Randomized Trial: Comparing Vaccine + GM-CSF vs GM-CSF only

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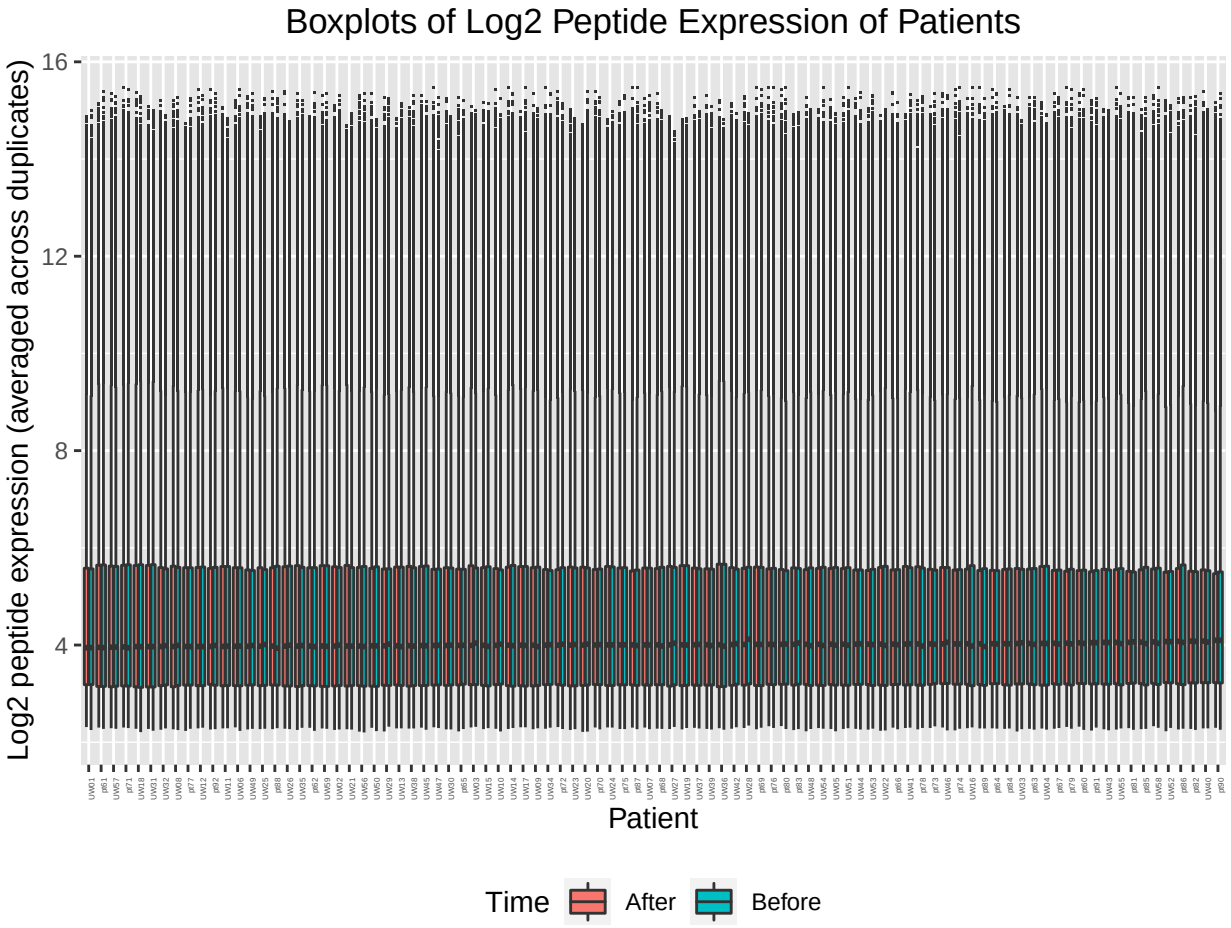
1 Introduction

Potluri et al. [2020] reported that people with early recurrent prostate cancer treated with vaccine + GM-CSF (pTVG-HP) had IgG to multiple peptides/proteins – not seen in patients treated with androgen deprivation. In this blinded randomized trial, 92 patients with the same stage of disease were treated with vaccine + GM-CSF (47 patients) or GM-CSF only (45 patients). We compared changes of peptide levels between these two groups of patients.

