Case Study

Using the cohort of patients who were diagnosed with Stage IV adenocarcinoma NSCLC, we will:

1. **Process data** into a analysis-ready matrix of events.
2. **Summarize genomic alteration frequencies** and analyze differences between males and females.

```r
nsclc_cohort <- create_analytic_cohort(
  data_synapse =
    nsclc_synapse_data$NSCLC_v2.0,
  stage_dx = c("Stage IV"),
  histology = "Adenocarcinoma",
  regimen_drugs =
    c("Carboplatin, Pemetrexed Disodium",
      "Cisplatin, Pemetrexed Disodium",
      "Bevacizumab, Carboplatin, Pemetrexed Disodium",
      "Bevacizumab, Cisplatin, Pemetrexed Disodium"),
  regimen_type = "Exact",
  regimen_order = 1,
  regimen_order_type = "within cancer",
  return_summary = TRUE
)
```

Characteristic	N = 241[1]
naaccr_sex_code	
Female	145 (60%)
Male	96 (40%)

[1] n (%)

Overview of Genomic Data

We will be processing and analyzing data on:

1) Mutations

```
mutations <- nsclc_synapse_data$NSCLC_v2.0$mutations_extended
```

2) Discrete Copy Number Alterations

```
cna <- nsclc_synapse_data$NSCLC_v2.0$cna
```

3) Fusions

```
fusions <- nsclc_synapse_data$NSCLC_v2.0$fusions
```

AACRA Data Guide: https://www.aacr.org/wp-content/uploads/2020/02/20200127_GENIE_Data_Guide_7.pdf

Processing Data

Issues When Processing Multi-Institutional Genomic Data

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   - Samples may be sequenced using different panels, therefore the non-overlapping genes have to be annotated as missing

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- Patients can have many NGS reports
- We can use `select_unique_ngs()` to select 1 sample per patient
- This function prioritizes characteristics of interest and breath of genes sequenced
- If patients only have one report, it will be returned regardless of criteria

Select One Sample Per Patient

1. **Multiple Samples Per Patient**
    - We often need to select a single sample per patient for analyses

```
nrow(nsclc_cohort$cohort_ngs)
```

[1] 262

```
nsclc_samp <- select_unique_ngs(
  data_cohort = nsclc_cohort$cohort_ngs,
  oncotree_code = "LUAD",
  sample_type = "Metastasis",
  min_max_time = "max"
)

nrow(nsclc_samp)
```

[1] 241

Format Data in Analysis-ready Matrix

sample_id	CREBBP	GLI2	KRAS	MAP3K1	PIK3C2B	PBRM1
GENIE-DFCI-004022-1313	0	0	0	0	0	NA
GENIE-DFCI-000013-8840	0	0	1	0	0	0
GENIE-DFCI-000136-6004	0	0	1	0	0	0
GENIE-DFCI-000215-8010	0	0	0	0	0	0
GENIE-DFCI-000381-9526	0	0	0	0	0	0

Format Data in Analysis-ready Matrix

- Next we want to get our genomic data in an **analysis-friendly format**.

sample_id	CREBBP	GLI2	KRAS	MAP3K1	PIK3C2B	PBRM1
GENIE-DFCI-004022-1313	0	0	0	0	0	NA
GENIE-DFCI-000013-8840	0	0	1	0	0	0
GENIE-DFCI-000136-6004	0	0	1	0	0	0
GENIE-DFCI-000215-8010	0	0	0	0	0	0
GENIE-DFCI-000381-9526	0	0	0	0	0	0

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- Next we want to get our genomic data in an **analysis-friendly format**.
- `create_gene_binary()` from {gnomeR} will give us a data frame of ***n* patients x *p* alterations**.

sample_id	CREBBP	GLI2	KRAS	MAP3K1	PIK3C2B	PBRM1
GENIE-DFCI-004022-1313	0	0	0	0	0	NA
GENIE-DFCI-000013-8840	0	0	1	0	0	0
GENIE-DFCI-000136-6004	0	0	1	0	0	0
GENIE-DFCI-000215-8010	0	0	0	0	0	0
GENIE-DFCI-000381-9526	0	0	0	0	0	0

