sipuleucel-T Trial: Comparing protein-level sip-T + pTVG-HP vs sip-T only

Tun Lee Ng and Michael A. Newton

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1 Introduction

This is a follow-up analysis after *"01_sipT.pdf"*. To recap, this sipT trial had patients who received sip-T alone (group A: sample size = 9) or sip-T followed by DNA vaccine pTVG-HP (group B: sample size = 9). Note that sip-T is a vaccine engineered with GM-CSF. Recall that in our first analysis reported in *"01_sipT.pdf"*, there is no significant evidence to suggest any difference between the two treatment groups in terms of changes in peptide-level antibody response.

In this follow-up analysis, we want to investigate any difference between the two treatment groups in terms of changes in **protein**-level antibody response. There are 2 approaches to determine if a protein is significant:

- **Approach 1**: if any peptide associated with a protein is statistically significant, then we consider the protein to be significant as well. A protein with no significant peptide is deemed not significant.

- **Approach 2**: Sum up peptide-level fluorescence to protein-level fluorescence, apply a log2 transformation on the fluorescence, and repeat the analysis that we did in *"01_sipT.pdf"*.

Approach 1 yields immediate conclusion, since we found no significant peptides in *"01_sipT.pdf"*. This document focuses on Approach 2. It is worth noting that Approach 2 might lead to conflicting conclusion, such as a protein may not turn up significant but at least one of its associated peptides is considered significant, or vice versa.

We would also like to investigate if there are individual proteins to which responses might have been generated by sip-T alone, compared with the JITC report, perhaps comprised of several peptides from different individuals. We checked that the 5680 significant peptides reported in Potluri et al. [2020] are associated with 1051 proteins.

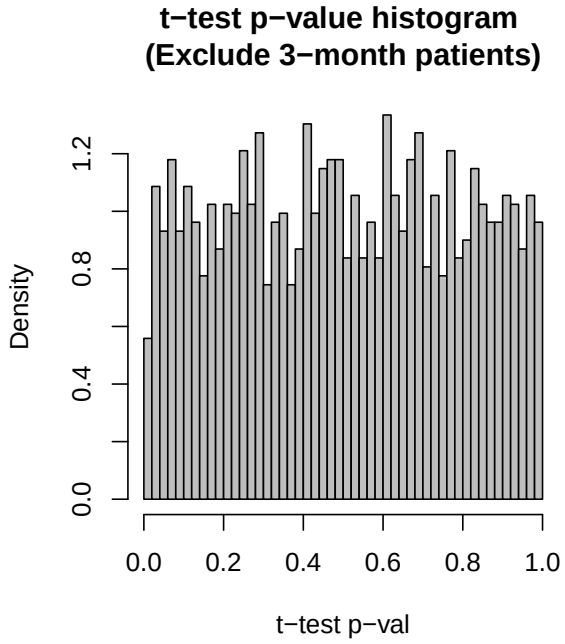
2 Statistical tests

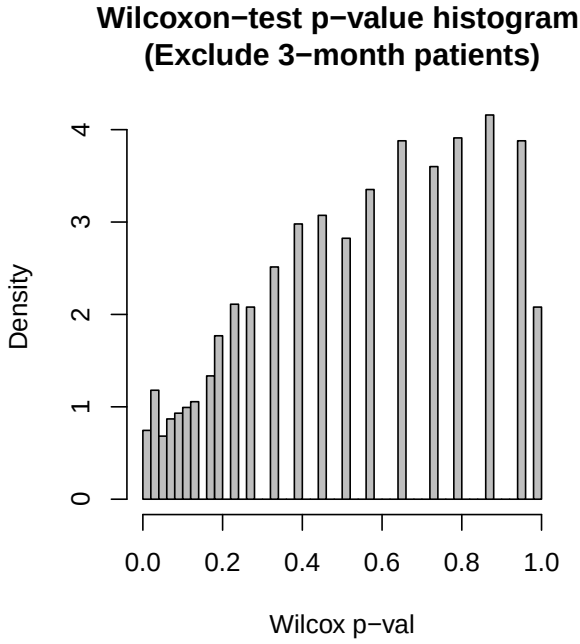
First, we repeat our calculations in Section 3 of *"01_sipT.pdf"*. Let $\mu_{A,p}$ and $\mu_{B,p}$ be the average difference (after - before) in log2 fluorescence levels, for the sip-T group (denoted A) and the sip-T + vaccine group (denoted B) respectively, for the p^{th} protein, where $p = 1, \cdots, 1611$. For each protein p, we test the following hypotheses