We collected blood at baseline and at 12 weeks and looked for antibodies to the same peptide array used in Potluri et al. [2020] with twice the density of spots – duplicates for every peptide on the same array (177,604 peptides $\times$ 2). We applied a log2 transformation of the peptide fluorescence data, and compute the mean (which, in this case, is equal to the median) log2 fluorescence levels of the same peptides. We verified that the peptide array data were normalized accordingly via the boxplots of mean (over duplicate peptides) log2 fluorescence level of all peptides for each patient. We then obtained the difference (after - before) of the log2 fluorescence levels for each patient and each peptide.

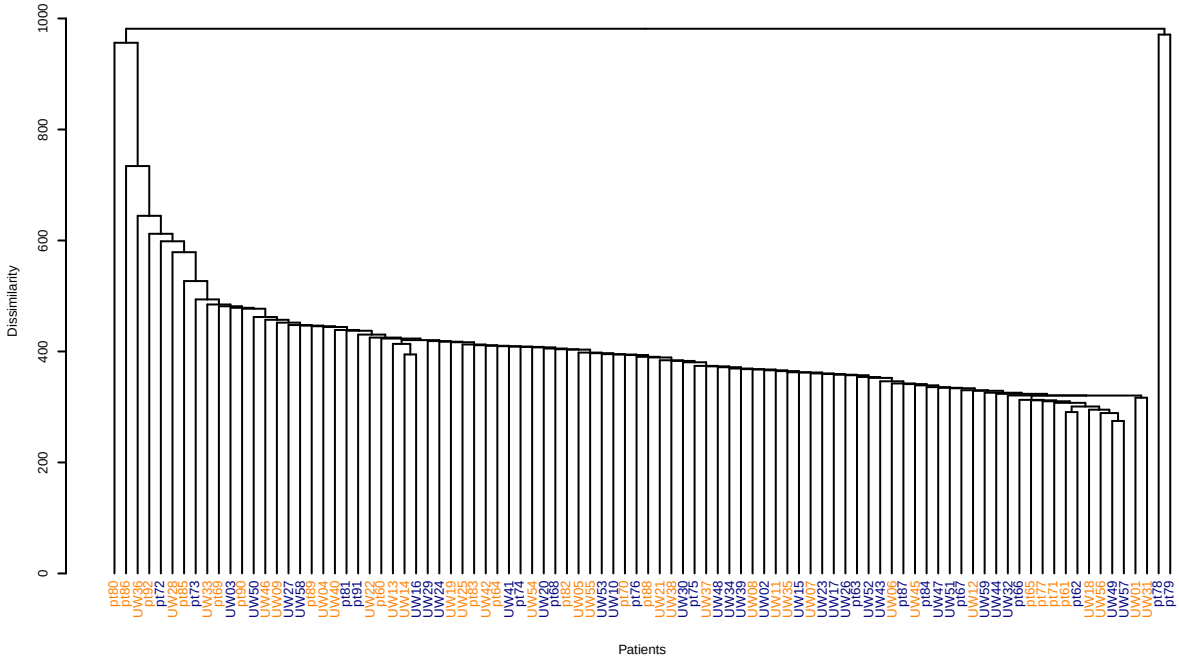


2 Preliminary Analysis

We visualize these log2(fluorescence) differences using:
* hierarchical clustering dendrogram
* PCA plot
* t-SNE plot [van der Maaten and Hinton, 2008]