Format Data in Analysis-ready Matrix

- Next we want to get our genomic data in an **analysis-friendly format**.
- `create_gene_binary()` from {gnomeR} will give us a data frame of ***n* patients x *p* alterations**.
- Alteration columns are denoted by the **gene name** if mutation (e.g. `TP53`) or **gene name + .Amp, .fus, .Del** (`TP53.Del`) for other alterations types.

sample_id	CREBBP	GLI2	KRAS	MAP3K1	PIK3C2B	PBRM1
GENIE-DFCI-004022-1313	0	0	0	0	0	NA
GENIE-DFCI-000013-8840	0	0	1	0	0	0
GENIE-DFCI-000136-6004	0	0	1	0	0	0
GENIE-DFCI-000215-8010	0	0	0	0	0	0
GENIE-DFCI-000381-9526	0	0	0	0	0	0

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- Next we want to get our genomic data in an **analysis-friendly format**.
- `create_gene_binary()` from {gnomeR} will give us a data frame of ***n* patients x *p* alterations**.
- Alteration columns are denoted by the **gene name** if mutation (e.g. `TP53`) or **gene name + .Amp, .fus, .Del** (`TP53.Del`) for other alterations types.
- Each cell will have `0` if no alteration, `1` if altered, or `NA` if that gene was not tested in that patient.

sample_id	CREBBP	GLI2	KRAS	MAP3K1	PIK3C2B	PBRM1
GENIE-DFCI-004022-1313	0	0	0	0	0	NA
GENIE-DFCI-000013-8840	0	0	1	0	0	0
GENIE-DFCI-000136-6004	0	0	1	0	0	0
GENIE-DFCI-000215-8010	0	0	0	0	0	0
GENIE-DFCI-000381-9526	0	0	0	0	0	0

Get Data in Standardized Format

2. **Data Formats & Gene Standards Often Inconsistent**
    - Column names, data formats and gene names may differ between studies or even within studies!

{gnomeR} functions are designed to work with standard alteration data formats used in common platforms like cBioPortal.

GENIE CNA and fusion data is slightly different than this standard, therefore we need to reformat it using:

- `gnomeR::reformat_fusions()`
- `gnomeR::pivot_cna_longer()`

Documentation on data formats: https://docs.cbioportal.org/file-formats/

Get Data in Standardized Format

Format fusions to follow the cBioPortal standard:

Before:

```
1 fusions
```

Hugo_Symbol	Tumor_Sample_Barcode	Fusion
TP53	GENIE-MSK-P-0004827-T01-IM5	TP53-intragenic
XPO1	GENIE-MSK-P-0004827-T01-IM5	XPO1-USP34 fusion
USP34	GENIE-MSK-P-0004827-T01-IM5	XPO1-USP34 fusion

After:

```
1 reformat_fusion(fusions)
```

sample_id	site_1_hugo_symbol	site_2_hugo_symbol	fusion
GENIE-MSK-P-0003446-T01-IM5	ALK	EML4	ALK-EML4
GENIE-MSK-P-0003863-T01-IM5	FLT4	NA	FLT4
GENIE-MSK-P-0004103-T01-IM5	BRAF	SND1	BRAF-SND1
GENIE-MSK-P-0004336-T01-IM5	CD74	ROS1	CD74-ROS1
GENIE-MSK-P-0004827-T01-IM5	TP53	NA	TP53
GENIE-MSK-P-0004827-T01-IM5	USP34	XPO1	USP34-XPO1

Get Data in Standardized Format

Format CNA to follow the cBioPortal standard:

Before:

```
1 cna[1:5, 1:3]
```

Hugo_Symbol	GENIE.DFCI.003908.234520	GENIE.DFCI.002183.6917
ABCB11	0	0
ABL1	0	0
ABL2	NA	NA
ACTB	NA	NA
ACTG1	NA	NA

After:

```
1 gnomeR::pivot_cna_longer(cna)
```

hugo_symbol	sample_id	alteration
APC	GENIE-DFCI-003908-234520	gain
ARID1B	GENIE-DFCI-003908-234520	loss
BCL2	GENIE-DFCI-003908-234520	loss
BCL6	GENIE-DFCI-002183-6917	gain
CARD11	GENIE-DFCI-002183-6917	gain

