**Introduction**

- The immune system initiates immune responses by pattern recognition receptors (PRRs).
- Pathogen associated molecular patterns (PAMPs) are recognized by mechanisms such as:
  - The retinoic acid-inducible gene I (RIG-I)-like receptor (RLR) family.
  - The toll-like receptor (TLR) family.
  - The DNA sensor cyclic GMP-AMP (cGAMP) synthase (cGAS).
- PAMPs induce activation of IRF3/7 and NF-kB signaling pathway, which results in induction of type I interferons (IFNs) and cytokines.
- Bulk RNA sequencing comparing tumors with mutant p53 and mutant p53 deletion suggest that mutant p53 deletion impacts all three mechanisms, however only the cGAS mechanism has been described in literature (Gosh et al.).

**Materials and Methods**

<table>
<thead>
<tr>
<th>PRR</th>
<th>PAMPS</th>
<th>Agonists</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR1/2</td>
<td>Gram – bacteria</td>
<td>Pam3CSK4</td>
</tr>
<tr>
<td>TLR2</td>
<td>Gram + bacteria</td>
<td>HKLM</td>
</tr>
<tr>
<td>TLR3</td>
<td>viral dsRNA</td>
<td>Poly I:C HMW</td>
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<td>TLR3</td>
<td>viral dsRNA</td>
<td>Poly I:C LMW</td>
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<tr>
<td>TLR4</td>
<td>LPS Gram - bacteria</td>
<td>LPS-EK</td>
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<tr>
<td>TLR5</td>
<td>bacterial flagellin</td>
<td>ST-FLA</td>
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<tr>
<td>TLR6/2</td>
<td>Gram +</td>
<td>FSL1</td>
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<tr>
<td>TLR7</td>
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<td>ssRNA-40</td>
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<tr>
<td>TLR9</td>
<td>bacterial DNA</td>
<td>ODN1826</td>
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**Results**

- Western blot results indicate that there is lower phosphorylation of TBK1 in mutant p53 cells compared to deleted mutant p53 under treatment of TLRs 9 and 3 (Fig.2).
- Induction of phosphorylation of –IKKα/β in mutant p53 cells under treatment of TLR3 (Fig.3)
- TNF α production below detection levels under treatment of TLRs 1-9 using ELISA (Fig.4).

**Future Work**

- We are currently testing cytokine expression of TNF-α, IFN-β, IFN-α and IL6 using RT-PCR.
- ELISA and Western will be conducted again to ensure reproducibility of the data.
- Longer of incubation period for ELISA
- Increase the number of cells per well to $3 \times 10^4$ as well as increasing the volume to 300ul per well.

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**Conclusions**

- TLR3 induces phospho-IKK α/b in mutant p53 proficient cells but not in mutant p53 deleted cells suggesting higher levels of NF-kB.
- Higher phospho-TBK1 signaling in cells without mut-p53 compared to cells with mut-p53.
- Levels of secreted TNF-α in the supernatant in both cell lines were below detection level via ELISA, suggesting that more cells per well are needed or longer incubation time after TLR treatment.

**References**