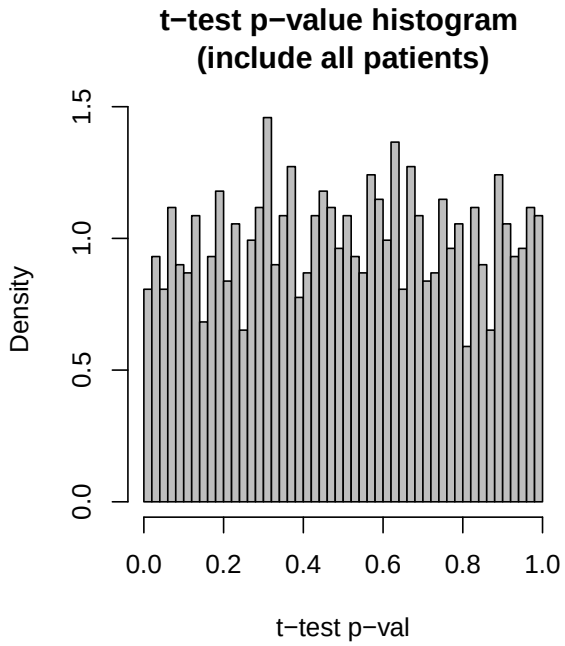
H_0: $\mu_{A,p} = \mu_{B,p}$
H_1: $\mu_{A,p} \neq \mu_{B,p}$

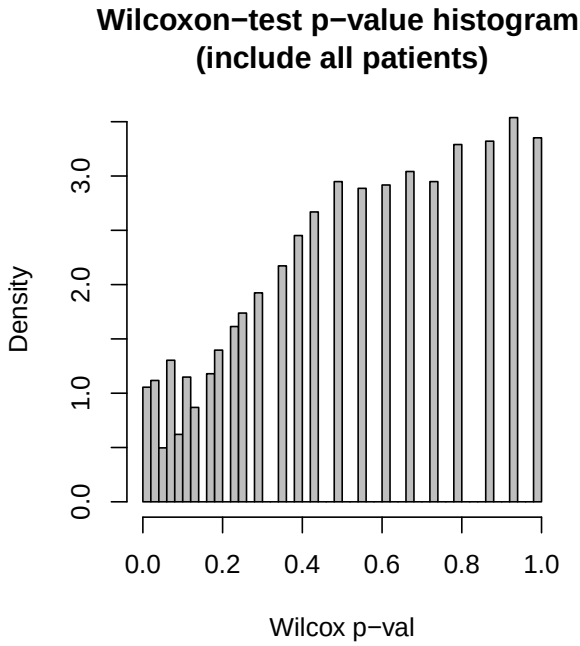
using both 2-sample t-test and Wilcoxon rank-sum test. We repeat the tests to include or exclude the patients (ID018 in group A and ID008 in group B) whose data was collected at different time points from the rest of the patients. Again, in all cases, there dont appear to be any signal about different antibody responses

between the two groups of patients. As expected, no proteins appears significant after Benjamini-Hochberg FDR control [Benjamini and Hochberg, 1995].