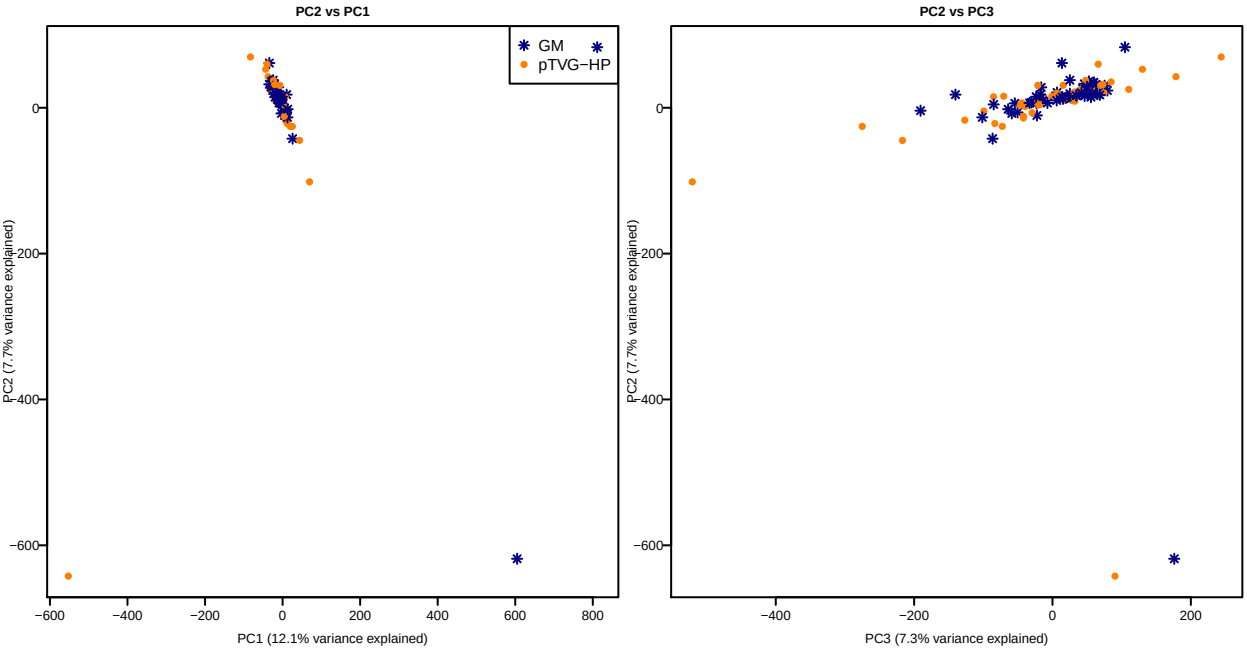
A small note on data-processing: In order to minimize character counts to distinguish the patients (data points) in the plots, I renamed the patient identifiers, as listed in "Patient_Rename.csv". For example, "UW pt 1" that appeared in "List of Sera Samples - phase 2 trial and replicates.xlsx" was renamed as "UW01"; whereas other patient records that appeared in "Sample_Submission_Sheet_PA-218.xlsx", such as "300015", was renamed as "pt60", and so on.