Documentation on data formats: https://docs.cbioportal.org/file-formats/

Get Data in Standardized Format

```
mutations <- nsclc_synapse_data$NSCLC_v2.0$mutations_extended
cna <- nsclc_synapse_data$NSCLC_v2.0$cna
fusions <- nsclc_synapse_data$NSCLC_v2.0$fusions
```

```
reformat_fusions <- gnomeR::reformat_fusion(fusions)

nrow(reformat_fusions)
```

[1] 538

```
reformat_cna <- gnomeR::pivot_cna_longer(cna)

nrow(reformat_cna)
```

[1] 38163

Format Data in Analysis-ready Matrix

Basic code:

```
gnomeR::create_gene_binary(
  mutation = mutations,
  cna = reformat_cna,
  fusion = reformat_fusions)
```

Format Data in Analysis-ready Matrix

- Data is now in standardized format and can be processed using {gnomeR} `create_gene_binary()`

Basic code:

```
gnomeR::create_gene_binary(
  mutation = mutations,
  cna = reformat_cna,
  fusion = reformat_fusions)
```

Format Data in Analysis-ready Matrix

- Data is now in standardized format and can be processed using {gnomeR} `create_gene_binary()`
- We will add additional arguments to `create_gene_binary()` to help address remaining data processing issues

Basic code:

```
gnomeR::create_gene_binary(
  mutation = mutations,
  cna = reformat_cna,
  fusion = reformat_fusions)
```

Cohort Inclusion

2. **Cohort Inclusion**
    - Samples with no alterations may be dropped when pulling raw genomic data

```
gnomeR::create_gene_binary(
  mutation = mutations,
  cna = reformat_cna,
  fusion = reformat_fusions
  samples = nsclc_samp$cpt_genie_sample_id)
```

The `samples` argument will ensure all study IDs have a row in resulting analysis data, even if they are not present in genomic files

Not All Patients Tested on Same Panel

4. **Multi-Institutional Studies Use Several Gene Panels**
    - Samples may be sequenced using different panels therefore the non overlapping genes have to be annotated as missing

```
gnomeR::create_gene_binary(
  mutation = mutations,
  cna = reformat_cna,
  fusion = reformat_fusions,
  samples = nsclc_samp$cpt_genie_sample_id,
  specify_panel = nsclc_panels)
```

The `specify_panels` argument can insert NAs when we know that gene was not tested for a specific set of patients.

Not All Patients Tested on Same Panel

To use `specify_panels`, we first need to create a data frame indicating which patient IDs were sequenced on which panels.

```
nsclc_panels <- data.frame(
  sample_id = nsclc_samp$cpt_genie_sample_id,
  panel_id = nsclc_samp$cpt_seq_assay_id) %>%
  mutate(panel_id = ifelse(!is.na(panel_id),
                           panel_id, "no"))

nsclc_panels %>% head()
```

sample_id	panel_id
GENIE-DFCI-000013-8840	DFCI-ONCOPANEL-2
GENIE-DFCI-000136-6004	DFCI-ONCOPANEL-2
GENIE-DFCI-000215-8010	DFCI-ONCOPANEL-2
GENIE-DFCI-000381-9526	DFCI-ONCOPANEL-2
GENIE-DFCI-000410-10003	DFCI-ONCOPANEL-2
GENIE-DFCI-000583-11175	DFCI-ONCOPANEL-2

Not All Patients Tested on Same Panel

Without Panel Annotation

```
binmat1 <- gnomeR::create_gene_binary(
  mutation = mutations,
  cna = reformat_cna,
  fusion = reformat_fusions,
  samples = nsclc_samp$cpt_genie_sample_id,
  specify_panel = "no")
```

sample_id	panel_id	GLI2	KRAS	PIK3C2B	PBRM1
GENIE-DFCI-004022-1313	DFCI-ONCOPANEL-1	0	0	0	0
GENIE-DFCI-000013-8840	DFCI-ONCOPANEL-2	0	1	0	0
GENIE-MSK-P-0002725-T01-IM3	MSK-IMPACT341	0	1	0	0
GENIE-MSK-P-0017722-T02-IM6	MSK-IMPACT468	0	0	0	0