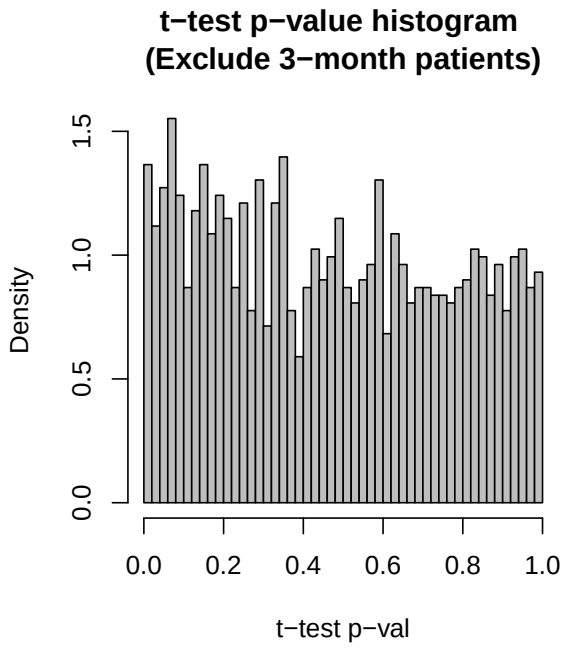


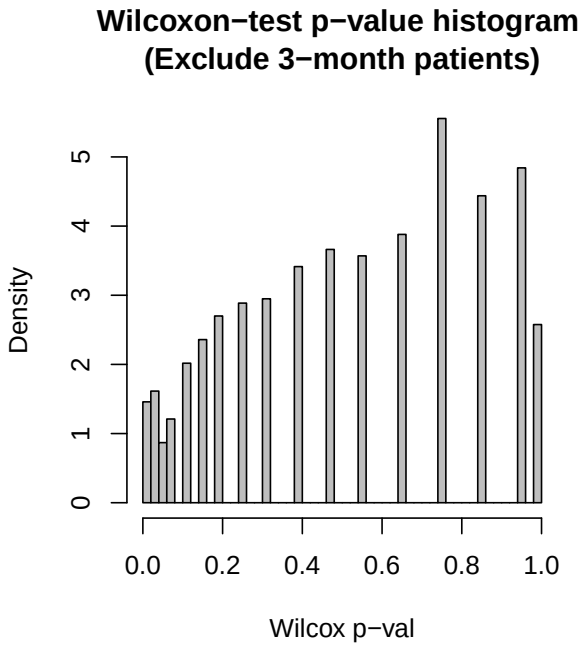


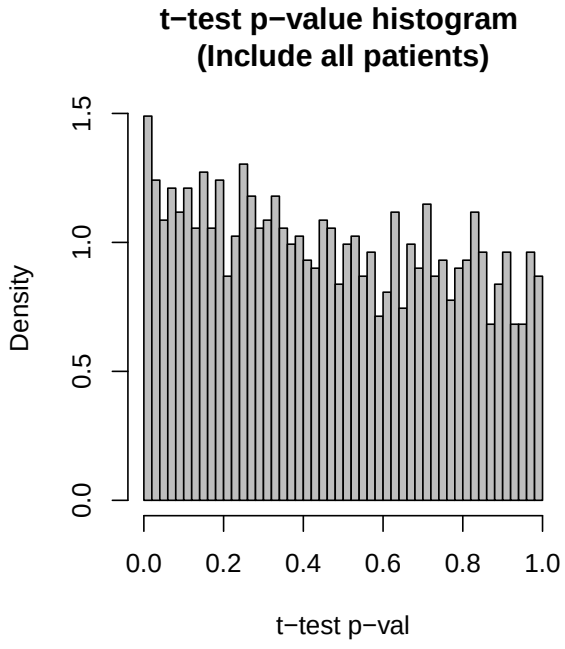
Next, we investigate if there are individual proteins to which responses might have been generated by sip-T alone. Specifically, we look at group A patients (getting sip-T only), and test if the mean fold changes for their proteins significantly changed following treatment, i.e. for $p = 1, \cdots, 1611$,

