

# DiseaseDiva

Us

10/24/2021

## Introduction

Prostate cancer is among one of the leading causes of death in males in the United States. The genomic study of metastatic prostate cancer has been limited due to tumor samples being obtained from autopsies as well as limited cohort pre-clinical models. Prospective studies are few and far between due to limitations in collecting adequate tumor tissue through biopsy. A paper published in 2015 prospectively analyzed tumor biopsies among affected individuals. Subsequently as of 2016, genomic data for prostate cancer has become more widely available due to the National Cancer Institutes (NCI) Genomics Data Commons (GDC).

In this project we will explore data from both the 2015 prospective cohort study along with data available from the NCI's Genomic Data Commons. This project aims to investigate three data sets, through mapping and transformation of the raw data through multiple platforms such as Excel, SQL and R, with the ultimate goal of optimizing downstream analysis of the prostate cancer cohorts. We will discuss various methods by which we wrangled the data to investigate underlying associations between individual case presentations and manifestation of disease.

A team of 73 researchers conducted a multi-institutional study that investigated clinical genomics of advanced prostate cancer specifically metastatic, castration resistant prostate cancer (mCRPC). mCRPC occurs in individuals who develop resistance to androgen deprivation therapies (ADT). The aim of this research was to facilitate precision medicine by developing a prospective whole-exome and transcriptome sequencing of tumor biopsies. Data was obtained from living affected individuals to compile a set of genomic alterations. The study was published in the journal "Cell" in May 2015.

The link to the publication can be found [here](#).

Data was extracted from the cBioPortal, a public cancer genomics site hosted and maintained by the Memorial Sloan Kettering Cancer Center. The sample included 150 individuals with metastatic prostate cancer along with 17 data points per individual, described below:

- **Patient ID:** unique patient identifier (number)
- **Sample ID:** unique biopsy sample identifier from clinical trial(number)
- **Mutation Count:** mutation rate for mCRPC defined as mutations per megabase or mutations/Mb (number)
- **Fraction Genome Altered:** fraction of mutant allele variants in biopsy (number)
- **Diagnosis Age:** patient's age when they were diagnosed (number)
- **Tumor Site:** physical site of tumor on human body from which biopsy was taken (string)
- **Abiraterzone (ABI) and Enzalutamide (ENZA) Exposure Status:** exposure to second-generation androgen deprivation therapies (binary: yes/no)
- **Center of Sequencing:** the international academic medical center where exome sequencing occurred (string)
- **Prior Treatment:** receipt of previous androgen deprivation therapy (binary:yes/no)
- **Site Sequenced:** site of exome sequencing: Broad or UM (string)
- **tumor content:** percentage of tumor captured in the biopsy (number)

The National Cancer Institute (NCI) maintains a unified repository of genomic data known as the Genomic Data Commons(GDC). Groundwork for this repository began in June 2015 with nearly 50,000 raw data sequences analyzed by June 2016. This data sharing platform uses an Open-Stack-based private cloud to unite several large-scale cancer genome research programs such as TCGA and OCG. The purpose of this repository is to standardize the data processing of these multi-dimensional data sets by using common bioinformatics pipelines that facilitate direct comparison of the data.

More about the National Cancer Institute’s Genomic Data Commons can be found [here](#).

Data was extracted from GDC Portal’s Exploration page. The “cleaned” version of the data sample included 493 individuals with metastatic prostate cancer along with 24 data points per individual as described below:

- **CaseUUID:** universally unique identifier assigned to every entity in the data model (string)
- **CaseID:**specific individual cases using the submitter ID from the genomic cancer research program (string)
- **Project:**The cancer research program from which the case originated from (string)
- **Primary Site:** The primary site of the cancer (string)
- **Gender:**“Gender is defined as a set of characteristics that distinguish persons based on their social roles” (string)
- **Files:** the quantity of files available for that case (number)
- **Seq:**Number of the sequencing reads(an inferred sequence of basepairs) (number)
- **Exp:**The transcriptome profiling (number)
- **SNV:**simple nucleotide variation (number)
- **CNV:**Copy number variation,the number of copies of a particular gene varies from one individual to the next (number)
- **Meth:**Number of DNA methylation that took place (number)
- **Clinical:** The number of clinical alterations (number)
- **Bio:**biospecimen (number)
- **Mutations:**The number of simple somatic mutations detected for that case(number)
- **Genes:** The quantity of genes affected by mutations for each case (number)
- **Slides:** the quantity of slides available for each case (number)
- **Program:** The type of program the subject is enrolled in “The Cancer Genome Atlas” or the “Count Me In” (string)
- **Disease Type:** This describes if it is Adenomas and Adenocarcinomas, the location where the cancer is formed (string)
- **Age at diagnosis:**Age at the time of diagnosis calculated from their day of birth to the day they were diagnosed with cancer (string)
- **Days to death:**The date from when they were diagnosed, and they died (string)
- **Vital Status:** the survival status of the person (string)
- **Primary Diagnosis:**Their initial pathological diagnoses of cancer (string)
- **Ethnicity:** “An individual’s self-identified social and cultural category, particularly whether they identify as Hispanic or Latino” (string)
- **Race:** “A taxonomic group that is a division of a species that is classified arbitrarily. It is characterized by shared heredity, physical attributes, and behavior, and in the case of humans, by common history, nationality, or geographic distribution” (string)

Using patient data from the 2015 prospective cohort study, we centered our project around three main aims:

First, we wanted to look at possible associations between geographic location and key characteristics of prostate cancer. More specifically, our analysis revolved around looking at links between patients’ sequencing site location and severity of tumor content, along with whether sequencing site region had any link to prior treatment status and exposure to second-generation androgen deprivation therapies. Through this aim, we sought to answer the question “Does geographic region affect severity of prostate cancer characteristics?” We specifically chose to focus on geographic region for this aim so we could draw conclusions on if patient location had any clinical implications when considering future treatments for prostate cancer, and answer

questions such as “Would it be beneficial to allocate more resources to certain regions of the country?” or “Are men in certain regions of the country who have had exposure to second-generation androgen deprivation therapies at more risk for developing prostate cancer?”

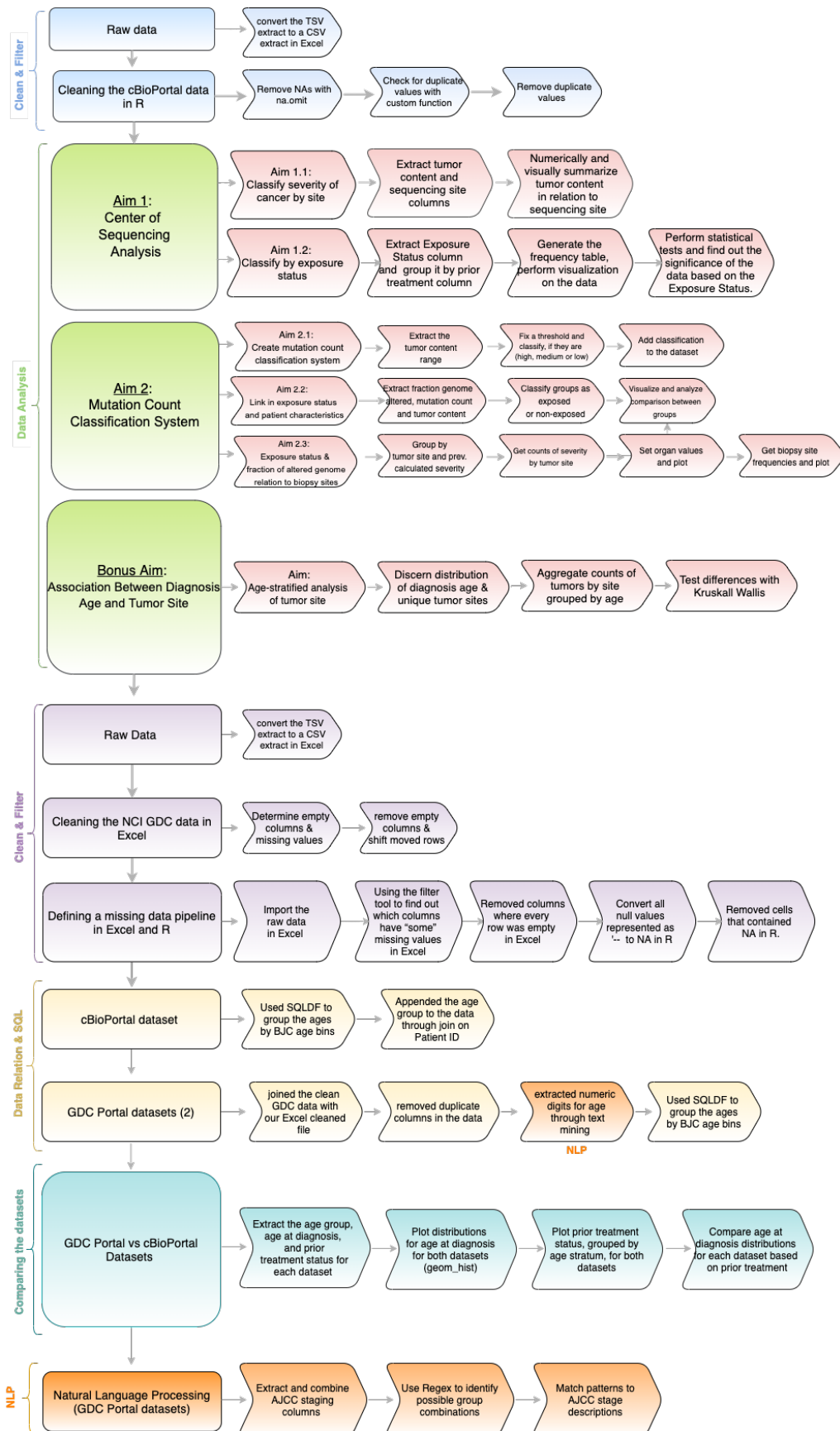
Our second aim focused on patient mutation counts and their relationship to biopsy sites, exposure to second-generation androgen deprivation therapies, and fraction of patient’s genome that was altered. According to a 2017 publication from Cell Martincorena, et al. , cancer will usually emerge in a patient after 1-10 mutations. Thus, we created a classification system to categorize mutation level as “high”, “medium”, or “low” based on the mutation count variable from the data set, and compare severity of mutation to other patient characteristics. Since the 2015 prospective cohort study was specifically interested in patients who developed metastatic, castration resistant prostate cancer (mCRPC), which occurs in individuals who develop resistance to androgen deprivation therapies (ADT), our second aim sought to look at correlations between mutation count and exposure to second-generation androgen deprivation therapies, along with fraction of patient’s genome altered and biopsy sites.

Our last aim, titled the “Bonus Aim” as we hadn’t originally planned to perform this analysis, studied possible associations between patient age at diagnosis and tumor site. We wanted to study this specific association to see if we could identify if earlier age at diagnosis corresponds to specific tumor site (likewise for older ages of diagnosis), since finding such an association could have clinical implications for when considering treatments for different age groups. To study this association, we performed an age stratified analysis based on age groups that were used by a population based study published in the British Journal of Cancer in 2013 read [here](#).

We also wanted to utilize the lesson on Natural Language Processing from class in our data sets, so we applied the principles of NLP to the second data set in order to better understand information that was given about cancer stages for each patient. In order to do this, we used Regex and information on cancer stages descriptions found [here](#).

## PIPELINE

Below is the pipeline for our project. It covers our cleaning process for both data sets, our process for working with relational data (with GDC data), how we performed Natural Language Processing on the GDC portal data sets, and what we did for each of our aims. We will be covering each of these points in detail over the course of this paper.



# Methods

## GITHUB

We used Github, a version-control software readily available on the web, to collaborate with each other throughout the project and efficiently add our individual contributions to our final paper. We created a repository titled “Data Wrangling Project”, which included our final Rmd file (this paper), a copy of our project pipeline image, our custom library package, and all relevant data sets throughout our analysis. We followed a standard protocol for adding and updating changes to our files – we used the git pull command for updating our files and we used the add, commit, and push commands for adding our individual changes to the repository. Utilizing Github allowed all of our group members to work on our individual aims at our own time and pace while keeping everyone else updated on our individual and overall progress. A link to our finals repository can be found [here](#).

## CUSTOM FUNCTIONS

Below are our custom functions that will be used throughout our data cleaning and joining processes.

Our first function, `string_NA`, checks for values in a data frame that are equal to the string " ’– “, and changes the value to "NA".

Our second function, `carbon.copy`, checks for columns that have the exact same values in a data frame. If two columns have the exact same values (case insensitive), the function will remove one of those columns.

Our third and final function `find_nonuniq`, prints out columns in a given data frame that do not contain a single unique value.

Our functions are included below:

```
library(DiseaseDivas)

## Convert to NAs function
string_NA <- function(data){
  data[data == "'– “] <- NA
  return(data)
}

# Remove a column if two columns are the exact same
carbon.copy <- function(df){
  return(df[!duplicated(as.list(df))])
}

# Print out all columns that have the same value for all rows
find_nonuniq <- function(dataset){
  cols_nonunique <- c()
  for(i in 1:ncol(dataset)){
    if(length(unique(dataset[,i])) <= 1){
      cols_nonunique[length(cols_nonunique) + 1] <- i #append the vector with the new columns
    }
  }
  print(cols_nonunique) #print the vector
}
```

We also compiled our functions into a simple library, titled `DiseaseDivas`, which is available on our [GitHub](#)

repository. We followed methods outlined by Dr. Joshua Levy from class on Thursday, October 14th, 2021. Link to the lecture can be found [here](#).

Below is an example of how to use our library:

```
rawdat<- read.csv("~/Desktop/Data-Wrangling-Project/diseasedivadata.csv")
dim(rawdat) # Check raw data dimensions
```

```
## [1] 150 20
```

```
example_dat = DiseaseDivas::carbon.copy(rawdat) # Use library to get rid of duplicate functions
dim(example_dat) # Check new non-duplicate data frame dimensions
```

```
## [1] 150 18
```

## Extracting the Data from the cBioPortal

The first data set was downloaded from the cBioPortal site using the “download clinical data for the selected cases” button extracting all columns.

The link to the data set can be found [here](#).

## Cleaning Our Data

The first data set was downloaded as a TSV file which required conversion in Excel to a CSV file. After importing the data into Excel using the Import Data From Text tool, the worksheet was saved as a csv file. The raw data contained 150 rows and 20 columns. As a team we worked through the exploratory investigation and cleaning of the data by utilizing GitHub to collaborate on an R Markdown file.

```
#Load data downloaded from
rawdat<- read.csv("~/Desktop/Data-Wrangling-Project/diseasedivadata.csv")
```

To gain an understanding for the types of variables we would be analyzing along with some of the numeric summaries for the continuous variables, we employed the summary function.

```
##      Study.ID      Patient.ID      Sample.ID
## Length:150      Length:150      Length:150
## Class :character Class :character Class :character
## Mode  :character Mode  :character Mode  :character
##
##
##
## Abiraterone..ABI..and.Enzalutamide..ENZA..Exposure.Status Diagnosis.Age
## Length:150                                         Min.    :40.00
## Class :character                                         1st Qu.:62.00
## Mode  :character                                         Median  :68.00
##                                         Mean    :66.84
##                                         3rd Qu.:72.00
##                                         Max.    :85.00
##                                         NA's    :3
```

```
## Cancer.Type          Cancer.Type.Detailed Center.of.sequencing
## Length:150           Length:150           Length:150
## Class :character     Class :character     Class :character
## Mode :character      Mode :character      Mode :character
##
##
##
## Fraction.Genome.Altered Mutation.Count      Oncotree.Code
## Min. :0.0072      Min. : 26.00      Length:150
## 1st Qu.:0.2285      1st Qu.: 55.25      Class :character
## Median :0.3570      Median : 71.50      Mode :character
## Mean :0.3767      Mean : 114.81
## 3rd Qu.:0.5432      3rd Qu.: 120.75
## Max. :0.8466      Max. :1255.00
##
## Prior.Treatment      Number.of.Samples.Per.Patient Sample.Type
## Length:150           Min. :1           Length:150
## Class :character     1st Qu.:1         Class :character
## Mode :character      Median :1         Mode :character
##                      Mean :1
##                      3rd Qu.:1
##                      Max. :1
##
## Sex                  Site.Sequenced      Somatic.Status      tumor.content
## Length:150           Length:150           Length:150           Min. :20.00
## Class :character     Class :character     Class :character     1st Qu.:44.00
## Mode :character      Mode :character     Mode :character     Median :62.50
##                      Mean :60.53
##                      3rd Qu.:75.00
##                      Max. :98.00
##
## Tumor.Site           Tumor.Type
## Length:150           Length:150
## Class :character     Class :character
## Mode :character      Mode :character
##
##
##
##
```

Preliminary findings indicate, mean age of diagnosis for prostate cancer is approximately 67 years of age. Average tumor content found in these biopsies appears to be 60%. Initial analysis revealed that there were several cells for which NA values were present. The following command was used to eliminate the NAs saving the new data to a data frame called complete\_dat.

```
#Remove NAs
complete_dat <- na.omit(rawdat)
```

Upon removal of the NAs, the data set reduced to 147 rows and 20 columns. Three patients from the original extract contained in rows 71 to 73 were therefore excluded from subsequent analyses due to the NAs being present in both the Diagnosis.Age column along with the Abiraterone (ABI) and Enzalutamide (ENZA) Exposure Status. These two columns are crucial in further analyses being as age is a related

factor for severity of disease. Furthermore, without information as to prior exposure status to the androgen deprivation therapies, assessment of mCRPC is incomplete.

Further analysis of the data revealed that several columns were found to have duplicated values. To assess whether the individual cases included in the sample were duplicated, we utilized the duplicated command to check the Sample ID column for repeat values. As shown below, none were found.

```
#Check for duplicates
duplicated(complete_dat$Sample.ID)

## [1] FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
## [13] FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
## [25] FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
## [37] FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
## [49] FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
## [61] FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
## [73] FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
## [85] FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
## [97] FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
## [109] FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
## [121] FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
## [133] FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
## [145] FALSE FALSE FALSE
```

Upon cursory glance the Patient ID and Sample ID column appeared to be populated with the exact same values per each row. To confirm this assumption we used the following syntax to check if the values between the two columns were unique. The syntax reads as follows, from the complete\_dat data frame check if Patient Id is not contained in Sample Id then output the results.

```
#Check if sample id is dup
complete_dat[!complete_dat$Patient.ID %in% complete_dat$Sample.ID, ]

## [1] Study.ID
## [2] Patient.ID
## [3] Sample.ID
## [4] Abiraterone..ABI..and.Enzalutamide..ENZA..Exposure.Status
## [5] Diagnosis.Age
## [6] Cancer.Type
## [7] Cancer.Type.Detailed
## [8] Center.of.sequencing
## [9] Fraction.Genome.Altered
## [10] Mutation.Count
## [11] Oncotree.Code
## [12] Prior.Treatment
## [13] Number.of.Samples.Per.Patient
## [14] Sample.Type
## [15] Sex
## [16] Site.Sequenced
## [17] Somatic.Status
## [18] tumor.content
## [19] Tumor.Site
## [20] Tumor.Type
## <0 rows> (or 0-length row.names)
```



The output above is empty indicating that all the Patient Ids are contained within the Sample Id. In other words the two columns are the same.