2.1 Hierarchical Clustering



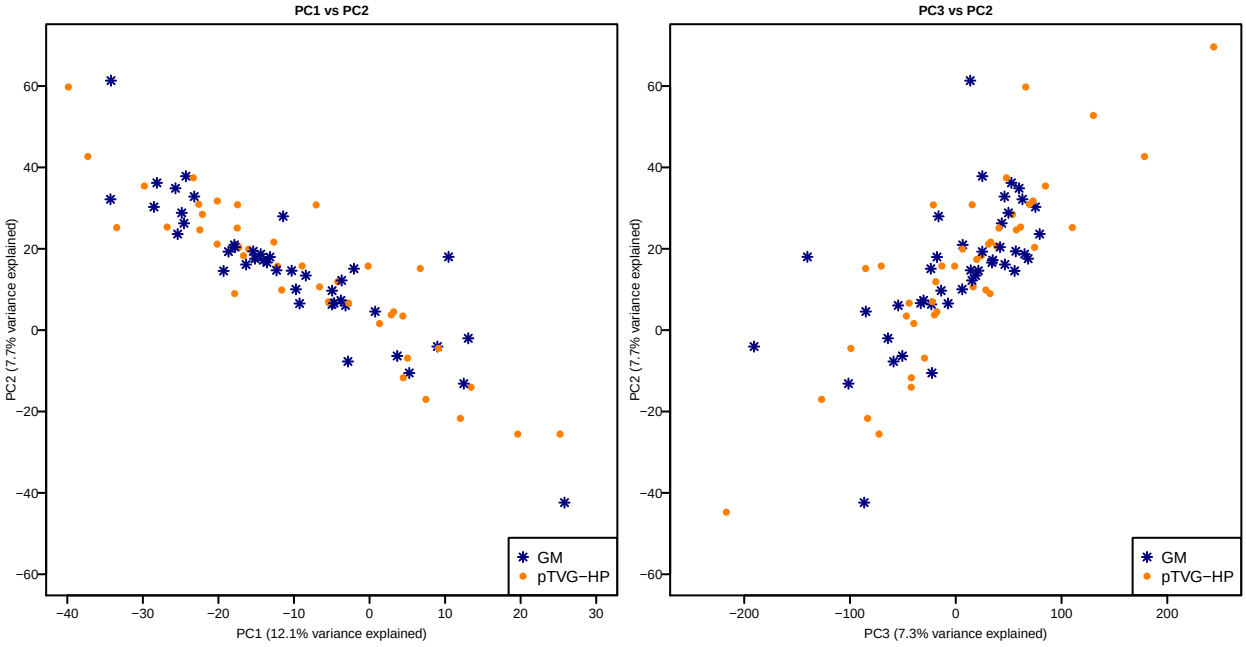
Patients such as “pt78”, “pt79”, “pt80” and “pt86” are clustered away from other patients. This pattern is consistent with the PCA plots displayed below.

2.2 PCA



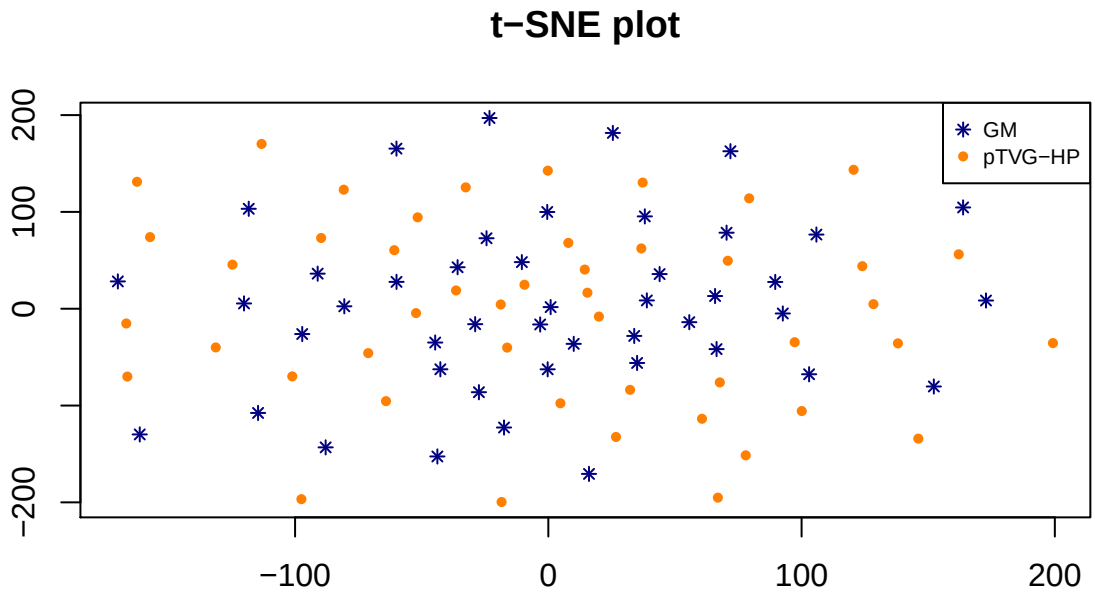
Most of the patients are cluttered together, except for the 4 aforementioned patients. We now zoom in this

crowd of patients.



Just like the dendrogram, it does not appear to have clear distinction between the two groups of patients, which we also observe in the t-SNE (t-distributed stochastic neighbor embedding) plot displayed below.