With Panel Annotation

```
binmat2 <- gnomeR::create_gene_binary(
  mutation = mutations,
  cna = reformat_cna,
  fusion = reformat_fusions,
  samples = nsclc_samp$cpt_genie_sample_id,
  specify_panel = nsclc_panels)
```

sample_id	panel_id	GLI2	KRAS	PIK3C2B	PBRM1
GENIE-DFCI-004022-1313	DFCI-ONCOPANEL-1	0	0	0	NA
GENIE-DFCI-000013-8840	DFCI-ONCOPANEL-2	0	1	0	0
GENIE-MSK-P-0002725-T01-IM3	MSK-IMPACT341	NA	1	NA	0
GENIE-MSK-P-0017722-T02-IM6	MSK-IMPACT468	NA	0	NA	0

Ensure Gene Names Are Consistent Across Studies

2. **Data Formats & Gene Standards Often Inconsistent**
    - Column names, data formats and gene names may differ between studies or even within studies.

```
no_recode <- gnomeR::create_gene_binary(
  samples = nsclc_samp$cpt_genie_sample_id,
  mutation = mutations,
  cna = reformat_cna,
  fusion = reformat_fusions,
  specify_panel = nsclc_panels,
  recode_aliases = "no")
```

```
recode <- gnomeR::create_gene_binary(
  samples = nsclc_samp$cpt_genie_sample_id,
  mutation = mutations,
  cna = reformat_cna,
  fusion = reformat_fusions,
  specify_panel = nsclc_panels,
  recode_aliases = "impact")
```

```
setdiff(names(no_recode), names(recode)) %>% head()
```

[1] “MRE11A” “RFWD2” “H3F3A” “FAM46C” “HIST1H3D” “WHSC1L1”

Process Data: Final Dataset

Let's run `create_gene_binary()` with the `samples`, `specify_panel` and `recode_aliases` arguments.

First we create `nsclc_panels`:

```
nsclc_panels <- data.frame(
  sample_id = nsclc_samp$cpt_genie_sample_id,
  panel_id = nsclc_samp$cpt_seq_assay_id) %>%
  mutate(panel_id = ifelse(!is.na(panel_id),
                           panel_id, "no"))
```

Then run `create_gene_binary()`:

```
gene_binary <- gnomeR::create_gene_binary(
  mutation = mutations,
  cna = reformat_cna,
  fusion = reformat_fusions,
  samples = nsclc_samp$cpt_genie_sample_id,
  specify_panel = nsclc_panels,
  recode_aliases = "impact")
```

Analyzing Data

Issues When Analyzing Multi-Institutional Genomic Data

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1. **Multiple Testing and False Positives**

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   - Many hypothesis tests done simultaneously can lead to high rate of false positive findings.

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- Very low prevalence genes are often not very informative.

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- Choose a threshold (e.g. 1% or 5 %) *a prior* to limit number of hypothesis tests and consider reporting a q-value (adjusted for multiple testing).

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2. **Limited Power To Detect Clinical Associations When Sparse Alterations**

- If biologically meaningful, you may want to summarize on alteration, gene or pathway Level

Case Study

- We will use the processed binary data frame data (`gene_binary`) to **summarize genomic alterations** overall in the cohort, and **by sex**.

- First, we need to join clinical data on `sex` to genomic data

Add Clinical Variable To Data

```r
# get patient IDs and sample IDs
patient_index <-  nsclc_cohort$cohort_ngs %>%
  select(record_id, cpt_genie_sample_id)

# Join sex data to patient ID index
select_clinical <- nsclc_cohort$cohort_pt_char %>%
  select(record_id, naaccr_sex_code) %>%
  left_join(patient_index)

# Join all to gene binary data
gene_binary <- gene_binary %>%
  left_join(select_clinical,
            by = c("sample_id"= "cpt_genie_sample_id
  select(-record_id)

gene_binary <- gene_binary %>%
  select(sample_id, naaccr_sex_code, everything())

gene_binary %>%
  select(naaccr_sex_code) %>%
  tbl_summary()
```

Characteristic	N = 241[1]
naaccr_sex_code	
Female	145 (60%)
Male	96 (40%)

[1] n (%)

Subset By a Prevalence Threshold

1. **Multiple Testing and False Positives**

- Use `subset_by_frequency(t)` to **subset genes** above a given threshold
- `t` indicates a **prevalence threshold** between 0 (`t = 0`) and 100% (`t = 1`)
- `other_vars` retains the clinical variable of interest in the resulting data set