Several columns appeared to be populated by the same value for each row. For example, the Cancer.Type column was entirely populated by the value “Prostate Adenocarcinoma”. To determine whether this assumption was indeed true we created the following for loop that cycled through each column to check that the values contained in the column were unique.

```
cols_nonunique=DDCustomPkg::find_nonuniq(complete_dat)
```

```
## [1] 1 6 7 11 13 14 15 17 20
```

```
remove_nonuniques <- complete_dat[,-cols_nonunique]
```

## Aim 1

### Aim 1.1: Classify severity of cancer by site.

We used the dplyr library for most of the data manipulation for Aim 1.1. To begin our analysis, we extracted the two columns of interest: “Center of sequencing”, which has the locations of where each patient sample was sequenced, and “Tumor content”, which contains the tumor content of each patient sample. Tumor content was classified as percentages that were defined by computational analysis, based on mutant allele variant fractions and zygosity shifts. We made a separate table using just these two columns from the filtered data and named it “aim\_1\_1”.

```
# Load dplyr library for data manipulation.  
library(dplyr)
```

```
##  
## Attaching package: 'dplyr'  
  
## The following objects are masked from 'package:stats':  
##  
## filter, lag  
  
## The following objects are masked from 'package:base':  
##  
## intersect, setdiff, setequal, union
```

```
# Extract columns needed for Aim 1.1 and make subtable  
aim_1_1 = complete_dat %>%  
  select(Center.of.sequencing, tumor.content)
```

The next step in our analysis was to create a classification system for tumor content and categorize each patient sample as either low, medium, or high tumor content level. The study we downloaded our raw data from only included patient samples whose tumor content was greater than 20%, so we made a simple classification system in which 20-45% tumor content is considered low, 46-70% tumor content is considered medium, 71-100% tumor content is considered high. To add this classification to our data frame, we created a new column named “TC\_Level” and added each classification for patient sample as a factor using if-else statements.

```

# 20 - 45 is low
# 46 - 70 is medium
# 71 - 98 is high

# Create and add classification system to data frame
aim_1_1$TC_level <- as.factor(ifelse(aim_1_1$tumor.content < 46, 'Low',
                                     ifelse(aim_1_1$tumor.content < 71, 'Medium', 'High'))))

```

The next step in our analysis was to group sequencing centers (aka the “Center of sequencing” column) into geographic regions. Sequencing centers in our data set included Dana-Farber Cancer Institute, Memorial Sloan Kettering Cancer Center, Karmanos Cancer Center, University of Michigan, University of Washington, and the Insitute of Cancer Research & The Royal Marsden. Geographic regions were grouped as shown below:

EAST COAST LOCATIONS: DFCI (Boston, MA) MSKCC (New York, NY)

MIDWEST LOCATIONS: Karmanos (Detroit, MI) U Michigan (Ann Arbor, MI)

WEST COAST LOCATIONS: U Washington (Seattle, WA)

INTERNATIONAL LOCATIONS: RM-ICR (London, UK)

To add the geographic regions to our data frame, we followed the same step used to add the tumor content classifications to the data frame – we created a factor using if-else statements and added the region for each patient sample, as shown below

```

# Add geographic region based on sequencing center
aim_1_1$Site_region <- as.factor(ifelse(aim_1_1$Center.of.sequencing == 'DFCI' | aim_1_1$Center.of.sequencing == 'Karmanos Cancer Insitute' | aim_1_1$Center.of.sequencing == 'U Washington', 'West Coast', 'International'))

```

Now that we have created a classification system for tumor content and grouped sequencing centers by geographic region, we started to look at the data numerically and visually. To get a better understanding of the distribution of the tumor content levels across the geographic region, we created a simple table looking at the mean tumor content across each sequencing region. We made this table using the dplyr and gtsummary packages. The gtsummary package contains the tbl\_summary() function which is very helpful when trying to create tables with different types of statistical information. We used piping to select the proper data columns from our original aim\_1\_1 subtable, and then we used the gtsummary package to design the table as shown below.

```

library(gtsummary)
# Create numeric summary of mean tumor content by sequencing region
raw_tc_tab = aim_1_1 %>%
  select(Site_region, tumor.content) %>%
  tbl_summary(by=Site_region, statistic = list(all_continuous() ~ "{mean} ({sd})"), label = tumor.content,
             modify_spanning_header( all_stat_cols() ~ "**Sequencing Region**") %>% modify_header(label = "")
raw_tc_tab

```

```

## Table printed with 'knitr::kable()', not {gt}. Learn why at
## http://www.danielsjoberg.com/gtsummary/articles/rmarkdown.html
## To suppress this message, include 'message = FALSE' in code chunk header.

```

	East Coast, N = 28	International, N = 30	Midwest, N = 48	West Coast, N = 41
Mean Tumor Content	63 (18)	58 (19)	62 (21)	58 (20)

From the table created above, we can see that the mean tumor content seems to be fairly equal among the geographic regions covered in the data. The mean tumor content ranges from 58% in the West Coast/internationally to 63% on the East Coast. Additionally, the standard deviations are also relatively similar across the regions, ranging from 18% to 21%.

Next, we tried to look at the data visually. We decided to make a bar plot to visually assess the distribution of the mean tumor content percentages across sequencing regions. In order to create my plot, we first used the tidyverse package to group my data by sequencing region, and then get the mean tumor content levels for each group. We made a new table, “raw\_tc\_df”, which contains the mean tumor content level by sequencing region that we could feed into ggplot to create our visualization.

Then, we used ggplot to create our boxplot. Using the data from “raw\_tc\_df”, we plotted the mean tumor content on the y axis against the sequencing regions on the x axis. We then used several features (geom\_text, scale\_fill\_brewer, theme, just to name a few) to improve the aesthetics of the plot.

```
library(tidyverse)
```

```
## -- Attaching packages ----- tidyverse 1.3.1 --
```

```
## v ggplot2 3.3.5      v purrr  0.3.4
## v tibble  3.1.5      v stringr 1.4.0
## v tidyr   1.1.4      v forcats 0.5.1
## v readr   2.0.1
```

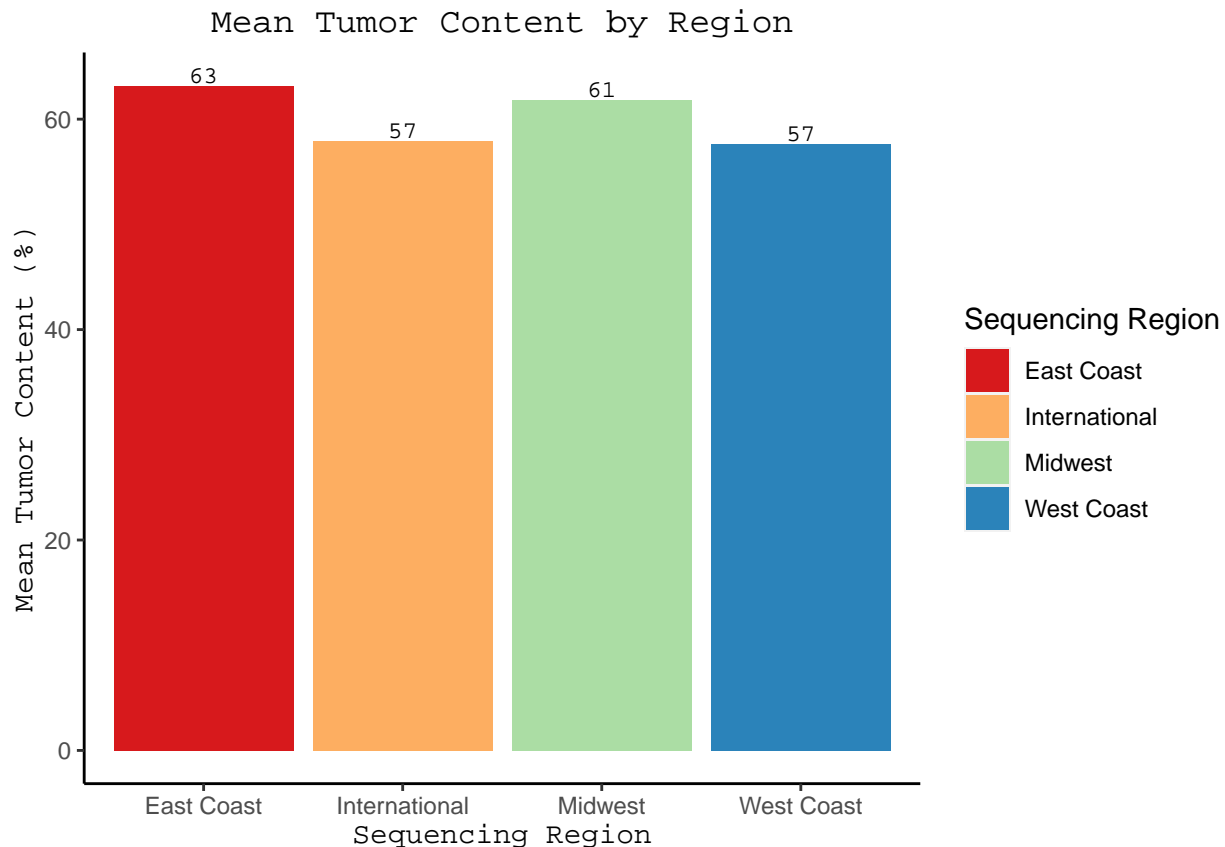
```
## -- Conflicts ----- tidyverse_conflicts() --
## x dplyr::filter() masks stats::filter()
## x dplyr::lag()     masks stats::lag()
```

```
# Create subtable which contains mean tumor content levels by sequencing region
```

```
raw_tc_df = aim_1_1 %>%
  group_by(Site_region) %>%
  summarise_at(vars(tumor.content), list(name = mean))
raw_tc_df <- raw_tc_df %>%
  rename(mean.tumor.content=name)
```

```
# Plot mean tumor content levels for each sequencing region
```

```
ggplot(data=raw_tc_df, mapping=aes(x=Site_region, y=mean.tumor.content, fill=Site_region)) +
  ggtitle("Mean Tumor Content by Region") + # Set title
  # Add mean tumor content values to the bars
  geom_text(aes(label = as.integer(mean.tumor.content)), vjust = -0.2, size = 3, position = position_dodge()) +
  geom_bar(stat='identity') +
  # Set color palette
  scale_fill_brewer(palette = "Spectral") +
  # Set legend title
  guides(fill=guide_legend(title="Sequencing Region")) +
  # Remove background grid, change fonts, make it pretty
  theme(panel.background = element_blank(), axis.line = element_line(colour="black"), plot.title = element_text(margin = 10)) +
  xlab("Sequencing Region") + ylab("Mean Tumor Content (%)" )
```



After visually assessing the mean tumor content levels by region, we can see that the tumor content levels do seem to be fairly equal among the geographic regions. Next, we decided to look at the distributions of tumor content levels (using my classification system) by center of sequencing. We made this table using the gtsummary package again. We also ran a Fisher's exact test and obtained a p-value of 0.9, suggesting that there is not a significant association between Sequencing Site and tumor content level.

```
# Make a table to look at tumor content levels across sequencing site
p1 = aim_1_1 %>%
  select(Center.of.sequencing, TC_level) %>%
  tbl_summary(by = TC_level,
              label = Center.of.sequencing ~ "Sequencing Site") %>%
  add_p() %>%
  modify_header(label = "") %>%
  modify_spanning_header(all_stat_cols() ~ "Tumor Content Level") %>%
  bold_labels()

p1
```

```
## Table printed with 'knitr::kable()', not {gt}. Learn why at
## http://www.danielsjoberg.com/gtsummary/articles/rmarkdown.html
## To suppress this message, include 'message = FALSE' in code chunk header.
```

	High, N = 50	Low, N = 44	Medium, N = 53	p-value
Sequencing Site				0.9
DFCI	2 (4.0%)	3 (6.8%)	3 (5.7%)	

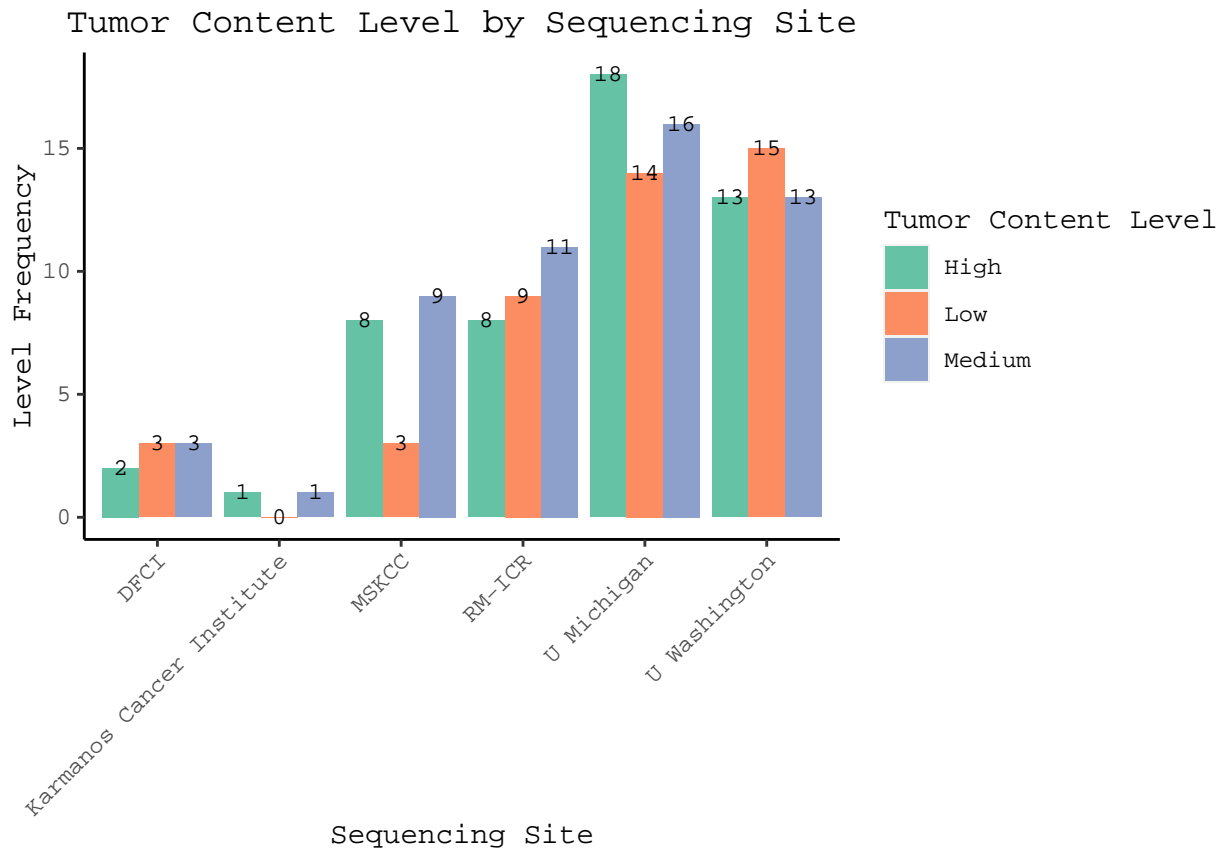
	High, N = 50	Low, N = 44	Medium, N = 53	p-value
Karmanos Cancer Institute	1 (2.0%)	0 (0%)	1 (1.9%)	
MSKCC	8 (16%)	3 (6.8%)	9 (17%)	
RM-ICR	8 (16%)	9 (20%)	11 (21%)	
U Michigan	18 (36%)	14 (32%)	16 (30%)	
U Washington	13 (26%)	15 (34%)	13 (25%)	

To get a better visual assessment of the tumor content levels by sequencing sites, we made a grouped bar plot using ggplot:

```
mid_tab=table(aim_1_1$TC_level, aim_1_1$Center.of.sequencing)
mid_df = data.frame(mid_tab)
mid_df
```

```
##      Var1              Var2 Freq
## 1    High             DFCI     2
## 2     Low             DFCI     3
## 3  Medium             DFCI     3
## 4    High Karmanos Cancer Institute 1
## 5     Low Karmanos Cancer Institute 0
## 6  Medium Karmanos Cancer Institute 1
## 7    High             MSKCC     8
## 8     Low             MSKCC     3
## 9  Medium             MSKCC     9
## 10   High             RM-ICR     8
## 11   Low             RM-ICR     9
## 12  Medium             RM-ICR    11
## 13   High            U Michigan    18
## 14   Low            U Michigan    14
## 15  Medium            U Michigan    16
## 16   High            U Washington    13
## 17   Low            U Washington    15
## 18  Medium            U Washington    13
```

```
grouped_plot_a = ggplot(data = mid_df, mapping = aes(x=Var2, y=Freq, fill=Var1)) +
  geom_bar(stat="identity", position = "dodge") +
  geom_text(aes(label = Freq), vjust = 0.5, size = 3, position = position_dodge(0.9), colour="black", fontface="bold") +
  scale_fill_brewer(palette = "Set2") + ggtitle("Tumor Content Level by Sequencing Site") +
  theme(panel.background = element_blank(),
        axis.line = element_line(colour="black"),
        plot.title = element_text(hjust=0.5, family="Courier"),
        axis.title.x = element_text(family="Courier"),
        axis.title.y = element_text(family="Courier"),
        axis.text.x = element_text(family="Courier", angle = 45, vjust = 1, hjust = 1),
        axis.text.y = element_text(family="Courier"),
        legend.text=element_text(family="Courier"),
        legend.title=element_text(family="Courier")) +
  guides(fill=guide_legend(title="Tumor Content Level")) + xlab("Sequencing Site") + ylab("Level Frequency")
grouped_plot_a
```



We then realized that this table might be misleading since some sequencing sites have a much larger sample size than others, so we decided to look at the tumor content levels by sequencing site region instead. This table was also made using gtsummary.

```
# Create a table to compare tumor content levels across sequencing site regions
library(dplyr)
aim_1_1 %>%
  tbl_cross(
    row = TC_level,
    col = Site_region,
    percent = "cell", label = TC_level ~ "Tumor Content"
  ) %>% modify_header(label = "") %>% modify_spanning_header(all_stat_cols() ~ "Sequencing Region") %>%
  add_p() %>% bold_labels()
```

```
## Table printed with 'knitr::kable()', not {gt}. Learn why at
## http://www.danielsjoberg.com/gtsummary/articles/rmarkdown.html
## To suppress this message, include 'message = FALSE' in code chunk header.
```

	East Coast	International	Midwest	West Coast	Total	p-value
<b>Tumor Content</b>						0.9
High	10 (6.8%)	9 (6.1%)	18 (12%)	13 (8.8%)	50 (34%)	
Low	6 (4.1%)	9 (6.1%)	14 (9.5%)	15 (10%)	44 (30%)	
Medium	12 (8.2%)	12 (8.2%)	16 (11%)	13 (8.8%)	53 (36%)	
<b>Total</b>	28 (19%)	30 (20%)	48 (33%)	41 (28%)	147 (100%)	

