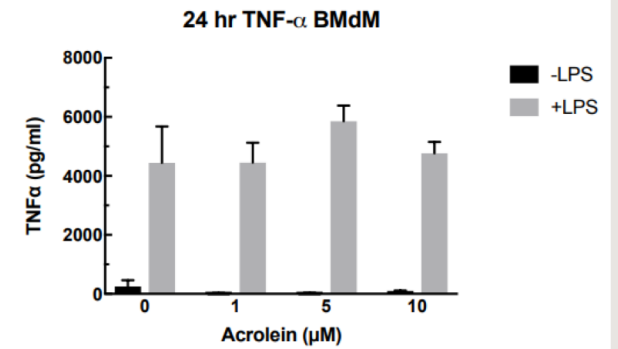
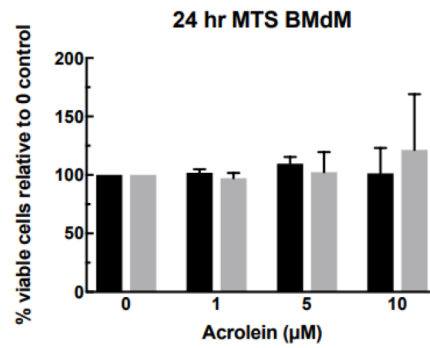
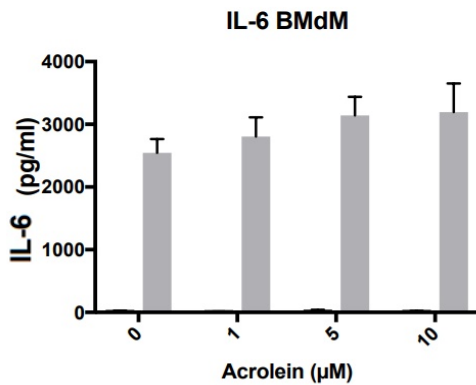


Researching Acrolein Exposure in Vitro

- Exposed mouse AM's to acrolein
- Ran MTS assay to determine cell viability
- Treated AM's with LPS
- Ran ELISA to determine if acrolein is inhibiting the cytokine signaling pathway
 - TNF alpha
 - IL-6
- Western blot to determine if Src Kinase is being inhibited

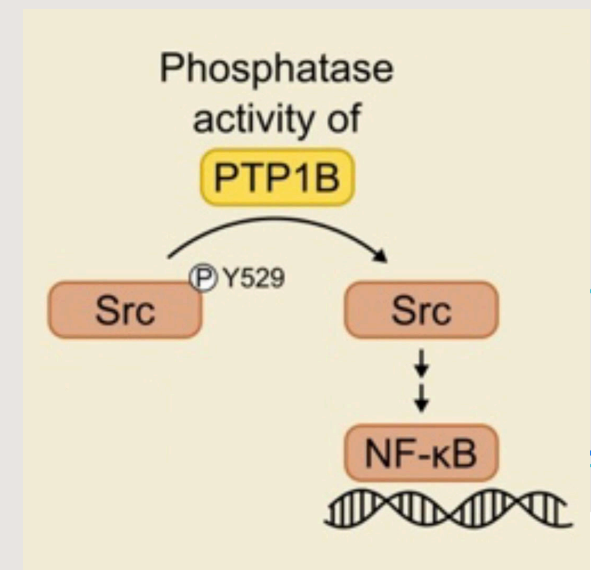


Results of in Vitro Exposure



Conclusion and Future Research

- Confirmed AM model is more sensitive to acrolein
 - Dephosphorylated src kinase not detectable in BMdM
 - AM model possible dose response in ELISA (TNF & IL-6)
- Confirmed dephosphorylated src kinase disappears in acrolein exposures
- Future research to determine the mechanism of src kinase and acrolein
 - Changes in ratio of phosphorylated to dephosphorylated src kinase
 - NF-kB activity
 - Improved dose response *in vitro* and *in vivo*



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