**Metabolomics & Lipidomics analysis plan and timeline**

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**Aqueous metabolites**

**Aim:** Exploratory analysis on a metabolomic dataset to examine (1) metabolic shifts that occur during tumor progression, (2) metabolic shifts that could explain tumor energy status, (3) if any metabolites correlate to tumor volume or if tumor volume can be explained by a combination of metabolites.

1. PCA for community trends and patterns - DONE
2. Two-way ANOVA for 2x2 factorial in tumor - DONE
   1. Visualizations – exp. 4/20
   2. Look for metabolites that change a lot
3. Three-way ANOVA for 2x2 factorial + Time factor in plasma - DONE
   1. Visualizations (boxplot, lineplot?) – exp. 4/20
   2. Look for metabolites that change a lot
4. Pathway analysis with KEGG numbers for broad metabolic shifts (ANOVA)
5. Correlation analysis or stepwise regression to predict tumor volume

**Hydrophobic metabolites (lipidomics)**

**Aim:**Exploratory analysis on an untargeted lipidomics dataset to examine the impact of exercise, weight restriction, or their combination has an effect on lipid groups, and to determine if any lipid groups correlate to tumor volume or if tumor volume can be explained by a combination of lipids. Tease out potential lipid groups or individual lipids that may warrant further mechanistic analyses.

1. Determine if any annotations are in both POS and NEG ion runs and if the values are similar – if similar, use POS values; if not similar, need to re-visit peak picking parameters.
   1. This is technically part of data cleaning, not analysis.
2. PCA for community trends & patterns
   1. All data - DONE
   2. Aggregated by group - DONE
   3. Within each group (this will be a LOT, each PCA x 53)
3. Two-way ANOVA for 2x2 factorial in tumor by functional group – exp. 4/20
   1. Visualizations
4. Three-way ANOVA for 2x2 factorial + Time factor in plasma – exp. 4/20
   1. Visualizations
5. Stepwise regression for most parsimonious model to predict tumor volume
6. PLSDA to predict group membership

**Timeline**

* April
  + Continue analyses
  + Outline a report for Metab Core to QC
    - Write data cleaning methodology
    - Outline stats & analysis plan
* May
  + Finish analyses
  + Write results section
    - Make final figures (color? B&w?)
* June
  + Work on discussion & biological inferences – input from Bill? Dr Shearer? Sherry?