Our next visualization is a boxplot comparing tumor content by sequencing site. We used the theme feature in ggplot to change the fonts and colors in the plot.

```
# Plot the tumor content percentages across sequencing site
aim_1_1 %>% select(Center.of.sequencing, tumor.content) %>%
  tbl_summary(by = Center.of.sequencing)
```

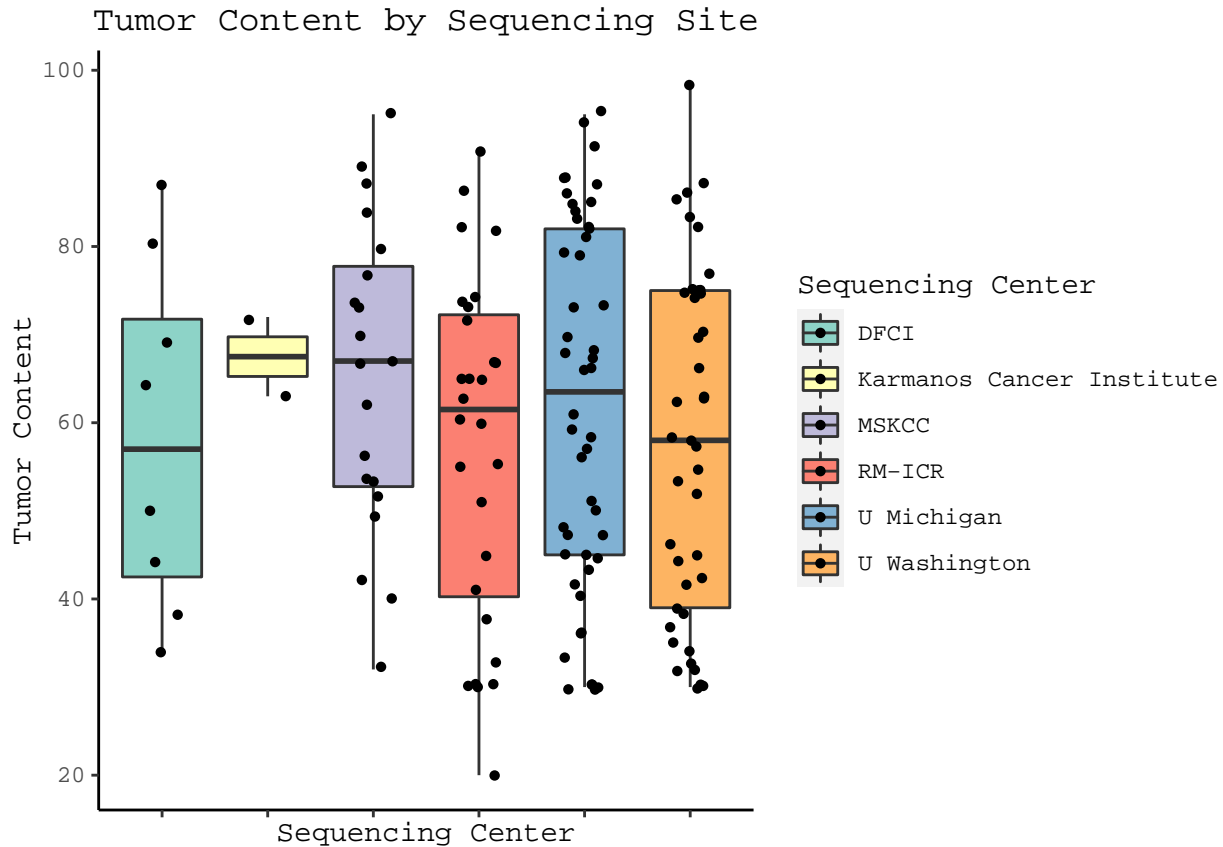
```
## Table printed with 'knitr::kable()', not {gt}. Learn why at
## http://www.danielsjoberg.com/gtsummary/articles/rmarkdown.html
## To suppress this message, include 'message = FALSE' in code chunk header.
```

Characteristics	MDACC, N = 8	Karmanos Cancer Institute, N = 2	MSKCC, N = 20	RM-ICR, N = 28	U Michigan, N = 48	U Washington, N = 41
tumor.content	57 (42, 72)	68 (65, 70)	67 (53, 78)	62 (40, 72)	64 (45, 82)	58 (39, 75)

```
xlabs <- paste(levels(aim_1_1$Center.of.sequencing), "\n(N=", table(aim_1_1$Center.of.sequencing), ")", sep="")

raw_plot = ggplot(data = aim_1_1, mapping = aes(x=Center.of.sequencing, y=tumor.content, fill=Center.of.sequencing)) +
  theme(panel.background = element_blank(),
        axis.line = element_line(colour="black"),
        plot.title = element_text(hjust=0.5, family="Courier"),
        axis.title.x = element_text(family="Courier"),
        axis.title.y = element_text(family="Courier"),
        axis.text.x=element_blank(),
        axis.text.y = element_text(family="Courier"),
        legend.text=element_text(family="Courier"),
        legend.title=element_text(family="Courier")) +
  scale_fill_brewer(palette = "Set3") +
  guides(fill=guide_legend(title="Sequencing Center")) +
  xlab("Sequencing Center") + ylab("Tumor Content") +
  ggtitle("Tumor Content by Sequencing Site") + geom_boxplot() + geom_jitter(shape=16, position=position_jitter(0.2))

raw_plot
```



Lastly, we plotted the tumor content levels by sequencing site region. We first made a subtable to count the frequencies of tumor content levels by site region, and then fed this table into ggplot. Once again, we utilized the theme feature to add labels to each bar and customize the fonts and colors in the graph.

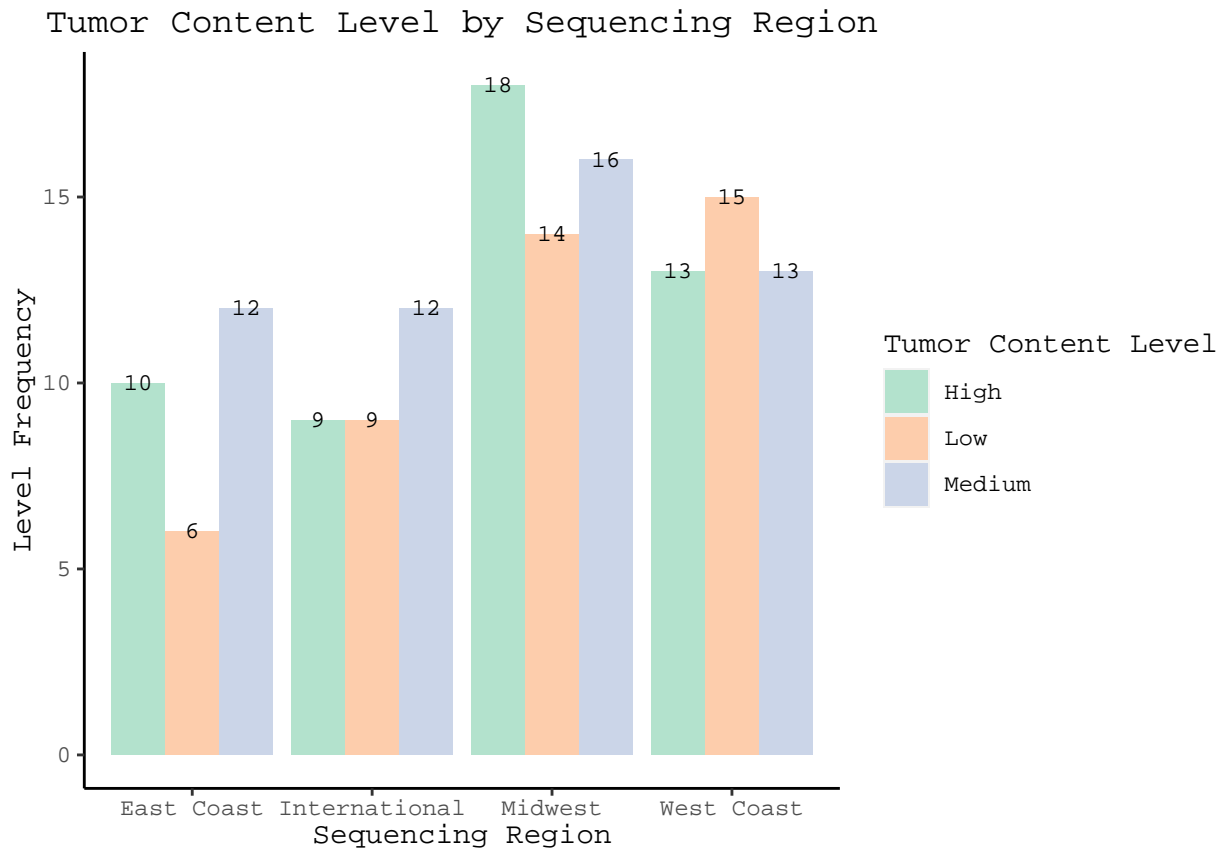
```
# Make table to count frequencies of tumor content levels by site region
tab = table(aim_1_1$TC_level, aim_1_1$Site_region)
tab1 = data.frame(tab)

# Stacked barplot
#barplot(tab, legend = TRUE, args.legend = list(x = "topleft",
#                                              inset = c(0.07, -0.1)))

# Grouped Barplot
grouped_plot = ggplot(data = tab1, mapping = aes(x=Var2, y=Freq, fill=Var1)) +
  geom_bar(stat="identity", position = "dodge") +
  geom_text(aes(label = Freq), vjust = 0.5, size = 3, position = position_dodge(0.9), colour="black", fontfamily="Courier") +
  scale_fill_brewer(palette = "Pastel2") + ggtitle("Tumor Content Level by Sequencing Region") +
  theme(panel.background = element_blank(),
        axis.line = element_line(colour="black"),
        plot.title = element_text(hjust=0.5, family="Courier"),
        axis.title.x = element_text(family="Courier"),
        axis.title.y = element_text(family="Courier"),
        axis.text.x = element_text(family="Courier"),
        axis.text.y = element_text(family="Courier"),
        legend.text=element_text(family="Courier"),
        legend.title=element_text(family="Courier")) +
  guides(fill=guide_legend(title="Tumor Content Level")) + xlab("Sequencing Region") + ylab("Level Frequency")
```



```
grouped_plot
```



And that completes the analysis for aim 1.1!

## AIM 1.2: Classify by exposure status (grouped by prior treatment) by site

Just like in Aim 1.1, we are going to use the library `dplyr` to perform data manipulation for this Aim. In order to know more about the exposure status, we select two columns: `Abiraterone..ABI..and.Enzalutamide..ENZA..Exposure.Status` which is a binary column for a patient's exposure to Abiraterone and Enzalutamide (contains Yes's and No's) and `Prior.Treatment` stores the information if the patient has ever received any prior Taxane treatment or not.

```
# Load dplyr library for data manipulation.
library(dplyr)
# Extract columns needed for Aim 1.2 and make sub-table
aim_1_2 = complete_dat %>%
  select(Center.of.sequencing, Exposure.Status = Abiraterone..ABI..and.Enzalutamide..ENZA..Exposure.Status,
         Prior.Treatment)
group_by(Prior.Treatment)

head(aim_1_2, 10)
```

```
## # A tibble: 10 x 3
## # Groups:   Prior.Treatment [2]
##   Center.of.sequencing Exposure.Status Prior.Treatment
##   <chr>                <chr>          <chr>
```

```
## 1 DFCI Yes No Prior Taxane Treatment
## 2 DFCI No No Prior Taxane Treatment
## 3 DFCI No No Prior Taxane Treatment
## 4 DFCI Yes No Prior Taxane Treatment
## 5 DFCI No No Prior Taxane Treatment
## 6 DFCI No No Prior Taxane Treatment
## 7 DFCI No No Prior Taxane Treatment
## 8 DFCI Yes No Prior Taxane Treatment
## 9 MSKCC Yes Had prior Taxane treatment
## 10 MSKCC Yes No Prior Taxane Treatment
```

In order to have few categories for finding the exposure status based on prior treatment found in different sequencing institutes, we need to club institutes into 4 main regions based on the data we have: East Coast, Midwest, West Coast and International. Similarly in Aim 1.1, we run the same if-else code for Aim 1.2.

```
aim_1_2$Site_region <- as.factor(ifelse(aim_1_2$Center.of.sequencing == 'DFCI' | aim_1_2$Center.of.sequencing == 'Karmanos Cancer Insitute' | aim_1_2$Center.of.sequencing == 'U Washington', 'West Coast', 'International'))
```

We now have grouped the Exposure status by Prior Treatment and have added the Sequencing region column into the data frame aim\_1\_2. Next, we calculate the mean of the all Exposure.Status counts based on the Sequencing Region and grouped by Prior.Treatment

```
library(gtsummary)
# Create numeric summary of mean tumor content by sequencing region
temp = aim_1_2 %>%
  select(Exposure.Status, Prior.Treatment, Site_region) %>%
  tbl_summary(by=Site_region, statistic = list(all_continuous() ~ "{mean} ({sd})"), label = Exposure.Status,
  modify_spanning_header( all_stat_cols() ~ "**Sequencing Region**") %>% modify_header(label = "Exposure.Status")
temp
```

```
## Table printed with 'knitr::kable()', not {gt}. Learn why at
## http://www.danielsjoberg.com/gtsummary/articles/rmarkdown.html
## To suppress this message, include 'message = FALSE' in code chunk header.
```

Exposure Status, grouped by Prior Treatment	East Coast, N = 28	International, N = 30	Midwest, N = 48	West Coast, N = 41
Mean of Exposure Status Prior.Treatment	17 (61%)	25 (83%)	16 (33%)	14 (34%)
Had prior Taxane treatment	9 (32%)	23 (77%)	19 (40%)	10 (24%)
No Prior Taxane Treatment	19 (68%)	7 (23%)	29 (60%)	31 (76%)

Next we perform visualizations on the same numbers we just got above and present the data in form of bar graphs with the help of ggplot2 package and used geom\_bar to plot the graph grouped by Prior.Treatment and have six graphs for six different Center.of.sequencing institutes. Furthermore, to plot the graph, we use the janitor and dplyr packages to store the tables based on these three variables.

```
library(janitor)

##
## Attaching package: 'janitor'
```

```
## The following objects are masked from 'package:stats':
##
##   chisq.test, fisher.test

summary_table = tabyl(aim_1_2, Exposure.Status, Prior.Treatment, Center.of.sequencing)
summary_table
```

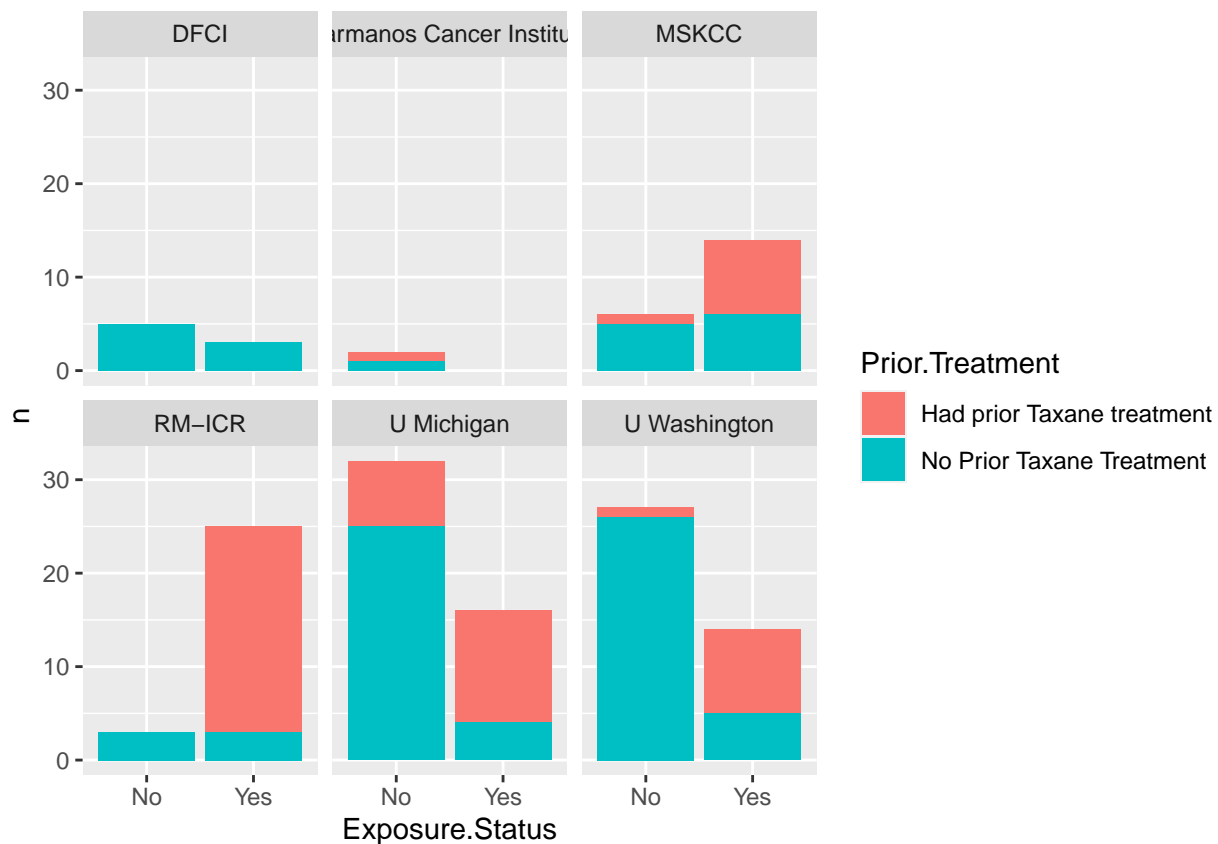
```
## $DFCI
## Exposure.Status Had prior Taxane treatment No Prior Taxane Treatment
##           No           0           5
##           Yes           0           3
##
## $'Karmanos Cancer Institute'
## Exposure.Status Had prior Taxane treatment No Prior Taxane Treatment
##           No           1           1
##           Yes           0           0
##
## $MSKCC
## Exposure.Status Had prior Taxane treatment No Prior Taxane Treatment
##           No           1           5
##           Yes           8           6
##
## $'RM-ICR'
## Exposure.Status Had prior Taxane treatment No Prior Taxane Treatment
##           No           0           3
##           Yes          22           3
##
## $'U Michigan'
## Exposure.Status Had prior Taxane treatment No Prior Taxane Treatment
##           No           7          25
##           Yes          12           4
##
## $'U Washington'
## Exposure.Status Had prior Taxane treatment No Prior Taxane Treatment
##           No           1          26
##           Yes           9           5
```

```
library(dplyr)
my_summary <- aim_1_2 %>%
  count(Center.of.sequencing, Exposure.Status, Prior.Treatment, sort = FALSE)
my_summary
```

```
## # A tibble: 19 x 4
## # Groups:   Prior.Treatment [2]
##   Prior.Treatment Center.of.sequencing Exposure.Status n
##   <chr>           <chr>           <chr>         <int>
## 1 Had prior Taxane treatment Karmanos Cancer Institute No           1
## 2 Had prior Taxane treatment MSKCC No           1
## 3 Had prior Taxane treatment MSKCC Yes           8
## 4 Had prior Taxane treatment RM-ICR Yes          22
## 5 Had prior Taxane treatment U Michigan No           7
## 6 Had prior Taxane treatment U Michigan Yes          12
## 7 Had prior Taxane treatment U Washington No           1
```

```
## 8 Had prior Taxane treatment U Washington Yes 9
## 9 No Prior Taxane Treatment DFCI No 5
## 10 No Prior Taxane Treatment DFCI Yes 3
## 11 No Prior Taxane Treatment Karmanos Cancer Institute No 1
## 12 No Prior Taxane Treatment MSKCC No 5
## 13 No Prior Taxane Treatment MSKCC Yes 6
## 14 No Prior Taxane Treatment RM-ICR No 3
## 15 No Prior Taxane Treatment RM-ICR Yes 3
## 16 No Prior Taxane Treatment U Michigan No 25
## 17 No Prior Taxane Treatment U Michigan Yes 4
## 18 No Prior Taxane Treatment U Washington No 26
## 19 No Prior Taxane Treatment U Washington Yes 5
```

```
library(ggplot2)
ggplot(my_summary, aes(Exposure.Status, n, fill = Prior.Treatment)) +
  geom_bar(stat = "identity") +
  facet_wrap(facets = vars(Center.of.sequencing))
```



