**4/2/19 Meeting with Pleuni, Ling, Collin, Tara, Chris, and Zandrea**

**R21 Study**

Questions to be addressed:

1. Is SIV population in LNs reflective of SIV populations in granulomas?
2. Are SIV populations different between granulomas (i.e., compartmentalization)?
3. Are granuloma SIV populations different than normal lung SIV population?

Analyses to be run by Pleuni's postdoc:

* Compare SIV population in draining LN to each granuloma
* Compare SIV population in individual granulomas
* Compare SIV population in granulomas to normal lung tissue
* Compare SIV population in different LNs

Additional correlations with granuloma/LN SIV RNA copy numbers:

* plasma SIV RNA copy numbers
* plasma SIV sequence diversity
* granuloma SIV sequence diversity
* granuloma and LN CFU counts and barcodes
* granuloma CD4 and CD8 counts
* plasma SIV-specific T cell responses?

Better to obtain more samples from the co-infected animals that we're already started sequencing:

* plasma (at necropsy)
* granulomas
* draining LN
* normal lung

We probably have enough SIV only animal data for comparison

ACTION ITEMS:

**PLEUNI:** 1) find a postdoc for analysis; 2) find out the best control for determining sequencing error rate

**TARA/LING/COLLIN:** come up with a list of additional samples from 16314, 20615, 31316, 3516, 30816, 3216

**LING/ZANDREA:** come up with money for additional sequencing runs (leftover Lin R01 money?)

**CHRIS:** 1) determine minimum template needed for SIVmac251 MiSeq; 2) run control sample for determining sequencing error rate of assay

**R01 Study**

**Major Question #1:** Why does plasma SIV viremia decrease transiently after Mtb infection?

* Hypothesis: increased SIV-specific T cell responses increase early after Mtb infection

Possible data to support hypothesis:

* Transient SIV-specific T cell responses early after Mtb infection (Collin's data)
  + graph plasma viremia and SIV-specific immunity over time for each animal
  + correlation of SIV plasma viremia and magnitude of SIV-specific reponse
  + show a lack of increase of SIV-specific response in Mtb only animals (and possible ART-treated animals?)
* Decrease in viral diversity in plasma (possibly tissues particularly in animals that were sacrificed early after Mtb infection? or week 5 BAL/LN samples?) at same time point or shortly thereafter

Additional questions:

* What about Mtb infection is driving this increase in SIV-specific T cell responses?
* Why is the effect transient?

**Major Question #2:** How does ART suppression affect TB disease outcome?

* compare to Mtb only group
* compare to ART untreated group

**Can we get SIVB670 only controls for comparison?**

* Money for a small group of animals that can be followed for ~30 weeks
* Plasma samples from week 8-10 and week 30-32 from Karen Norris' animals (no other infections or treatments)

ACTION ITEMS:

**PLEUNI:** 1) find a postdoc for analysis; 2) find out the best control for determining sequencing error rate; 3) determine sequence diversity scale (y-axis on graphs)

**CHRIS:** 1) should perform control for assay mutation rate when we figure out what the appropriate control is; 2) quantify week 5 BAL and LN samples (ideally also perform PrimerID MiSeq, SGS as alternative); 3) practice PrimerID MiSeq on tissue samples from Tara

**ZANDREA/LING:** write progress report (and make sure Cesar reads it); ask about supplement for additional SIV only and Mtb only animals