Subset By 40% Threshold

```
ncol(gene_binary)
```

[1] 1403

```
nscl_subset <- gene_binary %>%
  subset_by_frequency(t = .4, other_vars = naaccr_sex_code)

ncol(nscl_subset)
```

[1] 6

Subset by a Panel

Use `subset_by_panel()` to subset genes in a given targeted panel.

```
ncol(gene_binary)
```

[1] 1403

```
nscl_subset_panel <- gene_binary %>%
  subset_by_panel(panel_id = 'IMPACT300', other_vars = naaccr_sex_code)

ncol(nscl_subset_panel)
```

[1] 220

Summarize Alterations with tbl_genomic()

- `tbl_genomic()` is a wrapper function for `gtsummary::tbl_summary()` specifically designed for presenting genomic data
- You can use any **{gtsummary}** function on top of `tbl_genomic()` to customize the table (e.g. `bold_labels()`)

Summarize Alterations with tbl_genomic

Create a simple `tbl_genomic` object, then bold the labels.

```
nscl_subset %>%
  select(-naaccr_sex_code) %>%
  tbl_genomic() %>%
  bold_labels()
```

Characteristic	N = 241[1]
TP53	124 (51%)
GBA.Amp	17 (46%)
Unknown	204
KRAS	98 (41%)
JAZF1.Amp	15 (41%)
Unknown	204

[1] n (%)

Summarize Genes with tbl_genomic()

You may want to analyze on the **gene level** instead of the alteration level.

Use `summarize_by_gene()` first, then pass to `tbl_genomic()`:

> ⚠ **Note: `summarize_by_gene()` should come before passing to `subset_by_frequency()`**

```
1  tbl_gene <- gene_binary %>%
2    select(-naaccr_sex_code) %>%
3    summarize_by_gene() %>%
4    subset_by_frequency(t = .4) %>%
5    tbl_genomic()
6
7  tbl_gene
```

Characteristic	N = 241[1]
TP53	135 (56%)
GBA	17 (46%)
Unknown	204
PTK2B	17 (46%)
Unknown	204
KRAS	107 (44%)
WRN	38 (41%)
Unknown	149
JAZF1	15 (41%)
Unknown	204

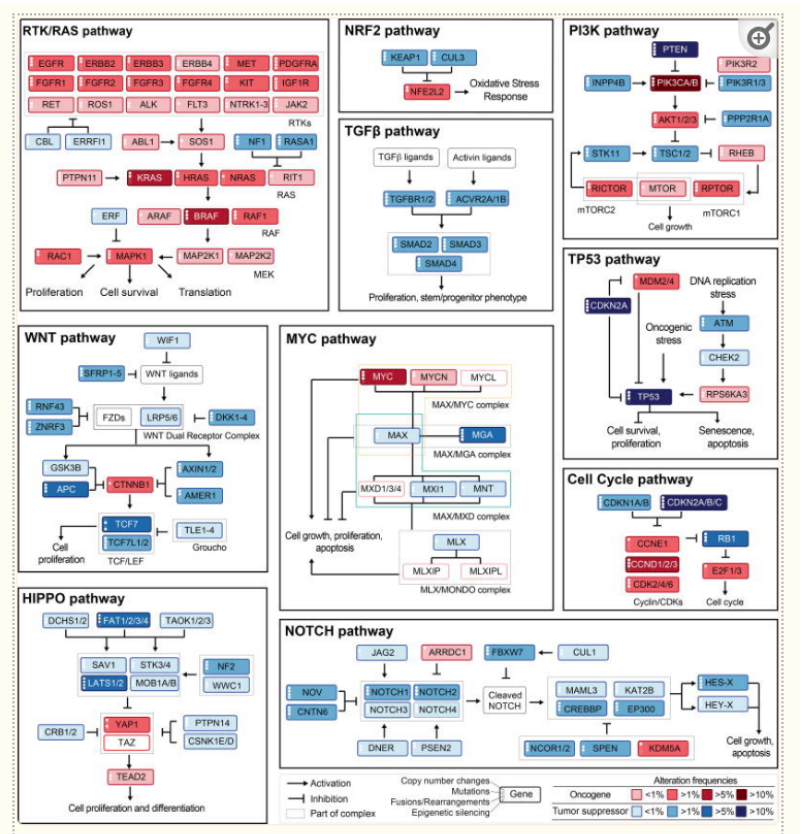
[1] n (%)

Summarize Oncogenic Pathways

2. **Limited Power To Detect Clinical Associations When Sparse Alterations**

- If biologically meaningful, you may want to summarize on Alteration, Gene or Pathway Level

Often we want to analyze alterations on the pathway level.