In order to get better visualizations, we decided to conglomerate the institutes into region, and find the Exposure.Status grouped by the Prior.Treatment column based on the Site\_region.

```
library(janitor)
summary.table.region = tabyl(aim_1_2, Exposure.Status, Prior.Treatment, Site_region)
summary.table.region
```

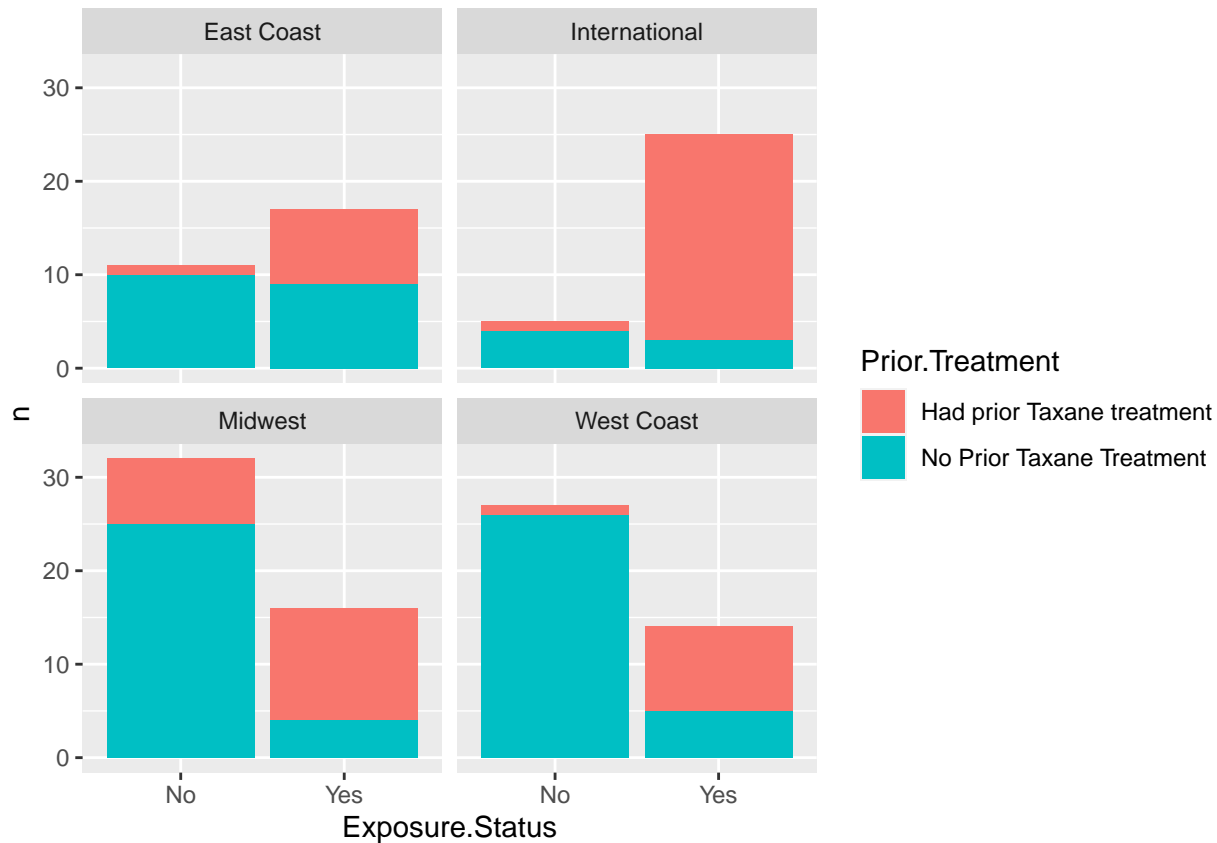
```
## $'East Coast'
```

```
## Exposure.Status Had prior Taxane treatment No Prior Taxane Treatment
##           No                      1                      10
##           Yes                     8                      9
##
## $International
## Exposure.Status Had prior Taxane treatment No Prior Taxane Treatment
##           No                      1                      4
##           Yes                     22                     3
##
## $Midwest
## Exposure.Status Had prior Taxane treatment No Prior Taxane Treatment
##           No                      7                      25
##           Yes                     12                     4
##
## $'West Coast'
## Exposure.Status Had prior Taxane treatment No Prior Taxane Treatment
##           No                      1                      26
##           Yes                     9                      5
```

```
library(dplyr)
my.summary.region <- aim_1_2 %>%
  count(Exposure.Status, Prior.Treatment, Site_region, sort = TRUE)
my.summary.region
```

```
## # A tibble: 16 x 4
## # Groups:   Prior.Treatment [2]
##   Prior.Treatment Exposure.Status Site_region      n
##   <chr>           <chr>           <fct>      <int>
## 1 No Prior Taxane Treatment No           West Coast    26
## 2 No Prior Taxane Treatment No           Midwest      25
## 3 Had prior Taxane treatment Yes           International 22
## 4 Had prior Taxane treatment Yes           Midwest      12
## 5 No Prior Taxane Treatment No           East Coast    10
## 6 Had prior Taxane treatment Yes           West Coast     9
## 7 No Prior Taxane Treatment Yes           East Coast     9
## 8 Had prior Taxane treatment Yes           East Coast     8
## 9 Had prior Taxane treatment No           Midwest       7
## 10 No Prior Taxane Treatment Yes           West Coast     5
## 11 No Prior Taxane Treatment No           International  4
## 12 No Prior Taxane Treatment Yes           Midwest       4
## 13 No Prior Taxane Treatment Yes           International  3
## 14 Had prior Taxane treatment No           East Coast     1
## 15 Had prior Taxane treatment No           International  1
## 16 Had prior Taxane treatment No           West Coast     1
```

```
library(ggplot2)
ggplot(my.summary.region, aes(Exposure.Status, n, fill = Prior.Treatment)) +
  geom_bar(stat = "identity") +
  facet_wrap(facets = vars(Site_region))
```



As seen in the graphs and tables, the distribution seems to be not normal and hence we decided to perform Fisher's exact test to calculate the p-value. We see that the p-value is less than 0.001, which means it is statistically significant across all confidence levels and hence there is an significant association between Sequencing Site and Exposure Status. Similarly we also get p-value less than 0.001 for Prior Treatment status and Exposure Status (using Pearson's Chi-Squared test), which means there is a statistically significant association between exposure status/sequencing site and exposure status/prior treatment status.

```
# Create numeric summary comparing exposure status to sequencing site and prior treatment status
# Run Fisher's exact test and Chi-squared for sequencing site and prior treatment status, respectively
p.test = aim_1_2 %>%
  select(Center.of.sequencing, Exposure.Status, Prior.Treatment) %>%
  tbl_summary(by = Exposure.Status, label = Center.of.sequencing ~ "Sequencing Site") %>%
  add_p() %>%
  modify_header(label = "") %>%
  modify_spanning_header(all_stat_cols() ~ "Exposure Status") %>%
  bold_labels()
p.test
```

```
## Table printed with 'knitr::kable()', not {gt}. Learn why at
## http://www.danielsjoberg.com/gtsummary/articles/rmarkdown.html
## To suppress this message, include 'message = FALSE' in code chunk header.
```

	No, N = 75	Yes, N = 72	p-value
<b>Sequencing Site</b>			<0.001
DFCI	5 (6.7%)	3 (4.2%)	

	No, N = 75	Yes, N = 72	p-value
Karmanos Cancer Institute	2 (2.7%)	0 (0%)	
MSKCC	6 (8.0%)	14 (19%)	
RM-ICR	3 (4.0%)	25 (35%)	
U Michigan	32 (43%)	16 (22%)	
U Washington	27 (36%)	14 (19%)	
<b>Prior.Treatment</b>			<0.001
Had prior Taxane treatment	10 (13%)	51 (71%)	
No Prior Taxane Treatment	65 (87%)	21 (29%)	

Now that we have completed our analysis for Aim 1, we can conclude that exposure status has shown evidence of association with prior treatment status (Taxane treatment) and sequencing site, and that sequencing site does not seem to be associated with severity of tumor content.

## Aim 2

### Aim 2.1 - Assign classifications of “high”, “medium” or “low” based on the intensity of mutation count

For Aim 2, we will be creating a mutation count classification system similar to the one made and utilized in Aim 1. To assess how we should divide the counts into “high”, “medium”, and “low”, let's first look at the distribution of Mutation Count:

```
finaldata<-remove_nonuniques
summary(finaldata$Mutation.Count)
```

```
##      Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##      26.0   55.0   71.0   107.4   120.5  1071.0
```

Based off of the summary above, we can divide the mutation count into the following levels of severity:

Low Mutation Count: 0-55 mutations Medium Mutation Count: 56-110 mutations High Mutation Count: >110 mutations

Now, let's add this severity classification to our data frame using our tried and true if-else statement and summarize the frequencies of mutation count severity levels:

```
# low = 0 - 55
# med = 56 - 110
# high = >110
finaldata%>%add_column(mu_class="Low")->finaldata
finaldata%>%mutate(mu_class=ifelse(`Mutation.Count`>55 & `Mutation.Count`<=110 , "Moderate", mu_class))->finaldata
finaldata%>%mutate(mu_class=ifelse(`Mutation.Count`>110, "High", mu_class))->finaldata
# Summarize frequencies of mutation count severity levels
table(finaldata$mu_class)
```

```
##
##      High      Low Moderate
##      41       38       68
```

Now that we have summarized the frequencies of mutation count severity levels, we can plot the frequencies in relation to biopsy sites.

For plotting the severity on a human graph we can use the package **gganatogram**, which is a package based on ggplot2 and allows for better visualization of organs and tissues. Here, we will be displaying mutation count data onto anatomical structures from the package. Essentially, we will be grouping our data set by biopsy site, and then calculating the mean mutation count for each site, and then use our mutation count classification system to set an appropriate value for visualizing the spread of severity across biopsy sites.

First we need to construct a data frame by renaming the biopsy sites exactly as built in the package **gganatogram**. Then, we can assign each tumor site a value corresponding to the severity level that matches the mutation count levels.

```
# Download all our gganatogram packages
#source("https://neuroconductor.org/neurocLite.R")
#neuro_install("gganatogram")
library(gganatogram)
```

```
## Loading required package: ggpolypath
```

```
library(gridExtra)
```

```
##
```

```
## Attaching package: 'gridExtra'
```

```
## The following object is masked from 'package:dplyr':
```

```
##
```

```
##      combine
```

```
# Calculate mean mutation count levels for each biopsy site
```

```
finaldata %>%
  group_by(Tumor.Site) %>%
  summarise(mean(Mutation.Count))
```

```
## # A tibble: 20 x 2
##   Tumor.Site      'mean(Mutation.Count)'
```

<chr>	<dbl>
1 Abdominal wall mass	1071
2 Bladder	79
3 Bone	112.
4 Chest Wall	216
5 Dura	71
6 glans penis	100
7 Liver	75.2
8 Lymph node	102.
9 Muscle	113
10 Neck Mass	58
11 Pelvic mass	82
12 Penile	134
13 Perirectal	154
14 Peritoneal nodule	86
15 Prostate	50
16 ProstateTURP	120



## 17 Psoas Mass	78
## 18 Retroperitoneum	61
## 19 Soft Tissue	101.
## 20 Thoracic epidural	55

We've successfully calculated the average mutation count for each biopsy site! Now, we can start the process to plot these on a human graph. First, note that the ranges for mutation count are much more variable than tumor content. Good thing we created a classification system for this! To ensure that our color scale doesn't range from 0-1000 mutations, which would cause most of the biopsy sites to be colored the same shade since majority of them would be on the lower end of the scale, we can utilize our classification system to normalize our mutation count values to a certain extent.

In the section below, we create a data frame that is formatted for the gganatogram package. It contains two columns – organ and value. Organ is the biopsy site, and value is the mutation count (note that the columns must be named like this in order to be fed into gganatogram).

To normalize the mutation count values, we will manually check which level of mutation each relevant biopsy site matches to, and then set that biopsy site's value to the maximum value of that level's range. For example, lymph node's mutation count average is 101, which falls into the medium severity level, which ranges from 56 to 110, so lymph node's value in our data frame will be 110.

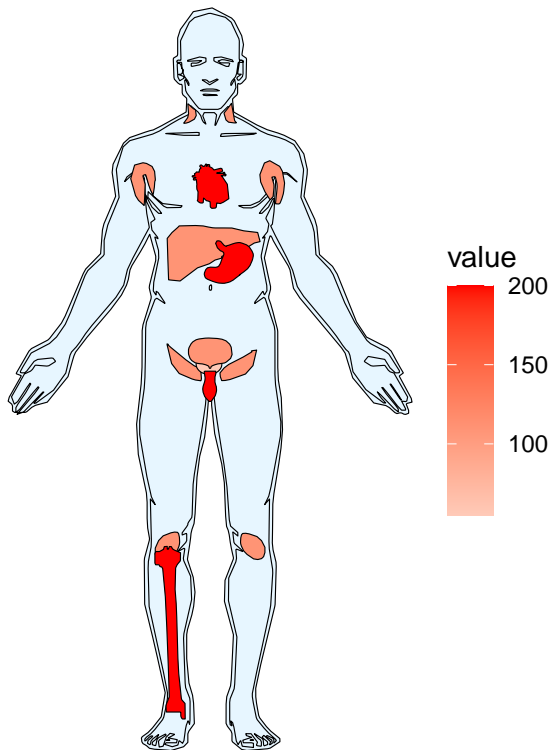
It is also worth noting that we can only plot the biopsy sites which are included in gganatogram's `hgMale_key` data frame due to the way the package works, but for the purposes of data wrangling we figured including the package and all its visually helpful plots is worth the loss of a couple of sites not included in the library.

```
# For reference, here is our mutation count classification system
# low = 0 - 55
# med = 56 - 110
# high = >110
```

```
biopsies <- data.frame(organ = c("lymph_node", "prostate", "liver", "penis", "bone", "heart", "stomach", "vagina"),
                      value = c(110, 55, 110, 200, 200, 200, 200, 110),
                      stringsAsFactors = TRUE)
```

```
temp_works <- gganatogram(data = biopsies, organism="human", sex="male",
                          fill="value", fillOutline = "#E7F6FF") + theme_void() +
  ggtitle("Mutation Count Severity for Biopsy Site") + coord_fixed() +
  scale_fill_gradient2(low = "grey", mid = "white",
                      high = "red", midpoint = .02)
temp_works
```

## Mutation Count Severity for Biopsy Site



Wow! We just made our first human plot that looks at mutation count severity levels by biopsy site! Thanks, `gganatogram`! As we can see from above the penis, bone, heart, and stomach biopsy sites seem to have the highest mutation count. This could be useful to look into in further studies to see if there could be any clinical implications in this area.

### Aim 2.2

This data set included a recording of patient exposure to Abiraterone and Enzalutamide (ABI and ENZA). ABI and ENZA are drugs commonly used in the treatment of prostate cancer. Both medications work as an anti-androgen drugs; ABI is an androgen synthase inhibitor, and ENZA inhibits androgen processes in the cell. Close to half of the participants in the study were either exposed or not exposed to ABI and ENZA in the fashion of a randomized control trial. In this aim we are trying to see if the participants in each arm of the study (exposed or not exposed) share similar characteristics, particularly among the Mutation Count, Fraction Genome Altered, and Tumor Content variables. To explore these characteristics of the data set, the data must first be split into those rows corresponding to participants who were exposed to ABI and ENZA and those who were not.

```
#Convert exposure status to numeric binary variable.
exposure_data <- remove_nonuniques
binary_exposure <- remove_nonuniques$Abiraterone..ABI..and.Enzalutamide..ENZA..Exposure.Status

binary_exposure[binary_exposure == "Yes"] = 1
binary_exposure[binary_exposure == "No"] = 0

exposure_data$Binary.Exposure <- binary_exposure # Add column for binary exposure

# Make separate tables for exposed vs non-exposed patients
exposed <- which(binary_exposure == 1)
```

```

non_exposed <- which(binary_exposure == 0)

exposed_dat <- exposure_data[exposed,]
nonexposed_dat <- exposure_data[non_exposed,]