2.3 t-SNE

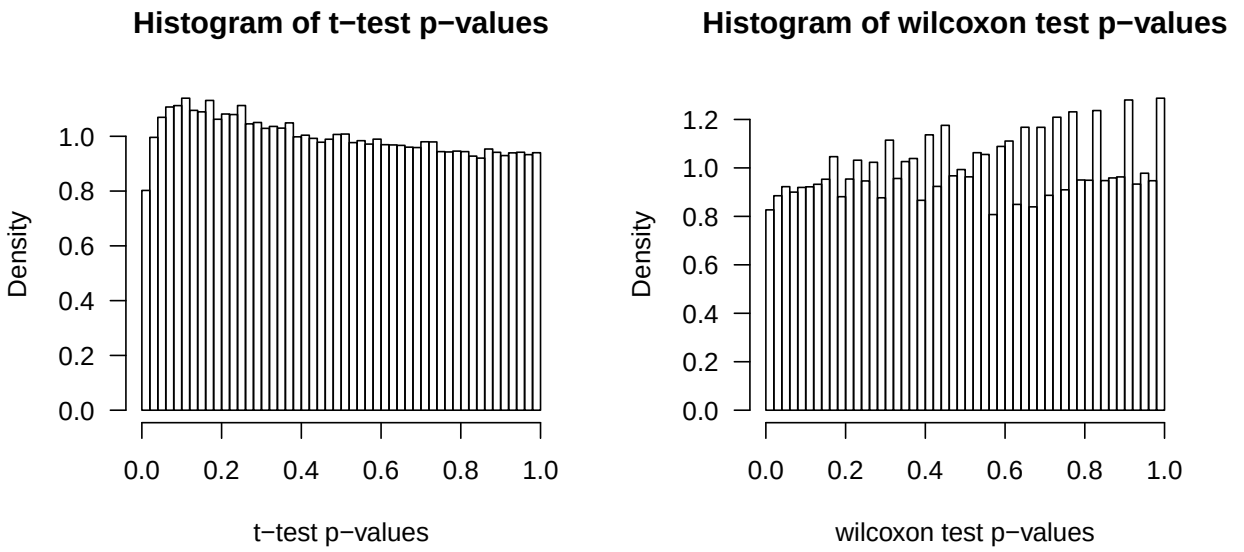


3 Statistical Tests

Let $\mu_{A,p}$ and $\mu_{B,p}$ be the average difference (after - before) in log2 fluorescence levels, for the vaccine+GM-CSF group (denoted A) and the GM-CSF only group (denoted B) respectively, for the p^{th} peptide, where $p = 1, \cdots, 177604$. For each peptide p, we test the following hypotheses

H_0: $\mu_{A,p} = \mu_{B,p}$
H_1: $\mu_{A,p} \neq \mu_{B,p}$

using both 2-sample t-test and Wilcoxon rank-sum test. We plot the p-value histograms as follows.



The shape of both p-value histograms is rather flat/uniform, suggesting a lack of clear signal or significant differences between the two groups of patients in terms of changes in peptide levels after treatment.

We apply the Benjamini-Hochberg (BH) method [Benjamini and Hochberg, 1995] on the t-test p-values to control for false discovery rate (FDR).