Summarize Oncogenic Pathways

2. **Limited Power To Detect Clinical Associations When Sparse Alterations**

- If biologically meaningful, you may want to summarize on Alteration, Gene or Pathway Level

{gnomeR} offers several default pathways that can be added with `add_pathways()`

```
1 paths <- gnomeR::pathways %>% names()
2 paths
```

[1] “RTK/RAS” “Nrf2” “PI3K” “TGFB” “p53”
[6] “Wnt” “Myc” “Cell cycle” “Hippo” “Notch”

Summarize Oncogenic Pathways

You can also add a custom pathway:

```
path_df <- gene_binary %>%
  add_pathways(custom_pathways = c("SPOP.mut", "FOXA1.mut"))

path_df %>% select("pathway_custom") %>%
  tbl_summary()
```

Characteristic	N = 241[1]
pathway_custom	2 (0.8%)

[1] n (%)

Note: You must specify `.mut`, `.Amp`, `.Del` for alterations `custom_pathways`

Summarize Alteration Pathways

```
path_df <- gene_binary %>%
  select(-naaccr_sex_code) %>%
  add_pathways()

path_df %>%
  select(contains("pathway")) %>%
  tbl_summary() %>%
  bold_labels()
```

Summarize Alteration Pathways

```r
path_df <- gene_binary %>%
  select(-naaccr_sex_code) %>%
  add_pathways()

path_df %>%
  select(contains("pathway")) %>%
  tbl_summary() %>%
  bold_labels()
```

Characteristic	N = 241[1]
pathway_RTK/RAS	217 (90%)
pathway_Nrf2	48 (20%)
pathway_PI3K	114 (47%)
pathway_TGFB	34 (14%)
pathway_p53	181 (75%)
pathway_Wnt	51 (21%)
pathway_Myc	70 (29%)
pathway_Cell cycle	90 (37%)
pathway_Hippo	31 (13%)
pathway_Notch	72 (30%)

[1] n (%)

Comparing Alteration Frequencies Across Clinical Data

We can easily compare frequencies by sex using the by argument:

```
tbl_gene <- gene_binary %>%
  subset_by_frequency(
    t = .4,
    other_vars = naaccr_sex_code) %>%
  tbl_genomic(by = naaccr_sex_code) %>%
  bold_labels()

tbl_gene
```

Comparing Alteration Frequencies Across Clinical Data

We can easily compare frequencies by sex using the by argument:

```
tbl_gene <- gene_binary %>%
  subset_by_frequency(
    t = .4,
    other_vars = naaccr_sex_code) %>%
  tbl_genomic(by = naaccr_sex_code) %>%
  bold_labels()

tbl_gene
```

Characteristic	**Overall**, N = 241[1]	**Female**, N = 145[1]	**Male**, N = 96[1]
TP53	124 (51%)	72 (50%)	52 (54%)
GBA.Amp	17 (46%)	8 (36%)	9 (60%)
Unknown	204	123	81
KRAS	98 (41%)	66 (46%)	32 (33%)
JAZF1.Amp	15 (41%)	9 (41%)	6 (40%)
Unknown	204	123	81

[1] n (%)

Comparing Alteration Frequencies Across Clinical Data

1. **Multiple Testing and False Positives**

```
gene_binary %>%
  subset_by_frequency(
    t = .4,
    other_vars = naaccr_sex_code) %>%
  tbl_genomic(
    by = naaccr_sex_code) %>%
  bold_labels() %>%
  add_p() %>%
  add_q()
```

Characteristic	Overall, N = 241[1]	Female, N = 145[1]	Male, N = 96[1]	p-value[2]	q-value[3]
TP53	124 (51%)	72 (50%)	52 (54%)	0.5	0.7
GBA.Amp	17 (46%)	8 (36%)	9 (60%)	0.2	0.3
Unknown	204	123	81		
KRAS	98 (41%)	66 (46%)	32 (33%)	0.059	0.2
JAZF1.Amp	15 (41%)	9 (41%)	6 (40%)	>0.9	>0.9
Unknown	204	123	81		