```

Next, the participants can be plotted by the two groups – exposure vs. no exposure – for comparing mutation counts, fraction of genome that was altered, and tumor content.

```

# Construct table used for plotting mutation count, fraction of genome altered, and tumor content by exposure status
emc <- exposed_dat %>% select(c("Mutation.Count", "Fraction.Genome.Altered", "tumor.content"))
nemc <- nonexposed_dat %>% select(c("Mutation.Count", "Fraction.Genome.Altered", "tumor.content"))

emc$Exposed <- "Exposed"
nemc$Exposed <- "Non-Exposed"

amc <- rbind(emc, nemc)

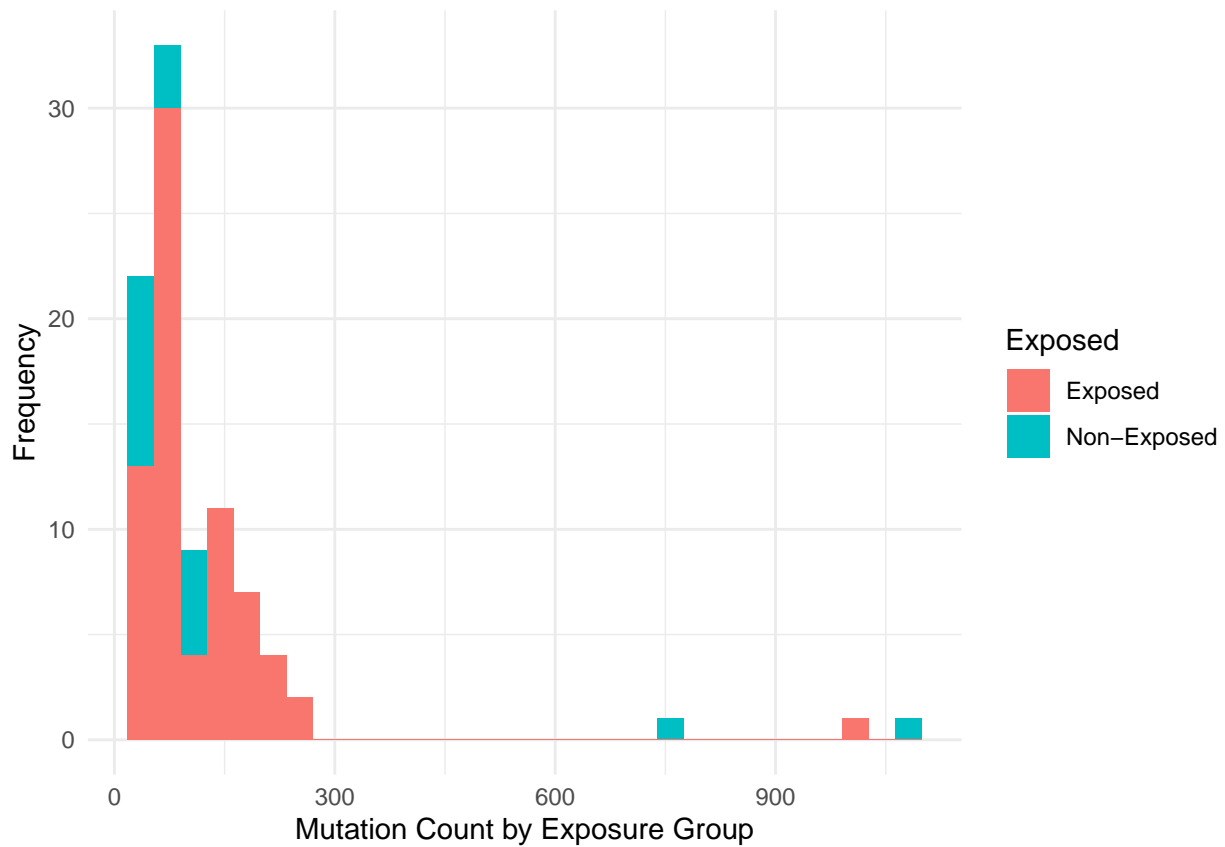
# Plot distributions of mutation count by exposure status
ggplot(amc,aes(x=Mutation.Count, fill=Exposed)) +
  geom_histogram(data=subset(amc,Exposed == 'Non-Exposed')) +
  geom_histogram(data=subset(amc,Exposed == 'Exposed')) +
  xlab("Mutation Count by Exposure Group") +
  ylab("Frequency")+
  theme_minimal()

```

```

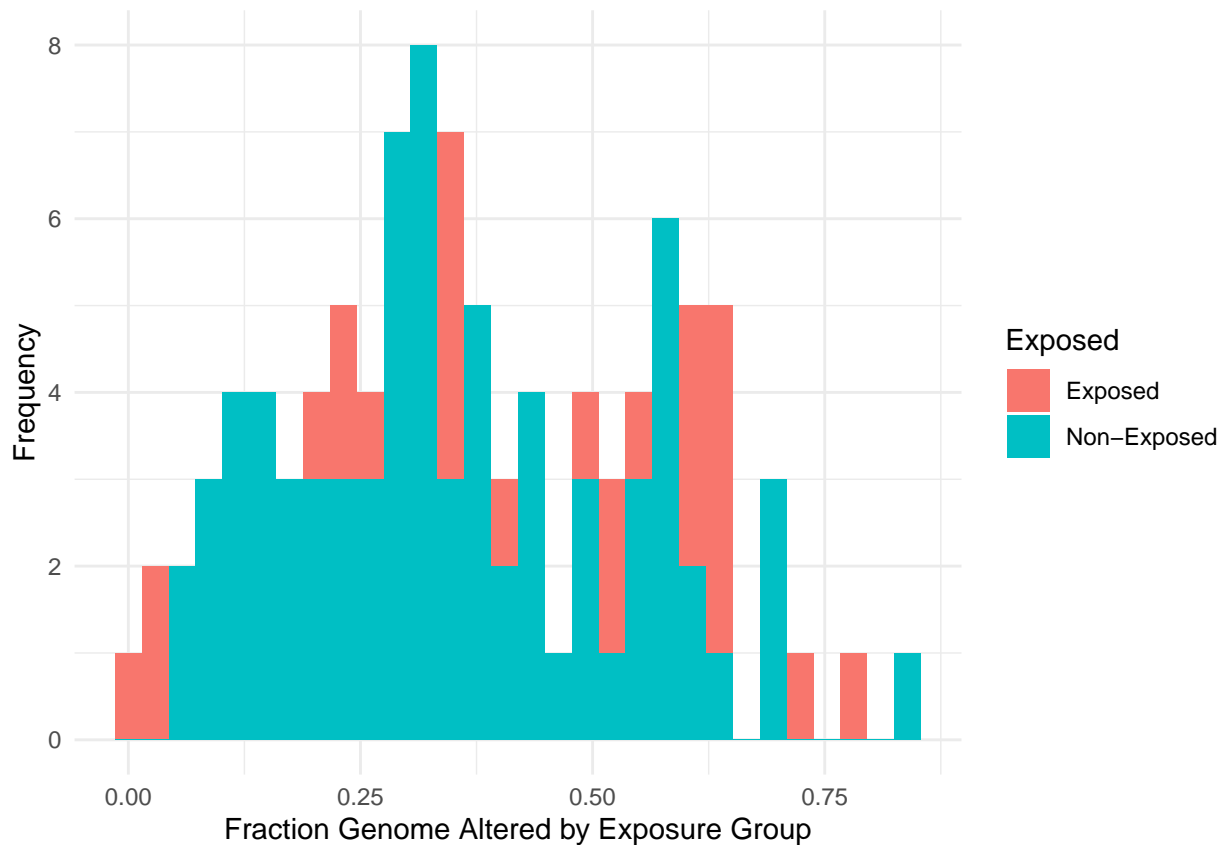
## 'stat_bin()' using 'bins = 30'. Pick better value with 'binwidth'.
## 'stat_bin()' using 'bins = 30'. Pick better value with 'binwidth'.

```



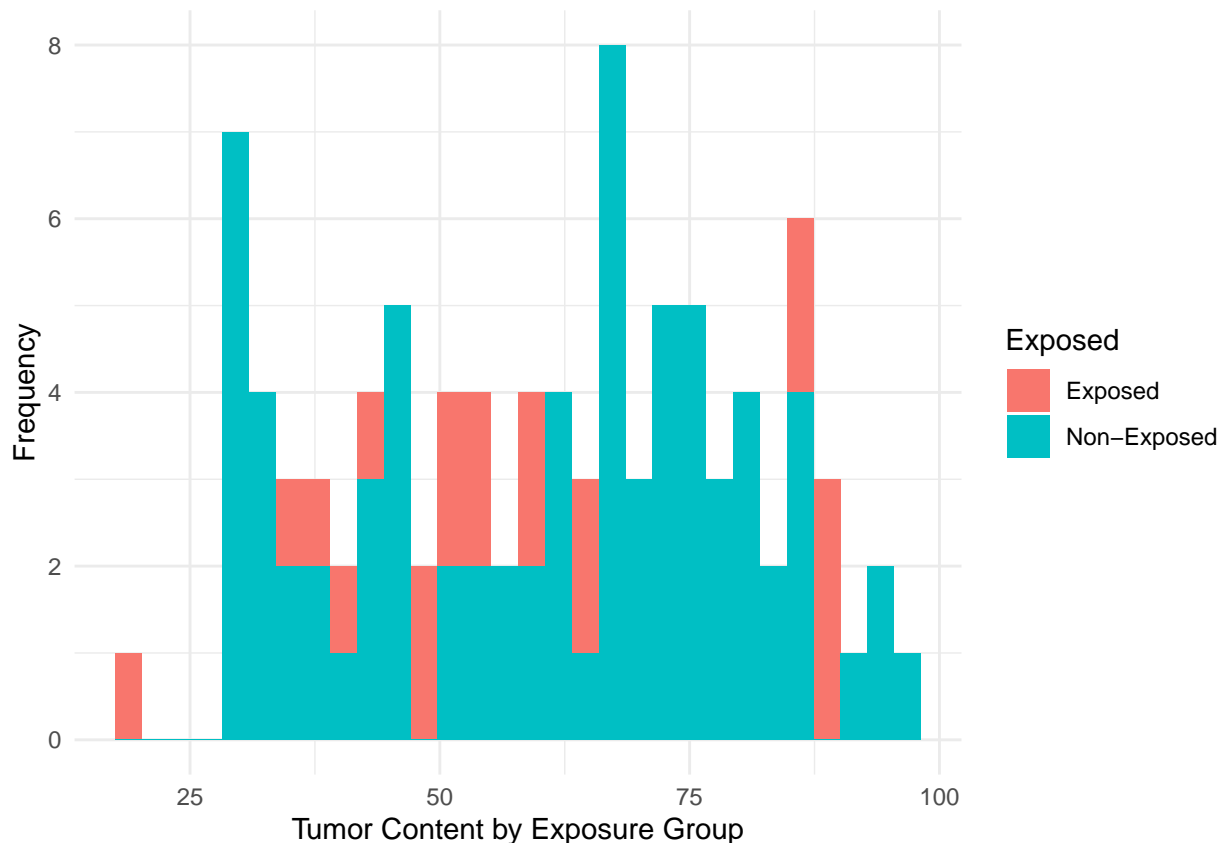
```
# Plot distributions of fraction genome altered by exposure status
ggplot(amc,aes(x=Fraction.Genome.Altered, fill=Exposed)) +
  geom_histogram(data=subset(amc,Exposed == 'Exposed')) +
  geom_histogram(data=subset(amc,Exposed == 'Non-Exposed')) +
  xlab("Fraction Genome Altered by Exposure Group") +
  ylab("Frequency")+
  theme_minimal()
```

```
## 'stat_bin()' using 'bins = 30'. Pick better value with 'binwidth'.
## 'stat_bin()' using 'bins = 30'. Pick better value with 'binwidth'.
```



```
# Plot distributions of tumor content by exposure status
ggplot(amc,aes(x=tumor.content, fill=Exposed)) +
  geom_histogram(data=subset(amc,Exposed == 'Exposed')) +
  geom_histogram(data=subset(amc,Exposed == 'Non-Exposed')) +
  xlab("Tumor Content by Exposure Group") +
  ylab("Frequency")+
  theme_minimal()
```

```
## 'stat_bin()' using 'bins = 30'. Pick better value with 'binwidth'.
## 'stat_bin()' using 'bins = 30'. Pick better value with 'binwidth'.
```



```
# Create numeric summary of characteristics by exposure status
colnames(amc) <- c("Mutation Count", "Fraction Genome Altered", "Tumor Content", "Exposed")

amc %>%
  tbl_summary(by = Exposed, label = Exposed ~ "Exposed") %>% add_p(test=everything()~"t.test")

## Table printed with 'knitr::kable()', not {gt}. Learn why at
## http://www.danielsjoberg.com/gtsummary/articles/rmarkdown.html
## To suppress this message, include 'message = FALSE' in code chunk header.
```

Characteristic	Exposed, N = 72	Non-Exposed, N = 75	p-value
Mutation Count	80 (60, 150)	64 (54, 96)	0.5
Fraction Genome Altered	0.39 (0.23, 0.56)	0.32 (0.22, 0.49)	0.12
Tumor Content	58 (42, 76)	66 (44, 75)	0.6

These visualizations and results show that the groups differ by values that are reasonably expected under random chance. We performed a Welch's two sample t-test (which assumes unequal variances between exposure arms), and obtained p-values of 0.5 (mutation count), 0.12 (fraction genome altered), and 0.6 (tumor content). These values all suggest that there is no association between exposure status and mutation count, fraction genome altered, and tumor content.

### Aim 2.3- Is Severity of Mutation Count Related to Frequency of Biopsy Sites?

To finish off aim 2, we will be checking if severity of Mutation Count is associated with frequency of biopsy sites sampled in the first data set. Let's first create a data frame that stores the frequency of each biopsy

site from our data set:

```
# Frequencies of biopsy site in first data set
aim_2_3 = data.frame(table(finaldata$Tumor.Site))
aim_2_3
```

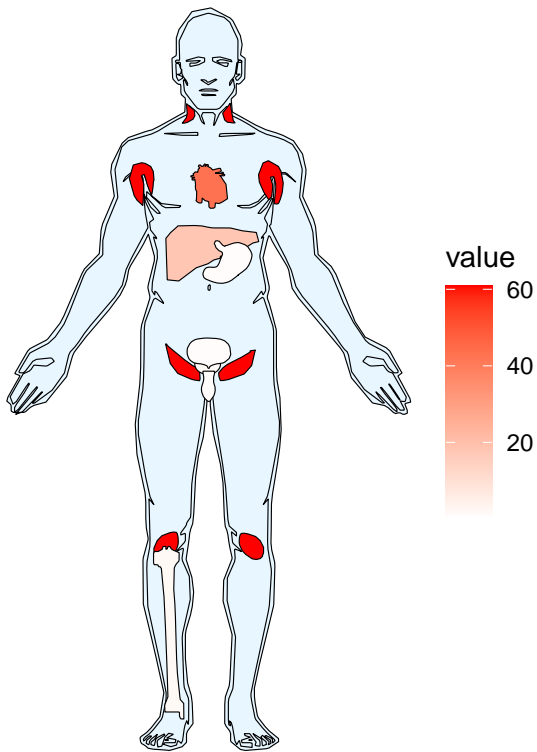
```
##           Var1 Freq
## 1 Abdominal wall mass    1
## 2           Bladder    1
## 3           Bone   43
## 4       Chest Wall    1
## 5           Dura    1
## 6       glans penis    1
## 7           Liver   18
## 8       Lymph node   61
## 9           Muscle    2
## 10          Neck Mass    1
## 11        Pelvic mass    1
## 12          Penile    1
## 13        Perirectal    1
## 14 Peritoneal nodule    1
## 15          Prostate    2
## 16        ProstateTURP    1
## 17          Psoas Mass    1
## 18      Retroperitoneum    1
## 19          Soft Tissue    7
## 20 Thoracic epidural    1
```

Now we can once again use the `gganatogram` package to plot the frequencies of each biopsy site on a human outline. Note, that since the package is picky about which organs we can use, we must once again manually create a data frame with relevant “organs” for the package’s plotting function and manually include the values (in this case, the frequency of the biopsy site):

```
# Create data frame for plotting frequencies of biopsy site
biopsies <- data.frame(organ = c("lymph_node", "prostate", "liver", "penis", "bone", "heart", "stomach", "
  value = c(61, 2, 18, 1, 2, 43, 1, 1), stringsAsFactors = TRUE)

# Plot frequency of biopsy sites
p2 <- gganatogram(data = biopsies, organism="human", sex="male",
  fill="value", fillOutline = "#E7F6FF") + theme_void() +
  ggtitle("Frequency for each Biopsy Site") + coord_fixed() + scale_fill_gradient2(low = "grey", mid = "
p2
```

## Frequency for each Biopsy Site

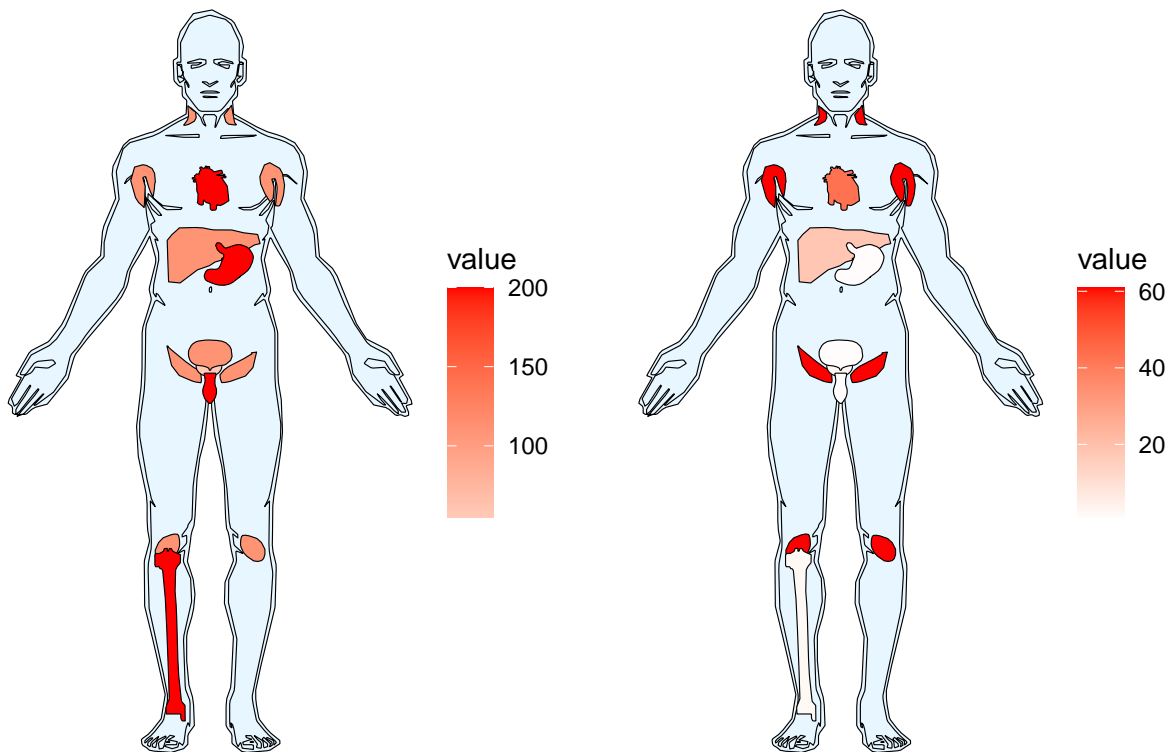


Now that we have created two human plots – one for mutation count severity by biopsy site, and one for frequency of each biopsy site from our data set, we can compare the two side-by-side:

```
# Compare tumor content severity for biopsy site to frequency for each biopsy site  
lay <- rbind(c(1,2), c(1,2))  
grid.arrange(temp_works, p2, layout_matrix = lay)
```



## Mutation Count Severity for Biopsy Site    Frequency for each Biopsy Site



Now

that we can compare severity of tumor content directly next to frequency of biopsy site, we can visually assess any possible correlation between the two. After comparing the two side-by-side, we can see that there does not seem to be any association between tumor content severity at a biopsy site and how frequently that biopsy site was sampled in this data set. And with that, we can complete our analysis for Aim 2!

## BONUS AIM

The last aim checks for an association between diagnosis age and tumor site. According to the Cleveland Clinic 1 in 6 men will be diagnosed with prostate cancer during the course of their lifetime. Research from the American Cancer Society suggests that 6 out of 10 cases of prostate cancer are diagnosed among men 65 years or older. As mentioned previously the mean age of diagnosis among this prospective cohort was 67 years of age. Prior research suggests that as men get older their risk of being diagnosed with prostate cancer increases. In addition, the publication in Cell suggests that bone tumors are the most common site of metastatic disease. This aim therefore intends to determine whether these two prior notions hold true among the data in question.