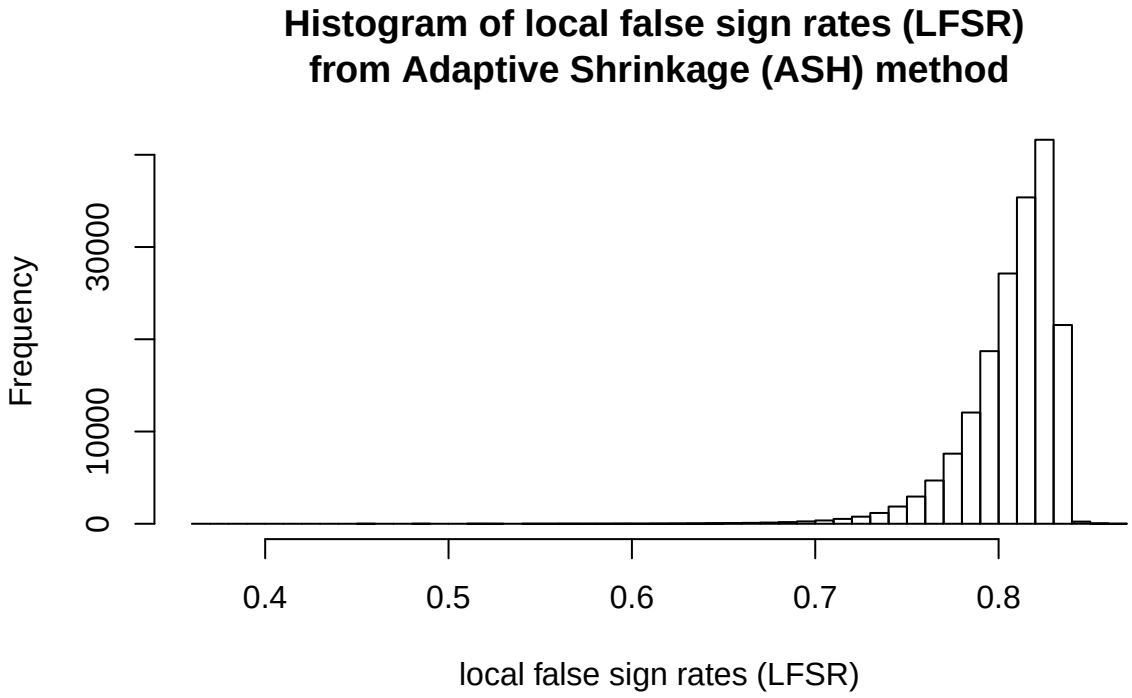
Threshold	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
Peptide counts	0	0	0	0	0	0	0	0	0	0

No peptides turn out to be significant (even at 50% FDR) based on t-tests after controlling for FDR. We arrive at the same result for the Wilcoxon BH-adjusted p-values.

Threshold	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	0.5
Peptide counts	0	0	0	0	0	0	0	0	0	0

We also deploy the Adaptive Shrinkage (ASH) analysis [Stephens, 2017], which is an empirical Bayes approach that adopts a unimodal prior that leads to shrinkage estimation adaptive to amount of signal (effect) and measurement precision (standard error). The ASH analysis also computes the `local false sign rate (LFSR)`: the probability of getting the sign of an effect wrong. It is analogous to to `local false discovery rate`, but measures confidence in the sign of each effect rather than confidence in each effect being non-zero. Whereas small (local) FDR indicates that we can be confident the estimated effect is non-zero, small LFSR indicates that we can be confident about the sign of the estimated effect. Of course, being confident in the sign of an effect implies that we are confident it is non-zero (but not the other way), hence the reason why LFSR is usually more liberal.

Specifically, for each peptide p, we measure its associated "effect" as $\hat{\mu}_{A,p} - \hat{\mu}_{B,p}$, and we take the standard error term in the 2-sample t-tests as the standard error for `lfsr` calculation done using the R package `ashr` [Stephens et al., 2019].. From the lfsr histogram, it seems that there is no significant difference between the two groups of patients.