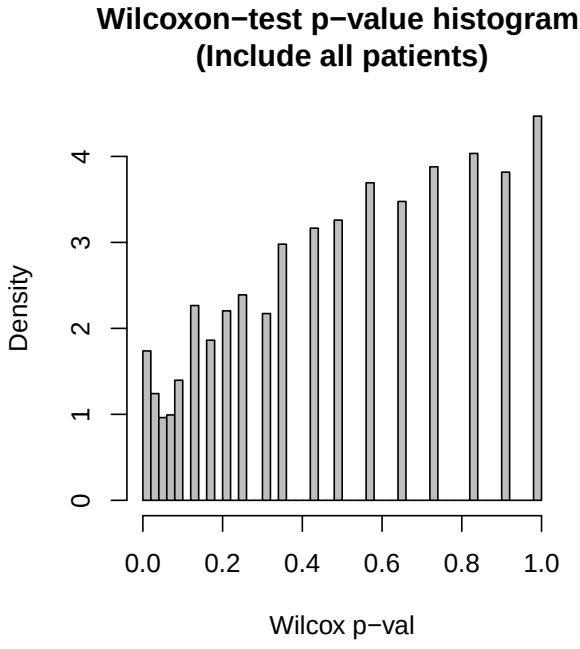
H_0: $\mu_{A,p} = 0$
H_1: $\mu_{A,p} \neq 0$

using both 2-sample t-test and Wilcoxon rank-sum test. Again, no proteins appears significant after Benjamini-Hochberg FDR control. The p-value histograms are displayed as follows.