To conduct the analysis we subset the data columns Diagnosis.Age and Tumor.Site into a data frame called age\_tumor. The numeric index of these columns was 4 and 11 respectively. Before proceeding with any analysis we conducted an exploratory investigation to understand the quantity of unique tumor sites present in the data set along with the spread of age at diagnosis.

```
age_tumor <- remove_nonuniques[c(4,11)] #subset the data
head(age_tumor)
```

```
##   Diagnosis.Age Tumor.Site
## 1           54      Bone
## 2           49 Lymph node
```

```
## 3          78 Lymph node
## 4          71 Lymph node
## 5          67 Lymph node
## 6          63      Bone
```

```
summary(unique(age_tumor$Tumor.Site)) #look at unique values for tumor.site
```

```
##      Length      Class      Mode
##           20 character character
```

According to the aforementioned summary and unique command, 20 unique tumor sites are present within this data. Next, we explored the distribution of diagnosis age to determine min, max, mean and median ages for this prospective prostate cohort.

```
summary(age_tumor$Diagnosis.Age)
```

```
##      Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##    40.00  62.00   68.00   66.84  72.00   85.00
```

Given the minimum age at diagnosis was 40 and the maximum age at diagnosis was 85 we needed to come up with a way to age-stratify the data in order to compare the various tumor sites with age at diagnosis. An article published in the British Journal of Cancer described a methodology for stratifying age within a population based study investigating prostate cancer. The researchers stratified age according to the following groups “40-59”, “60-69”, “70-79” and “80+ years”. Since this was a validated study we employed the same age-stratified bins for our analysis.

```
library(tidyverse) #load tidyverse to assist in analysis
#grouping the age by the BJC age-stratified bins using ifelse statements
age_tumor$agegrp <- ifelse(age_tumor$Diagnosis.Age >= 40 & age_tumor$Diagnosis.Age <=59, "40-59",
                          ifelse(age_tumor$Diagnosis.Age > 59 & age_tumor$Diagnosis.Age <= 69, "60-69",
                                ifelse(age_tumor$Diagnosis.Age > 69 & age_tumor$Diagnosis.Age <= 79, "70-79",
                                      ifelse(age_tumor$Diagnosis.Age >79, "80+", NA))))
```

After stratifying the data, we created a summary table to assess the aggregated counts of tumors by site grouped by age.

```
library(dplyr) #load dplyr to use gtsummary
#install.packages("gtsummary") #in order to create the graph you may need to install the gtsummary pkg
library(gtsummary) #load gtsummary to create the summary table
age_tumor %>%
  select(Tumor.Site, agegrp) %>%
  tbl_summary(by = agegrp, label = Tumor.Site ~ "Tumor Site") %>% #develop the table based on the selec
  add_p(test=everything()~"kruskal.test") #test for the differences among ranks
```

```
## Table printed with 'knitr::kable()', not {gt}. Learn why at
## http://www.danielsjoberg.com/gtsummary/articles/rmarkdown.html
## To suppress this message, include 'message = FALSE' in code chunk header.
```

Characteristic	40-59, N = 27	60-69, N = 54	70-79, N = 59	80+, N = 7	p-value
Tumor Site					0.9
Abdominal wall mass	0 (0%)	0 (0%)	1 (1.7%)	0 (0%)	
Bladder	0 (0%)	1 (1.9%)	0 (0%)	0 (0%)	
Bone	8 (30%)	17 (31%)	16 (27%)	2 (29%)	
Chest Wall	0 (0%)	1 (1.9%)	0 (0%)	0 (0%)	
Dura	1 (3.7%)	0 (0%)	0 (0%)	0 (0%)	
glans penis	1 (3.7%)	0 (0%)	0 (0%)	0 (0%)	
Liver	3 (11%)	8 (15%)	6 (10%)	1 (14%)	
Lymph node	9 (33%)	19 (35%)	31 (53%)	2 (29%)	
Muscle	0 (0%)	1 (1.9%)	1 (1.7%)	0 (0%)	
Neck Mass	0 (0%)	1 (1.9%)	0 (0%)	0 (0%)	
Pelvic mass	0 (0%)	1 (1.9%)	0 (0%)	0 (0%)	
Penile	0 (0%)	1 (1.9%)	0 (0%)	0 (0%)	
Perirectal	0 (0%)	1 (1.9%)	0 (0%)	0 (0%)	
Peritoneal nodule	1 (3.7%)	0 (0%)	0 (0%)	0 (0%)	
Prostate	2 (7.4%)	0 (0%)	0 (0%)	0 (0%)	
ProstateTURP	0 (0%)	0 (0%)	1 (1.7%)	0 (0%)	
Psoas Mass	0 (0%)	1 (1.9%)	0 (0%)	0 (0%)	
Retroperitoneum	1 (3.7%)	0 (0%)	0 (0%)	0 (0%)	
Soft Tissue	1 (3.7%)	2 (3.7%)	2 (3.4%)	2 (29%)	
Thoracic epidural	0 (0%)	0 (0%)	1 (1.7%)	0 (0%)	

As evidenced by the output of the table, several of the aggregate counts of tumors by site were either 0 or 1 in most cases, which is not that significant. Five sites however did demonstrate counts greater than 1 and those include, Bone, Liver, Lymph Node, Prostate and Soft Tissue. To assess whether the differences among the age stratified ranks were significant by tumor site we conducted a Kruskal-Wallis Rank Sum Test. The output of which can be found in the last column of this table. The value of p was reported as 0.9 and assuming an alpha significance level of 0.05 this value is not significant. Thus, we conclude that there is no significant difference exhibited among the age stratified ranks by tumor site.

We found the quantities of the aggregate counts for the following tumor sites: Bone, Liver, Lymph Node, Prostate and Soft Tissue to be interesting so we further subsetted the data to explore the distribution of tumors. Additionally, we wanted to further assess whether bone, as mentioned earlier, was indeed the site of greatest metastatic disease.

```
#calculate the frequency of tumors by age group
```

```
freq <- age_tumor %>% group_by(agegrp,Tumor.Site) %>% summarise(Freq=n())
```

```
## 'summarise()' has grouped output by 'agegrp'. You can override using the '.groups' argument.
```

```
freq <- rename(freq, "Count of Tumors"=Freq) #rename the frequency column
```

```
freq_gt1 <-freq[which(freq$`Count of Tumors`>1),] #subset the data to include the five columns of inter
```

```
library(ggplot2) #load in ggplot to create graphical summary
```

```
library(viridis) #load in viridis for pretty color palette
```

```
## Loading required package: viridisLite
```

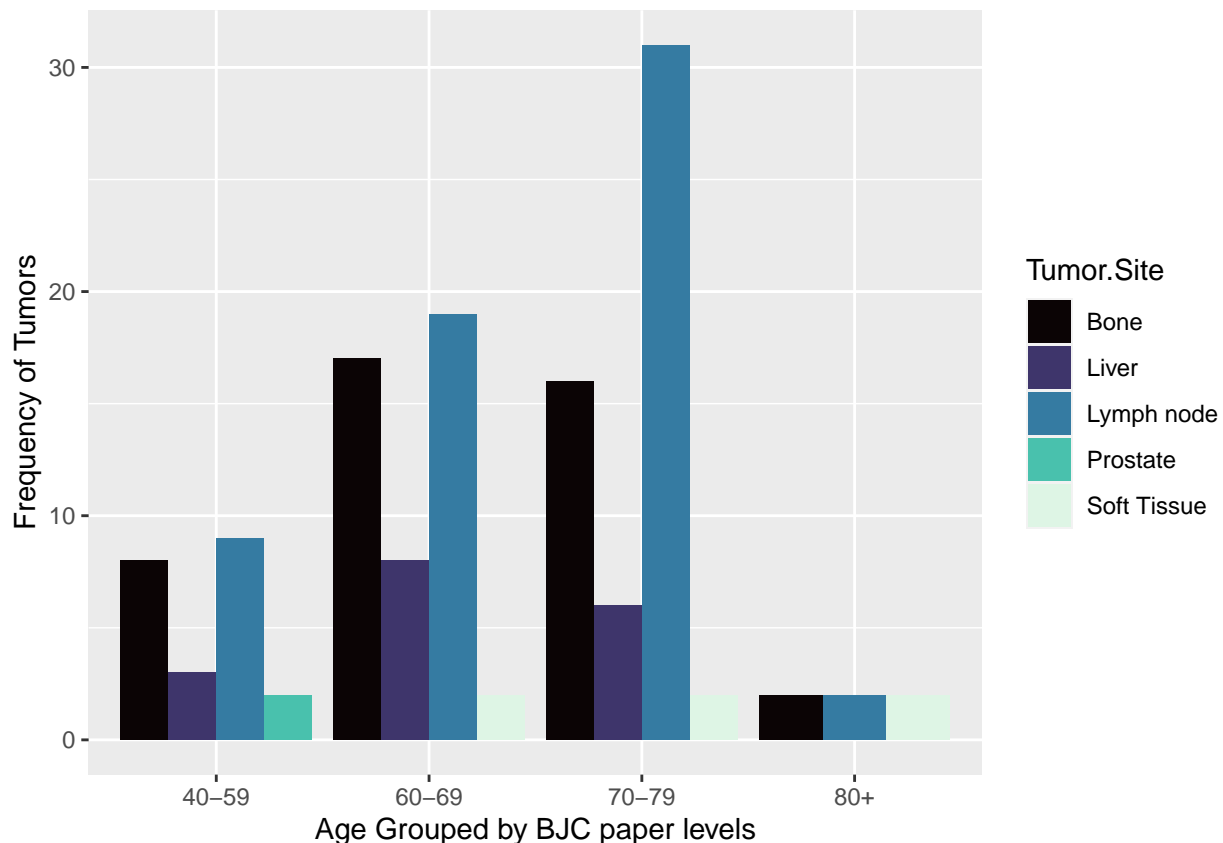
```
#create a bar chart of the data
```

```
ggplot(freq_gt1, aes(agegrp,`Count of Tumors`)) +
```

```
  labs(x="Age Grouped by BJC paper levels", y="Frequency of Tumors") +
```

```
  geom_bar(aes(fill=`Tumor.Site`),stat="identity",position="dodge") +
```

```
  scale_fill_viridis_d(option = "mako")
```



Curiously enough, bone was not the most common site of metastatic disease among this prospective prostate cohort. In fact, the lymph nodes were shown to have a greater quantity of tumors amounting to 61 tumors across the entire cohort as opposed to 43 among bone. Here from this graph we can also see that the “60-69” and “70-79” groups experience the most tumors proportionally, as would be expected given that as men age the likelihood of developing prostate cancer increases. If time permitted we would have further calculated the incident rate ratios per age group for the five tumor sites where frequency was greater than 1 to understand the proportional burden of disease experienced by each age group.

Overall, this aim confirms that as men age incidence of prostate cancer increases with metastasis of disease occurring more frequently in the lymph nodes and bone. This data was limited given that the population was a bit skewed toward the older age groups (60-79) with very few cases representing those older than 80. More information as to prognosis and vital status beyond age 80 would be beneficial in determining whether the assumption of age and incidence is valid.

## Natural Language Processing

There is no actual natural language in these data sets to process, but the principles and tools of natural language processing can be utilized to extract more information from the data. The second data has four columns corresponding to the AJCC staging classification of each patient in the data set. To help better understand what these classifications mean, we are adding a new column that includes a written description of each of the AJCC staging classifications that are provided. To do this, we can use Regex to identify AJCC stage patterns to match them to their written description. The written descriptions of each stage come from this webpage: <https://www.cancer.org/cancer/prostate-cancer/detection-diagnosis-staging/staging.html>

```
SampleRevised<-read.csv("~/Desktop/Data-Wrangling-Project/SampleRevised.csv")
```

```

library(stringr)
staging_dat <- SampleRevised
#Extract relevant columns
ajcc_cols <- staging_dat %>% select(c("ajcc_clinical_m", "ajcc_clinical_t", "ajcc_pathologic_n", "ajcc_

#Combine classifiers into one string
ajcc_cols$combined <- str_c(ajcc_cols$ajcc_clinical_m, ajcc_cols$ajcc_clinical_t, ajcc_cols$ajcc_pathol

#Take out effective NAs
ajcc_cols$combined <- str_remove(ajcc_cols$combined, "('--)+")

#Classification descriptions (from website given above)
classifications = c("The cancer has not spread to nearby lymph nodes or elsewhere in the body. The Grade
                    "The cancer has not spread to nearby lymph nodes [N0] or elsewhere in the body [M0].
                    "The cancer has not spread to nearby lymph nodes [N0] or elsewhere in the body [M0].
                    "It has not spread to nearby lymph nodes [N0] or elsewhere in the body [M0]. The Grade
                    " The cancer has spread to nearby lymph nodes [N1] but has not spread elsewhere in the
                    "It has spread to other parts of the body, such as distant lymph nodes, bones, or other
                    "Not classified")

#Patterns to identify classification from AJCC info
regs = c("MO.T[0|2a].*N0", "MO.T[12][abc].*N0", "MO.T[12].N0", "MO.T[34].N0", "MO.T.*N1", "M1.T.*N.*",

#Label row indices with groups
group1psa10 <- which(grepl(regs[1], ajcc_cols$combined))
group1psa20 <- which(grepl(regs[2], ajcc_cols$combined))
group2psa20 <- which(grepl(regs[3], ajcc_cols$combined))
groups14 <- which(grepl(regs[4], ajcc_cols$combined))
nodegroup <- which(grepl(regs[5], ajcc_cols$combined))
metastizedgroup <- which(grepl(regs[6], ajcc_cols$combined))
nogroup <- which(grepl(regs[7], ajcc_cols$combined))

#Create a column containing group description
ajcc_cols$AJCC_Stage_Description <- ajcc_cols$combined

ajcc_cols$AJCC_Stage_Description[nogroup] <- classifications[7]
ajcc_cols$AJCC_Stage_Description[metastizedgroup] <- classifications[6]
ajcc_cols$AJCC_Stage_Description[nodegroup] <- classifications[5]
ajcc_cols$AJCC_Stage_Description[groups14] <- classifications[4]
ajcc_cols$AJCC_Stage_Description[group2psa20] <- classifications[3]
ajcc_cols$AJCC_Stage_Description[group1psa20] <- classifications[2]
ajcc_cols$AJCC_Stage_Description[group1psa10] <- classifications[1]

#Lets view the first 6 classification descriptions.
head(ajcc_cols$AJCC_Stage_Description)

```

```

## [1] "The cancer has not spread to nearby lymph nodes or elsewhere in the body. The Grade Group is 1
## [2] "The cancer has not spread to nearby lymph nodes or elsewhere in the body. The Grade Group is 1
## [3] "Not classified"
## [4] "Not classified"
## [5] " The cancer has spread to nearby lymph nodes [N1] but has not spread elsewhere in the body [M0]

```

```
## [6] " The cancer has spread to nearby lymph nodes [N1] but has not spread elsewhere in the body [M0]
```

```
#Add this column to rest of data  
staging_dat$AJCC_Stage_Description <- ajcc_cols$AJCC_Stage_Description
```

## Extract the Data from National Cancer Institute

We downloaded the second cancer data set from the National Cancer Institute GDC Data Portal for all the clinical cases including all the columns.

The link can be found here.

*##Excel Cleaning for Second Data Set* The data was downloaded as a TSV file so it needed to be imported into Excel to change the format to a CSV or .XLSX file. The raw data contained 960 rows and 156 columns. Upon further investigation it was evident that several of the columns were empty or had values missing. In order to determine the columns with no data present along with those which had missing values we used the Filter tool in Excel. Missing values were those marked as ‘-’ in the cells.

The raw data contained 112 empty columns for which no data was available and 33 columns with missing values. The 112 empty columns were then removed from the analysis by simple deletion in Excel and a new sheet was created, “Sample”. This new sheet “Sample” contains 44 columns.

Exported the “Sample” data as a standalone CSV file.

```
sample <- read.csv("~/Downloads/cleanedsample.csv")
```

## Dealing with Missing Values

```
temp.NA <- sample  
temp.NA[temp.NA == "'--"] <- NA
```

```
#changing '-- Null values to NA  
temp.NA1 <- subset(temp.NA, temp.NA$classification_of_tumor != "NA" & temp.NA$last_known_disease_status  
#temp.NA1
```

```
#install.packages("VIM")  
#explored the relationship between the missing variables sorted by Case ID  
library(VIM)
```

```
## Loading required package: colorspace
```

```
## Loading required package: grid
```

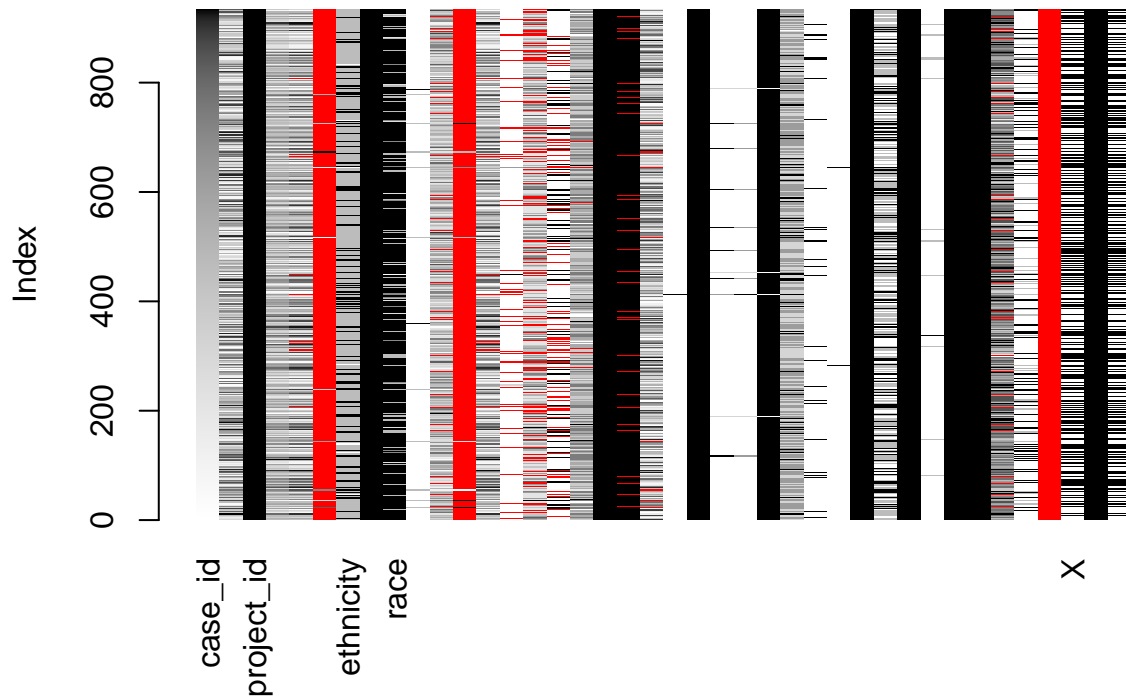
```
## VIM is ready to use.
```

```
## Suggestions and bug-reports can be submitted at: https://github.com/statistikat/VIM/issues
```