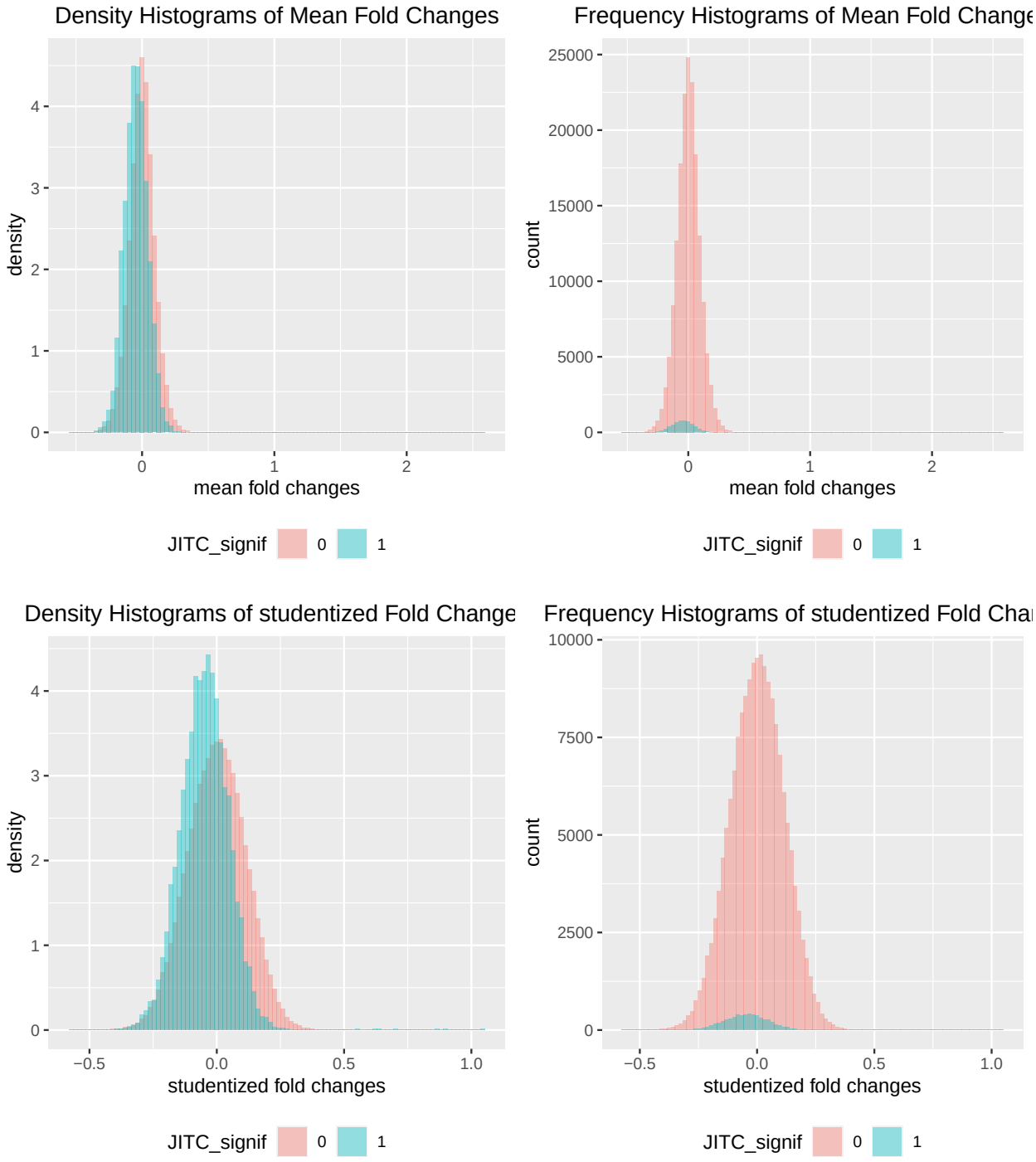
4 Compare with JITC results

All previous analyses indicate that there appears to be no evidence of significant difference between the two treatment groups in terms of peptide fold changes (before and after treatment).