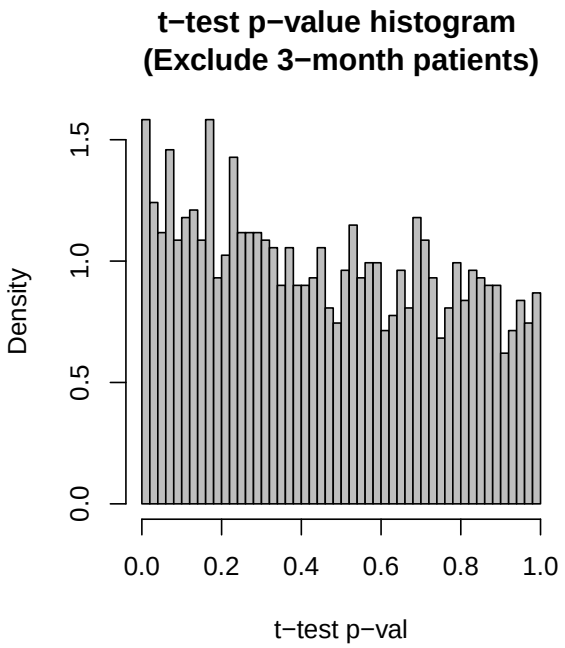


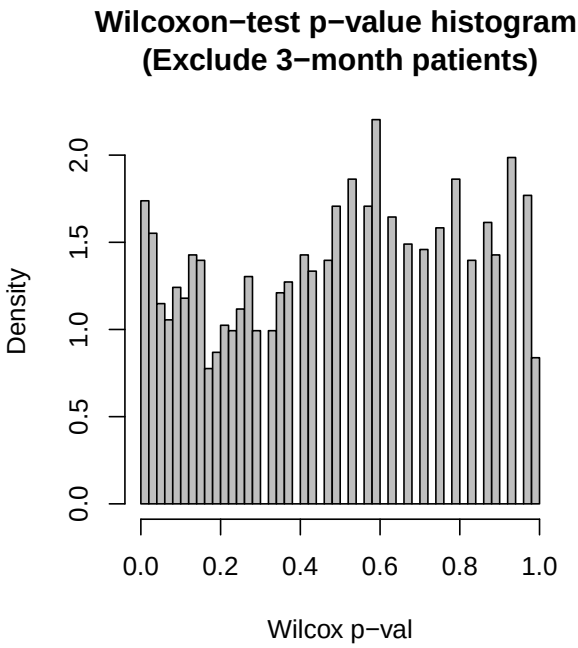


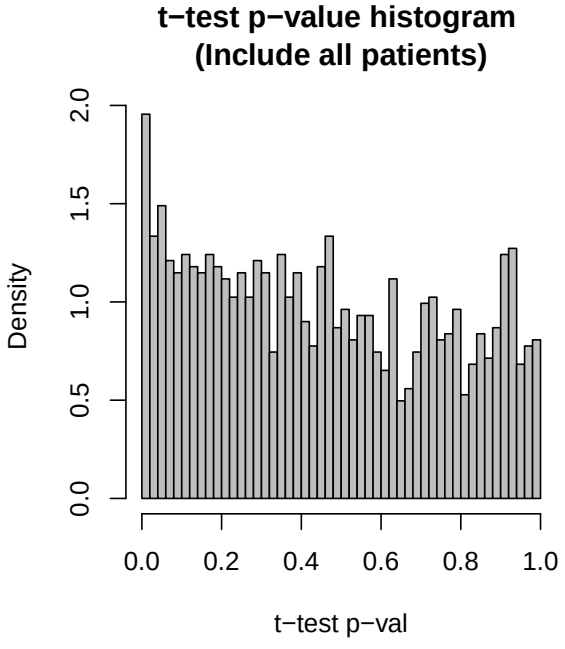
Finally, since our previous tests indicate that there is no significance difference in antibody response between groups A and B, we put these 2 groups of patients together, and test if μ = average difference (after - before) in log2 fluorescence levels for all patients is significantly changed post treatment, i.e. for $p = 1, \cdots, 1611$, we test

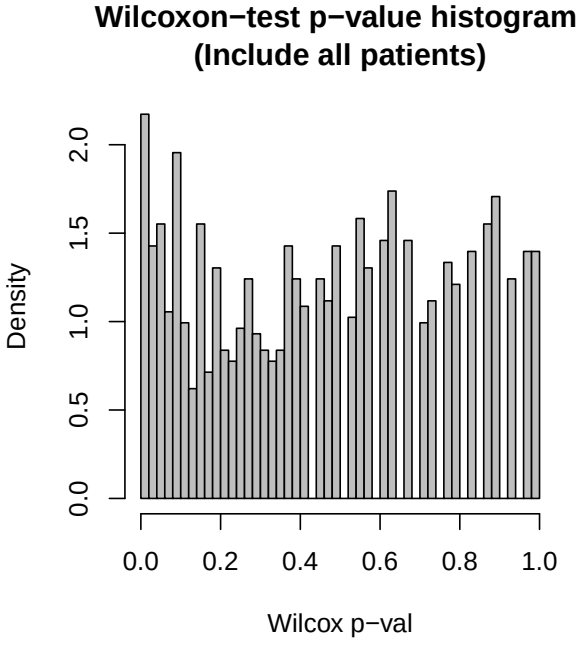
H_0: $\mu_p = 0$
H_1: $\mu_p \neq 0$

using both 2-sample t-test and Wilcoxon rank-sum test. The p-value histograms are displayed as follows.









After excluding the 3-month patients, no proteins appear to be significant at even 20% FDR. However, if we include all patients anyway, there are a couple of proteins that make the 5% FDR cutoff.

We tabulate the protein counts at various FDR thresholds based on BH-adjusted t-test p-values (including all patients).

We also tabulate the protein counts at various FDR thresholds based on BH-adjusted Wilcoxon p-values (including all patients).

FDR threshold	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.1
Peptide counts	0	0	0	1	1	1	1	1	1	1

FDR threshold	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.1
Peptide counts	0	0	0	0	2	2	2	2	2	2

The 2 proteins that make the 5% FDR cutoff based on Wilcoxon BH-adjusted p-values are

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## [1] "1141_EIF4A3_9775" "232_ACPP_55"
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The first protein was among the 1051 proteins associated with 5680 significant peptides reported in JITC, the second one was not.

References

Yoav Benjamini and Yosef Hochberg. Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society. Series B (Methodological)*, 57(1):289–300, 1995.

Hemanth K. Potluri, Tun Lee Ng, Michael A. Newton, Jin Zhang, Christopher A. Maher, Peter S. Nelson, and Douglas G. McNeel. Antibody profiling of patients with prostate cancer reveals differences in antibody signatures among disease stages. *Journal for ImmunoTherapy of Cancer*, 8(2), 2020. doi: 10.1136/jitc-2020-001510.