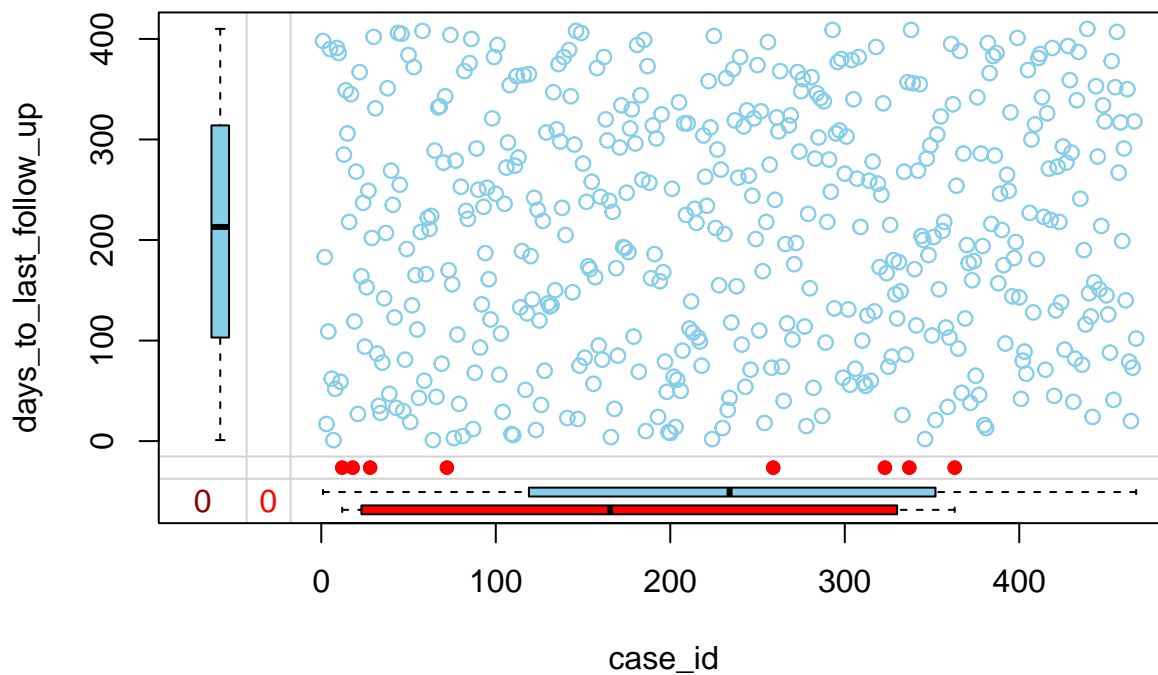
```
##  
## Attaching package: 'VIM'
```

```
## The following object is masked from 'package:datasets':
##
##   sleep
```

```
matrixplot(temp.NA1,sortby=1)
```



```
#created a margin plot to observe how the missing values in days to last follow up compare with each case
marginplot(temp.NA1[,c(1,20)])
```



```
#install.packages("sqldf")
library(sqldf)
```

```
## Loading required package: gsubfn
```

```
## Loading required package: proto
```

```
## Loading required package: RSQLite
```

```
library(tidyverse)
new_sample <- read.csv("~/Desktop/Data-Wrangling-Project/removed_dups.csv")
new_sample <- subset(new_sample, select=-c(36:39))
dim(new_sample)
```

```
## [1] 493 35
```

```
GDC <- read.csv("~/Desktop/Data-Wrangling-Project/Dataset2DD.csv")
GDC <- rename(GDC, case_id=Case.UUID)
dim(GDC)
```

```
## [1] 493 24
```

```
dataset_1 <- remove_nonuniques
dataset_1 <- rename(dataset_1, ID=Patient.ID)
dataset_1 <- rename(dataset_1, Age=Diagnosis.Age)
dataset_1sql <- sqldf("SELECT ID, case when Age >=40 and Age <= 59 then '40-59' when Age >59 and Age <=
                        case when Age >=40 and Age <= 59 then '40-59' when
                        Age >59 and Age <= 69 then '60-69'when
                        Age >69 and Age <= 79 then '70-79'
                        else '80+' end")
names(dataset_1sql)[2]<-"agegrp"
join_data1_sql <- sqldf("SELECT * FROM dataset_1 JOIN dataset_1sql on dataset_1.ID=dataset_1sql.ID")

#age check
age_ds1 <- select(join_data1_sql,"Age","agegrp")

joined_data <- sqldf("SELECT * FROM new_sample JOIN GDC on new_sample.case_id=GDC.case_id")
dim(joined_data)
```

```
## [1] 493 59
```

extracting numeric digits as text mining

```
z <- substr(joined_data$Age.at.diagnosis, 1,2)
z
```

```
## [1] "63" "58" "47" "70" "61" "68" "66" "70" "50" "61" "60" "57" "58" "57" "64"
## [16] "55" "65" "69" "66" "67" "--" "59" "51" "65" "58" "53" "65" "66" "67" "67"
## [31] "60" "69" "44" "64" "64" "54" "64" "72" "55" "47" "73" "61" "64" "53" "56"
## [46] "59" "60" "59" "57" "57" "60" "57" "61" "66" "66" "58" "63" "60" "65" "53"
```



```
## [61] "60" "67" "53" "63" "50" "68" "59" "63" "54" "65" "64" "--" "53" "69" "69"
## [76] "53" "58" "63" "65" "62" "62" "64" "57" "70" "75" "62" "64" "56" "49" "61"
## [91] "49" "69" "58" "71" "62" "70" "73" "64" "58" "50" "65" "59" "69" "68" "60"
## [106] "67" "68" "51" "50" "65" "55" "63" "63" "56" "55" "61" "66" "66" "55" "56"
## [121] "58" "50" "58" "63" "66" "54" "66" "61" "68" "69" "55" "65" "54" "58" "61"
## [136] "62" "64" "63" "63" "72" "46" "59" "65" "56" "55" "62" "62" "58" "70" "71"
## [151] "55" "66" "65" "65" "58" "57" "57" "62" "68" "52" "54" "67" "66" "48" "55"
## [166] "62" "63" "69" "58" "51" "61" "60" "64" "59" "55" "58" "65" "85" "68" "65"
## [181] "64" "59" "64" "72" "56" "67" "60" "56" "53" "65" "55" "59" "64" "69" "57"
## [196] "67" "66" "65" "65" "50" "63" "64" "--" "76" "57" "66" "47" "62" "60" "75"
## [211] "59" "58" "72" "56" "63" "56" "48" "53" "72" "49" "62" "60" "63" "62" "77"
## [226] "--" "63" "56" "65" "61" "73" "73" "53" "69" "46" "49" "64" "57" "69" "61"
## [241] "62" "61" "59" "53" "66" "48" "--" "55" "68" "67" "65" "62" "51" "68" "59"
## [256] "60" "52" "66" "58" "58" "57" "51" "68" "57" "60" "71" "49" "67" "72" "73"
## [271] "57" "66" "66" "61" "63" "59" "64" "52" "64" "56" "70" "52" "70" "54" "68"
## [286] "48" "58" "48" "64" "71" "68" "61" "61" "65" "57" "58" "65" "64" "49" "57"
## [301] "62" "61" "63" "73" "68" "66" "70" "62" "57" "72" "66" "61" "63" "70" "53"
## [316] "68" "63" "71" "59" "58" "59" "66" "56" "51" "61" "60" "68" "56" "51" "66"
## [331] "59" "78" "63" "57" "51" "60" "69" "65" "63" "52" "52" "56" "63" "54" "68"
## [346] "64" "67" "63" "64" "56" "70" "66" "66" "67" "62" "62" "50" "52" "--" "55"
## [361] "55" "53" "59" "57" "63" "67" "77" "61" "46" "71" "55" "56" "67" "65" "62"
## [376] "57" "67" "57" "54" "66" "59" "67" "59" "56" "71" "70" "66" "60" "64" "66"
## [391] "55" "62" "63" "54" "72" "58" "68" "58" "47" "57" "74" "69" "66" "64" "47"
## [406] "61" "56" "61" "52" "55" "55" "55" "41" "73" "61" "61" "64" "53" "72" "61"
## [421] "60" "68" "66" "72" "67" "54" "56" "64" "59" "71" "63" "68" "47" "--" "70"
## [436] "46" "68" "44" "67" "61" "56" "66" "57" "62" "66" "53" "70" "49" "58" "56"
## [451] "53" "51" "55" "66" "60" "57" "61" "56" "55" "67" "53" "69" "63" "54" "48"
## [466] "64" "53" "65" "60" "60" "46" "70" "57" "62" "--" "56" "55" "43" "72" "67"
## [481] "53" "62" "70" "58" "74" "59" "57" "72" "68" "--" "55" "65" "63"
```

```
joined_data$Age <- cbind(z)
joined_data$Age <- as.numeric(joined_data$Age)
```

```
## Warning: NAs introduced by coercion
```

```
joined_data[36] <- NULL
view(joined_data)
joined_data <- rename(joined_data, ID=case_id)
```

```
cols_dups <- c()

for(i in 1:ncol(joined_data)){
  if(length(unique(joined_data[,i])) <= 1){
    cols_dups[length(cols_dups) + 1] <- i
  }
}

print(cols_dups)
```

```
## [1] 8 31 33 38 39
```

```
remove_dups <- joined_data[,-cols_dups]
dim(remove_dups)
```

```
## [1] 493 54
```

```
## removing some duplicated manually
remove_dups[52]<-NULL #removes the ethnicity duplicate
remove_dups[52] <- NULL #removes the age duplicate
dim(remove_dups)
```

```
## [1] 493 52
```

```
dataset_2 <- remove_dups
dataset_2sql <- sqldf("SELECT ID, case when Age >=40 and Age <= 59 then '40-59' when Age >59 and Age <=
                        case when Age >=40 and Age <= 59 then '40-59' when
                        Age >59 and Age <= 69 then '60-69'when
                        Age >69 and Age <= 79 then '70-79'
                        else '80+' end")
names(dataset_2sql)[2]<-"agegrp"
dataset_2$agegrp <- cbind(dataset_2sql[2])
```

## CONCLUSION

### Summary

Our analysis aimed to understand the associations between patient characteristics and severity of prostate cancer through various data wrangling procedures we learned throughout the quarter. After utilizing three platforms for our cleaning and wrangling processes (R, SQL, and Excel), and creating numerical summaries along with visual representations of our data through R packages such as gtsummary and ggplot, we have come to the following conclusions:

1. After performing a Chi-squared test on the relationships between geographic location and severity of tumor content (%), the data set provides no evidence of association between geographic location and severity of tumor content (%).
2. After running a Fisher's exact test (for Sequencing Site) and a Pearson's Chi Squared test (Prior Treatment Status), there is evidence of a statistically significant association between Sequencing Site and Exposure Status along with Prior Treatment Status and Exposure Status.
3. As men age, incidence of prostate cancer increases with metastasis of disease occurring more frequently in the lymph nodes and bone.
4. From visual assessments, there does not seem to be an association between exposure status and mutation group, fraction of genome altered, or tumor content.

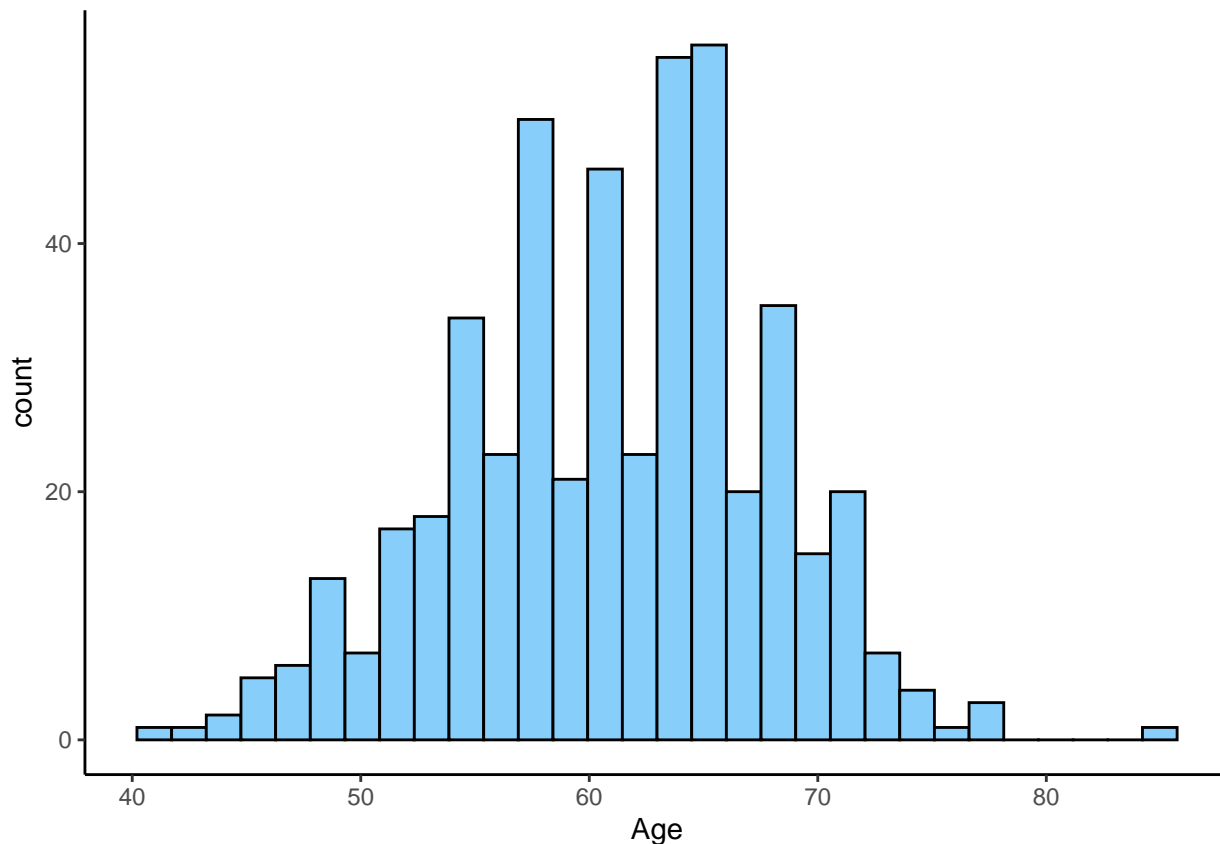
### Next Steps

After joining the two data sets from GDC via SQL, we noticed that our initial data set from cBioPortal and the new data set both had data on patient's age at diagnosis as well as whether the patient had prior treatment. As a basic step to check similarities between patients across both data sets, we plotted the distributions of age at diagnosis for both data sets.

```
# Plot age at diagnosis distributions for both data sets
ggplot(data.frame(dataset_2), aes(x=Age)) +
  geom_histogram(colour="black",fill="lightskyblue") +theme_classic()
```

```
## 'stat_bin()' using 'bins = 30'. Pick better value with 'binwidth'.
```

```
## Warning: Removed 9 rows containing non-finite values (stat_bin).
```

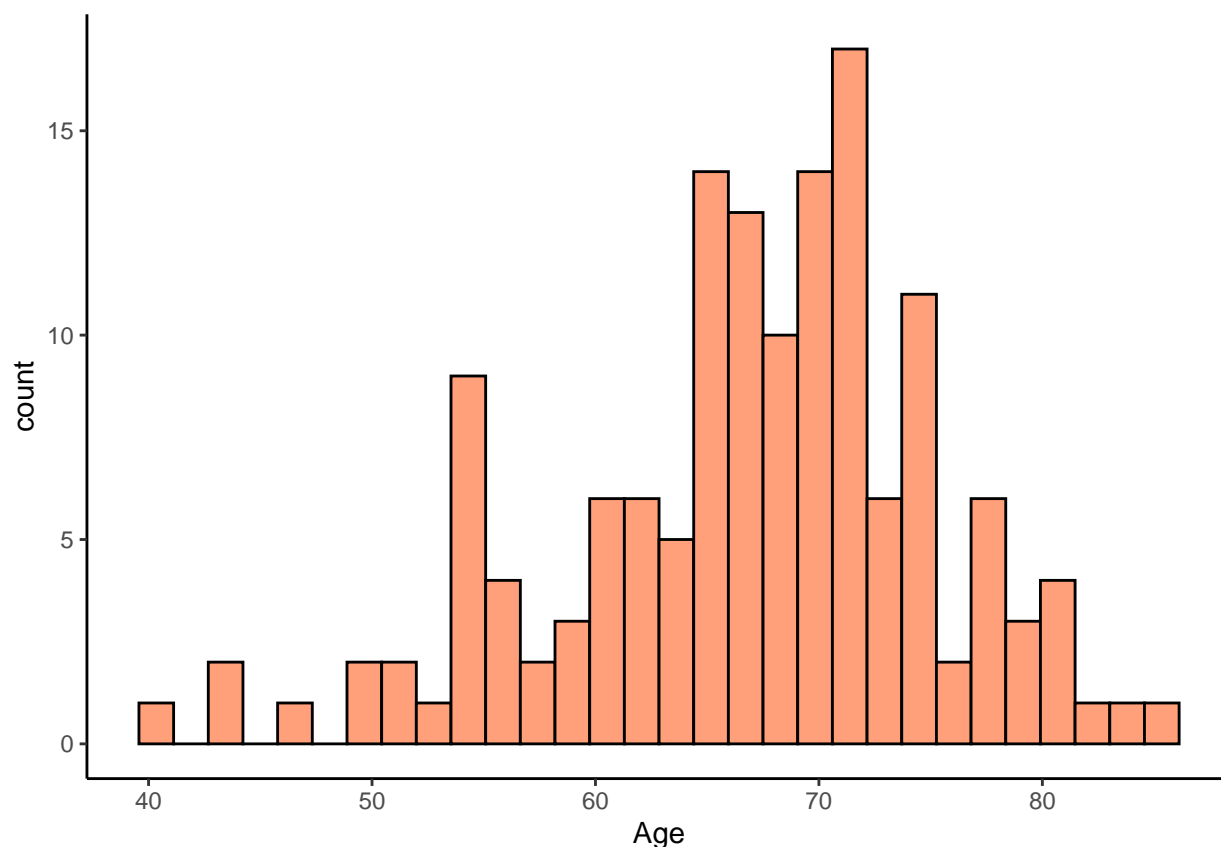


```
summary(dataset_2$Age)
```

```
##      Min. 1st Qu.  Median    Mean 3rd Qu.    Max.     NA's
##      41.00  56.00   61.00   61.05  66.00   85.00         9
```

```
ggplot(data.frame(dataset_1), aes(x=Age)) +
  geom_histogram(colour="black",fill="lightsalmon") + theme_classic()
```

```
## 'stat_bin()' using 'bins = 30'. Pick better value with 'binwidth'.
```



```
summary(dataset_1$Age)
```

```
##      Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##  40.00   62.00   68.00   66.84   72.00   85.00
```

After assessing the plots above, we noticed that the mean age at diagnosis for our first data set (cBioPortal) was slightly later than the mean age at diagnosis for our second data set (GDC). After realizing this, we wanted to see if patients from the cBioPortal had higher rates of prior treatment status than patients from the GDC data. In order to check this while stratifying for age groups, we plotted prior treatment status for patients from each data set based on our age groups.