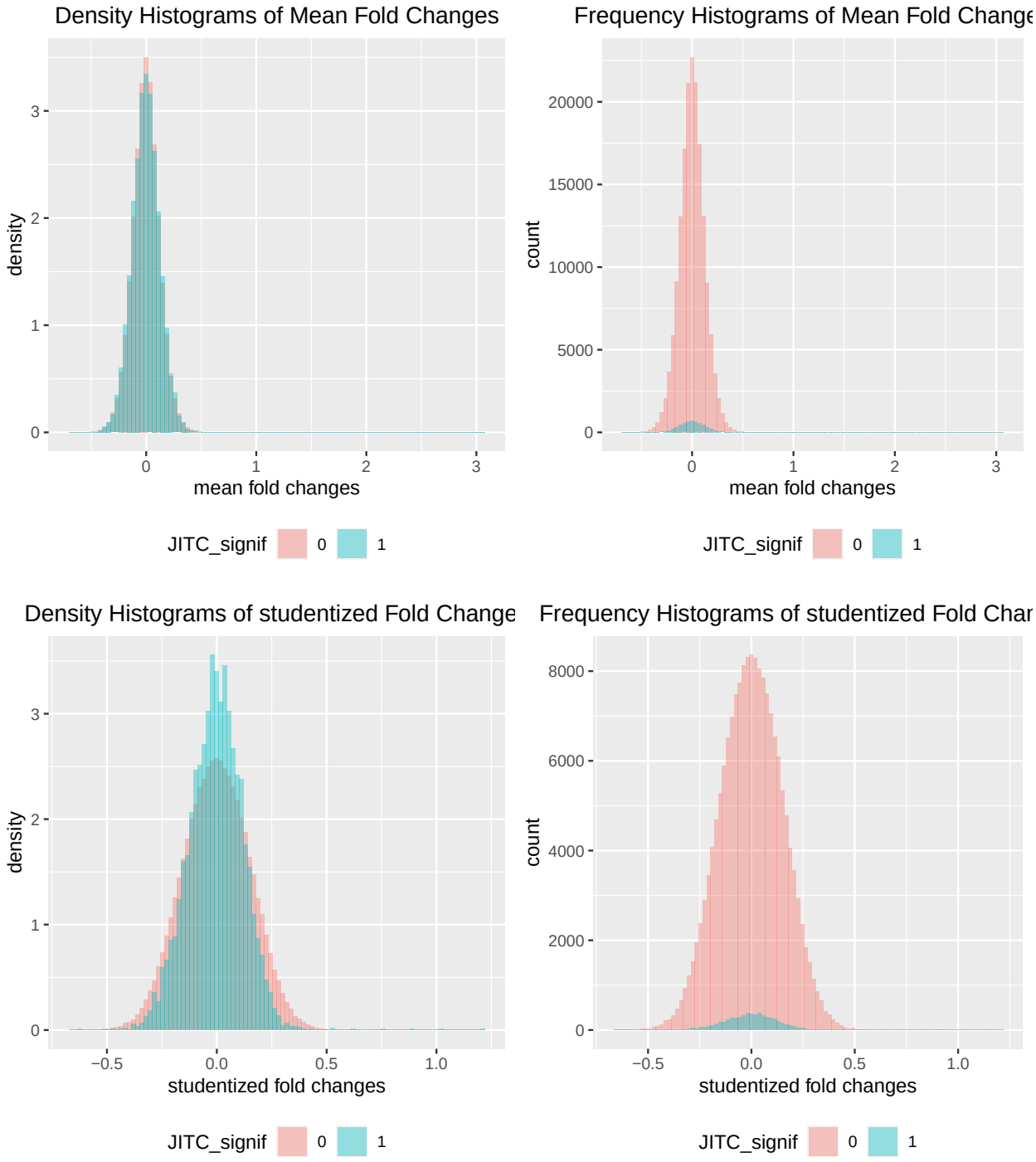
We tabulate
* the overall mean and studentized (across all 94 patients) fold changes
* the respective mean and studentized fold changes for the 2 treatment groups
* t-test p-values
* Wilcoxon p-values

for all 177,604 peptides in Excel spreadsheet "fold_change_all_peptides.csv". Meanwhile, Potluri et al. [2020] reported 5680 significant peptides associated with the comparison between the vaccine + GM-CSF (PAP) group and the androgen-deprivation (ADT) group. The spreadsheet "fold_change_all_peptides.csv" has a column that marks these 5680 significant peptides reported in the JITC paper.

We now plot the frequency histogram and density histograms of the overall mean fold changes and overall studentized fold changes between the 5680 JITC-reported peptides and all the other remaining peptides.



We also plot the frequency histogram and density histograms of the mean fold changes and overall studentized fold changes of the vaccine + GM-CSF group between the 5680 JITC-reported peptides and all the other remaining peptides.



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