Final Project Report

**Introduction**

In this report, we will explore three figures in a paper written by Kersten et al. The original data will be used to re-plot these figures using plotting and data analysis libraries in Python. We will also use statistical tests to find significant values in each of the datasets.

**Section 1**

In this dataset plotted in Fig. 1, we have the levels of expression of four genes in a type of macrophage present in tumors. The expression is measured in the control, anti-CD4 treatment, and anti-CD8 treatment for each gene.

Chart, box and whisker chart

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Figure 1:Quantification of H2kb, MHC-II, CD11c, and CD206 expression in tumors after isotype, anti-CD4, and anti-CD8 treatment. \*p < 0.05, \*\* p < 0.01

Here, our null hypothesis is that the treatment groups are different from the control. Since we must compare independent groups that are not normally distributed, we use the Mann-Whitney U test. Significant results were found for the anti-CD8 population, where there was a decrease in H2kb, MHC-II and CD11c expression, and an increase in CD206 expression.

**Section 2**

In this dataset plotted in Fig. 2, we have the levels of expression of a gene in three different types of cells. There are two sets of treatment groups, one with cells from B16ChOVA and one from the B16F10 tumors.

Our null hypothesis is that the three groups had an equal variance. This is rejected by performing the Levene test. The second null hypothesis is that none of the groups are significantly different from the control group. This was rejected by a one-way ANOVA test. Finally, to determine which group shows significant differences from the control group, we perform a multi-comparison Tukey test. The results of this test are shown in Fig. 2 below. The TAM and CD103+DC population from B16ChOVA tumors showed consistent significant differences from the control.

**Chart, box and whisker chart

Description automatically generated**Figure 2: Flow cytometric analysis of CD44, IRF4, PD-1, and TOX expression in T cells co-cultured for 72 h with *in vitro*-generated BMDCs and TAMs or CD103+ DCs isolated from B16ChOVA or B16F10 tumors. (.1 represents B16F10 tumors) \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, \*\*\*\*p<0.0001

**Section 3**

In this dataset plotted in Fig. 3, we have the levels of expression of a gene in five different types of cells. The expression is measured in normoxic (%21 O­­2) and hypoxic (%1.5 O­­2) conditions for each cell.

Chart, waterfall chart

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Figure 3: Quantification of PD-1+ expression on activated T cells after co-culture with BMDCs ± SL8, and TAMs isolated from B16ChOVA or B16F10 tumors. \*p < 0.05, \*\*\*\*p<0.0001

Here, our null hypothesis is that the treatment groups are different from the control. Since we must compare independent groups that are not normally distributed, we use the Mann-Whitney U test. Significant results were found for the BMDC+SL8 population showing a great variation from the control, and 2x TAM (B16ChOVA) population showed a smaller variation from the control.

**References**

Kersten, K., Hu, K. H., Combes, A. J., Samad, B., Harwin, T., Ray, A., Rao, A. A., Cai, E., Marchuk, K., Artichoker, J., Courau, T., Shi, Q., Belk, J., Satpathy, A. T., & Krummel, M. F. (2022). Spatiotemporal co-dependency between macrophages and exhausted CD8+ T cells in cancer. Cancer Cell, 40(6), 624-638.e9. https://doi.org/10.1016/j.ccell.2022.05.004