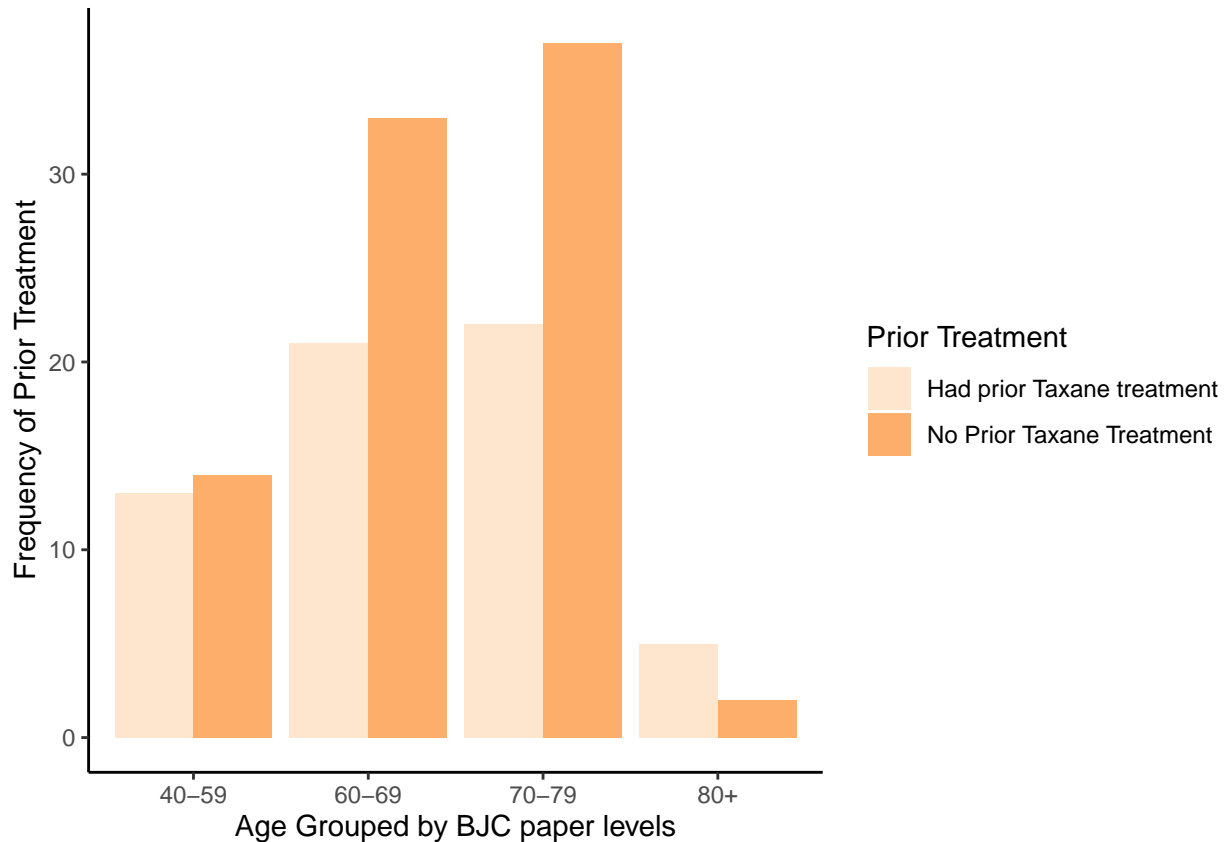
```
# Plot prior treatment status for both data sets
join_data1_sql[1] <- NULL
freq_1treat <- join_data1_sql %>% group_by(agegrp, Prior.Treatment) %>% summarise(Freq=n())
```

```
## 'summarise()' has grouped output by 'agegrp'. You can override using the '.groups' argument.
```

```
dat2 <- dataset_2
dat2[dat2 == "'--"] <- NA
dat2 <- dat2[!is.na(dat2$prior_treatment),]
freq_2treat <- dat2 %>% group_by(agegrp, prior_treatment) %>% summarise(Freq=n())
```

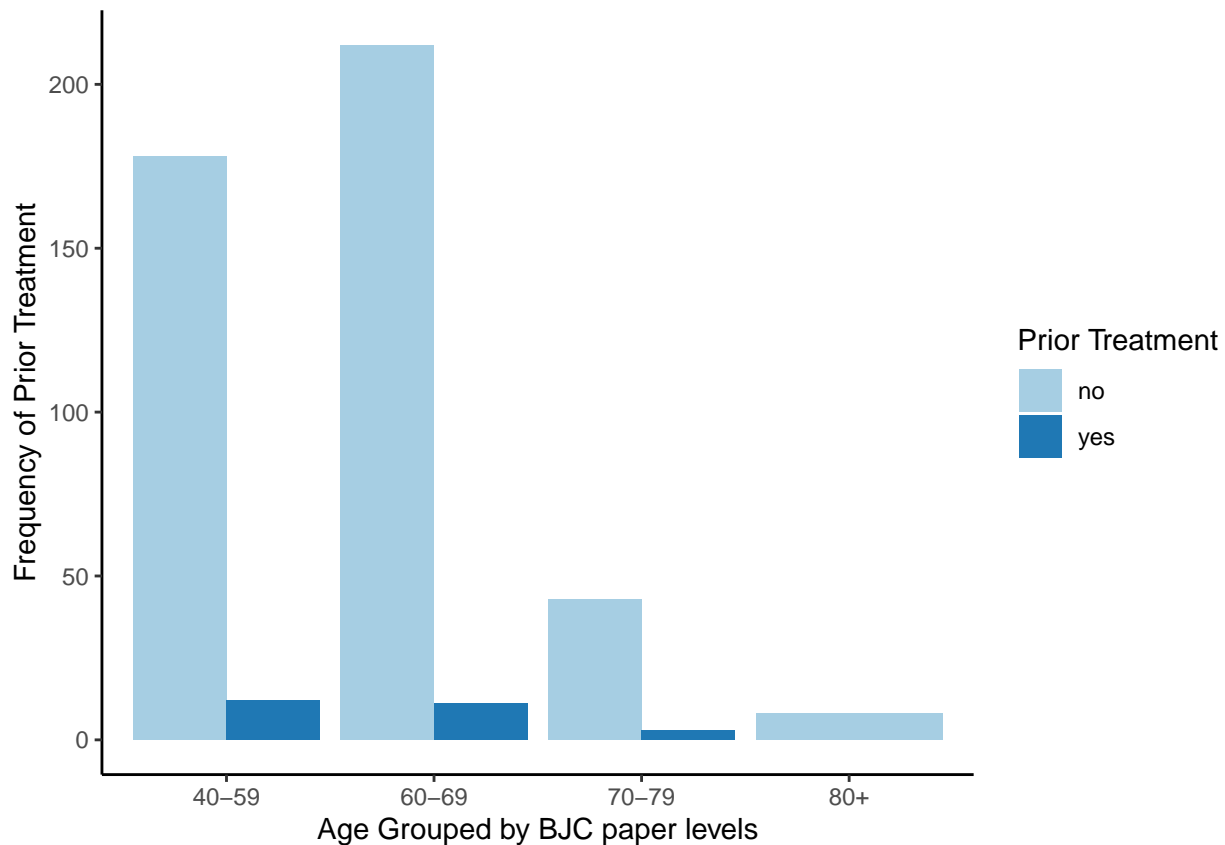
```
## 'summarise()' has grouped output by 'agegrp'. You can override using the '.groups' argument.
```

```
ggplot(data.frame(freq_1treat), aes(x=agegrp, y=Freq)) +
  labs(x="Age Grouped by BJC paper levels", y="Frequency of Prior Treatment", ) +
  geom_bar(aes(fill=Prior.Treatment), stat="identity", position="dodge") +
  guides(fill=guide_legend(title="Prior Treatment"))+
  scale_fill_brewer(palette="Oranges") +
  theme_classic()
```



```
x <- c("40-59", "40-59", "60-69", "60-69", "70-79", "70-79", "80+")
freq_2treat$AGEgrp <- cbind(x)

ggplot(data.frame(freq_2treat), aes(x=AGEgrp, y=Freq)) +
  labs(x="Age Grouped by BJC paper levels", y="Frequency of Prior Treatment", ) +
  geom_bar(aes(fill=prior_treatment), stat="identity", position="dodge") +
  guides(fill=guide_legend(title="Prior Treatment"))+
  scale_fill_brewer(palette="Paired") +
  theme_classic()
```



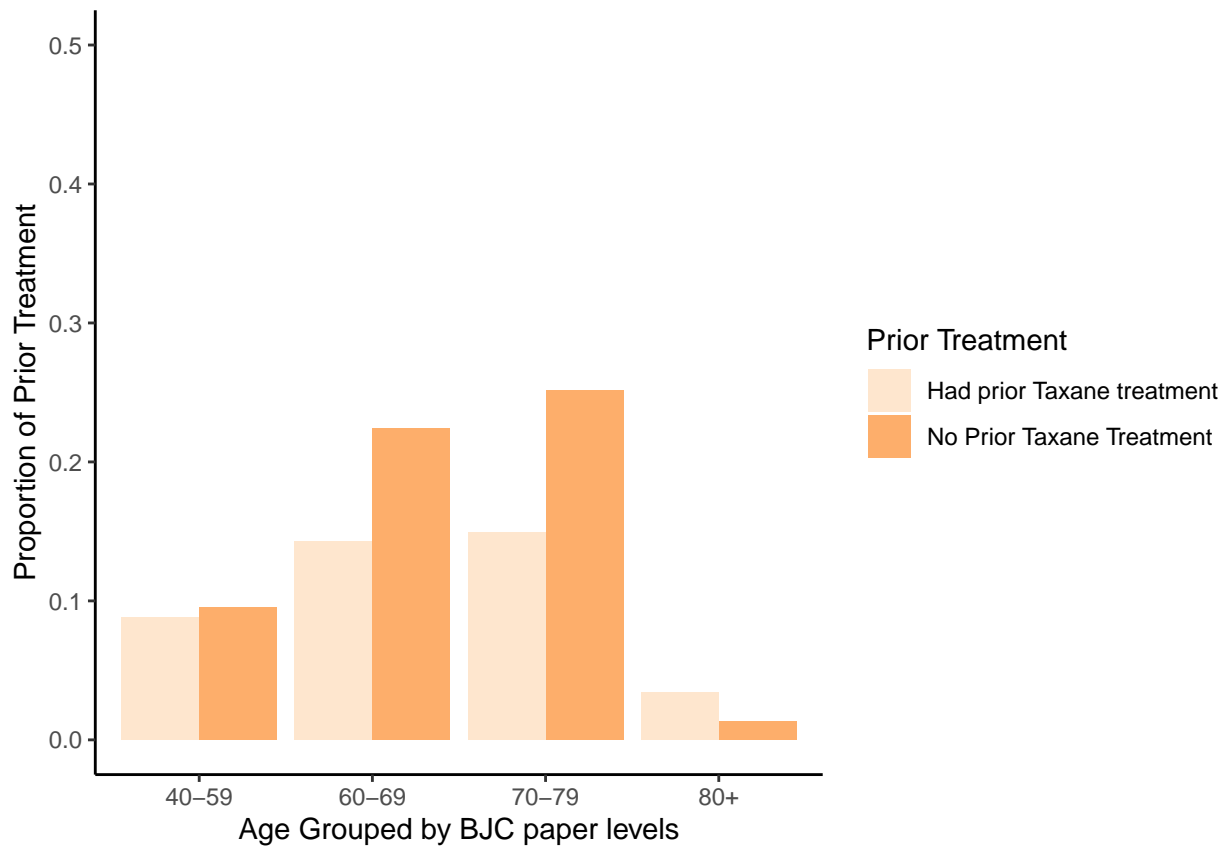
The first thing we noticed is that these graphs are slightly misleading since we are looking at frequencies instead of proportions. The cBioPortal data set frequencies range from 0-40, while the GDC data set frequencies range from 0 to >200. Let's try to make these graphs more comparable.

```
# Make proportions
freq_1treat$Prop = freq_1treat$Freq / sum(freq_1treat$Freq)

# Make proportions
freq_2treat$Prop = freq_2treat$Freq / sum(freq_2treat$Freq)

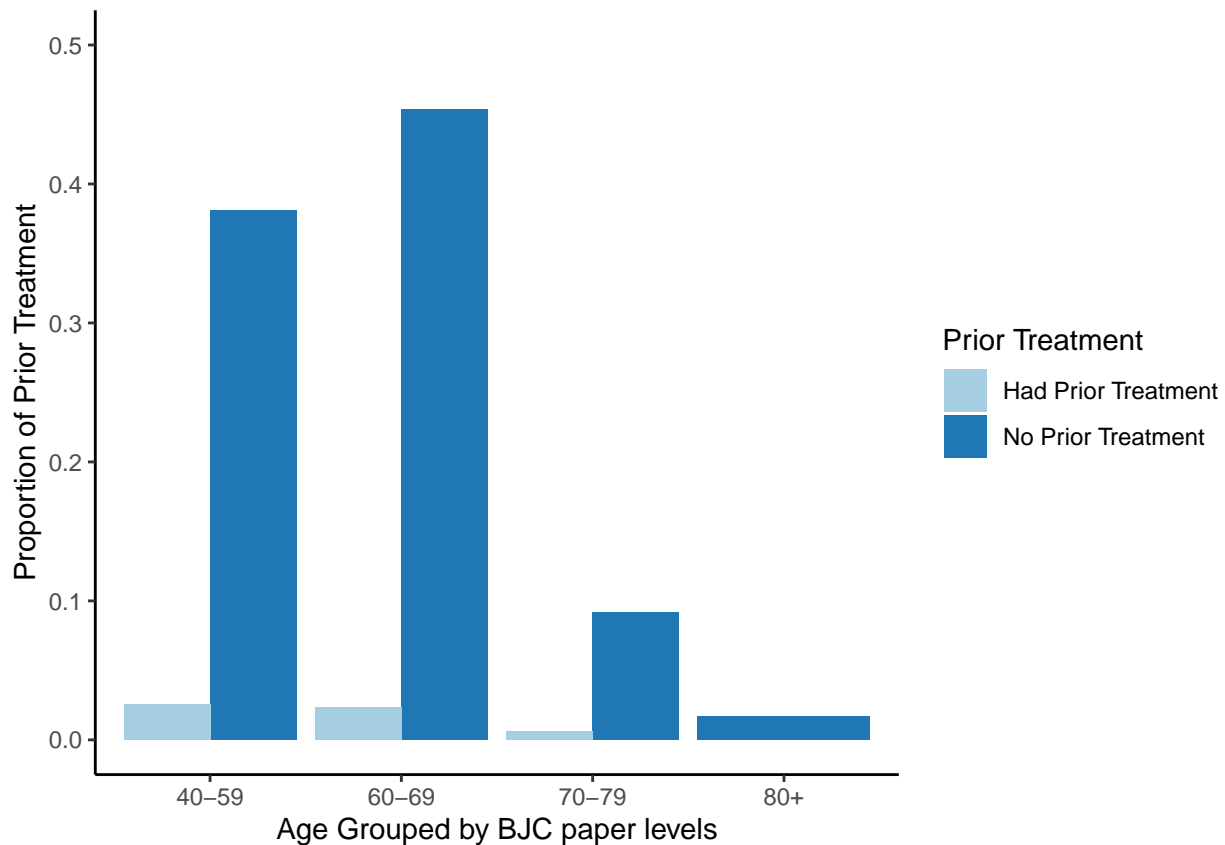
# Fix colors
freq_2treat$UpdatedPT <- ifelse(freq_2treat$prior_treatment == 'yes', 'Had Prior Treatment', 'No Prior Treatment')

ggplot(data.frame(freq_1treat), aes(x=agegrp, y=Prop)) +
  labs(x="Age Grouped by BJC paper levels", y="Proportion of Prior Treatment", ) +
  geom_bar(aes(fill=Prior.Treatment), stat="identity", position="dodge") +
  guides(fill=guide_legend(title="Prior Treatment")) +
  scale_fill_brewer(palette="Oranges") +
  theme_classic() + ylim(0, 0.5)
```



```
x <- c("40-59", "40-59", "60-69", "60-69", "70-79", "70-79", "80+")
freq_2treat$AGEgrp <- cbind(x)

ggplot(data.frame(freq_2treat), aes(x=AGEgrp, y=Prop)) +
  labs(x="Age Grouped by BJC paper levels", y="Proportion of Prior Treatment", ) +
  geom_bar(aes(fill=UpdatedPT), stat="identity", position="dodge") +
  guides(fill=guide_legend(title="Prior Treatment"))+
  scale_fill_brewer(palette="Paired") +
  theme_classic() + ylim(0, 0.5)
```



Great! Now that we have the proportions and equal y axis ranges, we can see that the gap between having prior treatment versus no prior treatment is relatively smaller in the patients from the first data set compared to patients from the second data set. Additionally, the proportion of patients who received prior treatment in the first data set seems to be higher across all age groups compared to the second data set.

This would be a great place to start further analysis if we had more time. We could look more into how prior treatment affects diagnosis age, if prior treatment has been linked to later diagnosis age, and where we would be able to find more data to test this association. One thing to note is that the first data set's patients were specifically studied on prior Taxane treatment, while the patients in the second data set did not have a specific treatment mentioned. So, we could also look at Taxane treatment effectiveness in preventing prostate cancer versus other treatments. In addition to investigating diagnosis age's relationship to prior treatment status, we had also noticed that the second data set had a "vital status" column which gives us information on the survival status of the patient. This information was missing from our first data set, so another great next step would be to track the incidence of disease in our second data set (from GDC), and by using our vital status column, we would calculate the patient mortality ratio for the GDC data set.

## Limitations

Though we were able to derive several conclusions from our data sets, there were a few limitations we faced. For starters, the data set we got from cBioPortal did not have well-defined variables or information on certain variables were calculated, what the units of measurements were, etc. For example, we initially struggled to find information on how tumor content and mutation count were measured, what the units of measurements were, and what ranges of each measurement were considered significant. When we tried to do some background research through search engines such as Google, we weren't able to find any helpful information, which leads us to our second limitation: prostate cancer data is very niche, and there were not many resources readily available for us to use to get a better understanding on some of the variables we were dealing with. Nevertheless, after reading through the data set's attached paper for understanding tumor



content and looking at other academic papers to better understand how mutation count works, we were able to successfully interpret our variables of interest.

## **Looking Forward - Clinical Implications**

If we were to use our analysis to make conclusions about the clinical implications of the prostate cancer data we analyzed throughout this project, our first piece of advice would be to further study associations between sequencing site and exposure status, along with prior treatment status and exposure status since we obtained significant results when studying these associations. Additionally, it would be worth looking into how age plays into certain factors such as prior treatment status and tumor site.