[1] n (%)

[2] Pearson's Chi-squared test

[3] False discovery rate correction for multiple testing

Comparing Alteration Frequencies Across Clinical Data

```
tbl_gene <- gene_binary %>%
  subset_by_frequency(
    t = .4,
    other_vars = naaccr_sex_code) %>%
  tbl_genomic(by = naaccr_sex_code) %>%
  bold_labels() %>%
  add_p() %>%
  add_q()

tbl_gene
```

Characteristic	Overall, N = 241[1]	Female, N = 145[1]	Male, N = 96[1]	p-value[2]	q-value[3]
TP53	124 (51%)	72 (50%)	52 (54%)	0.5	0.7
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JAZF1.Amp	15 (41%)	9 (41%)	6 (40%)	>0.9	>0.9
Unknown	204	123	81		

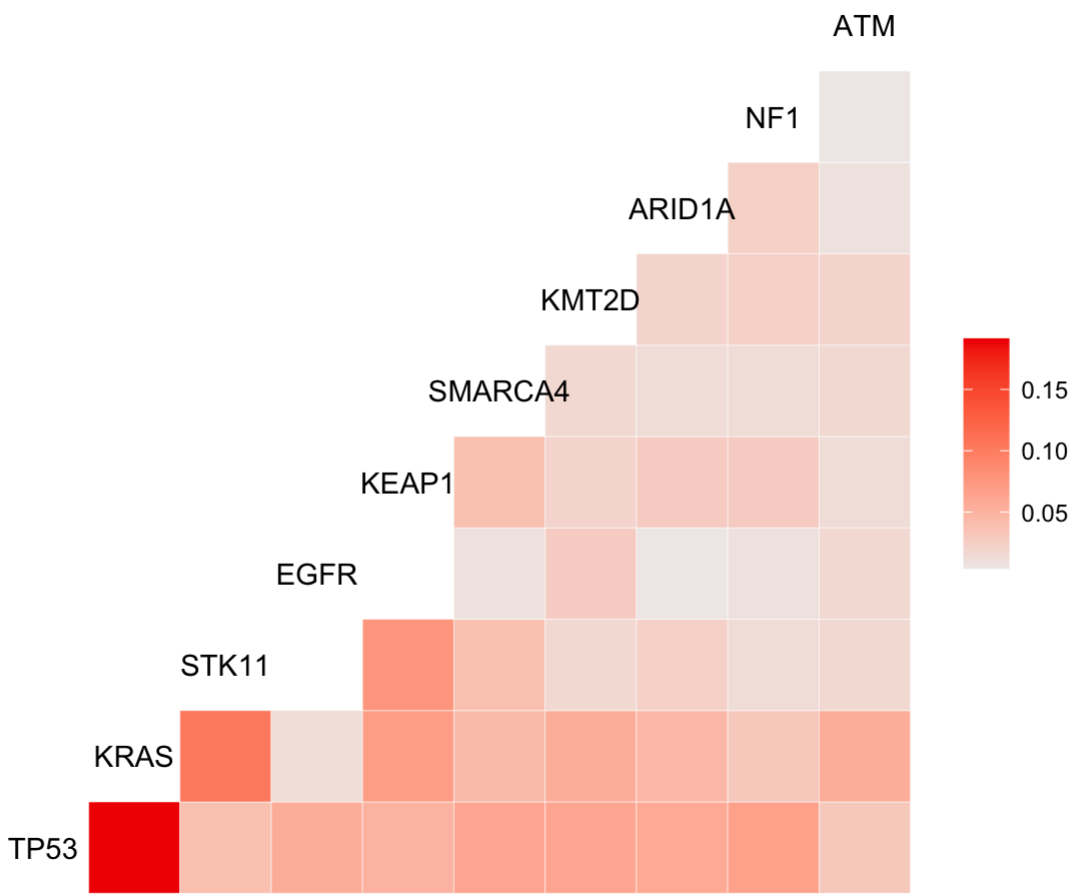
[1] n (%)

[2] Pearson's Chi-squared test

[3] False discovery rate correction for multiple testing

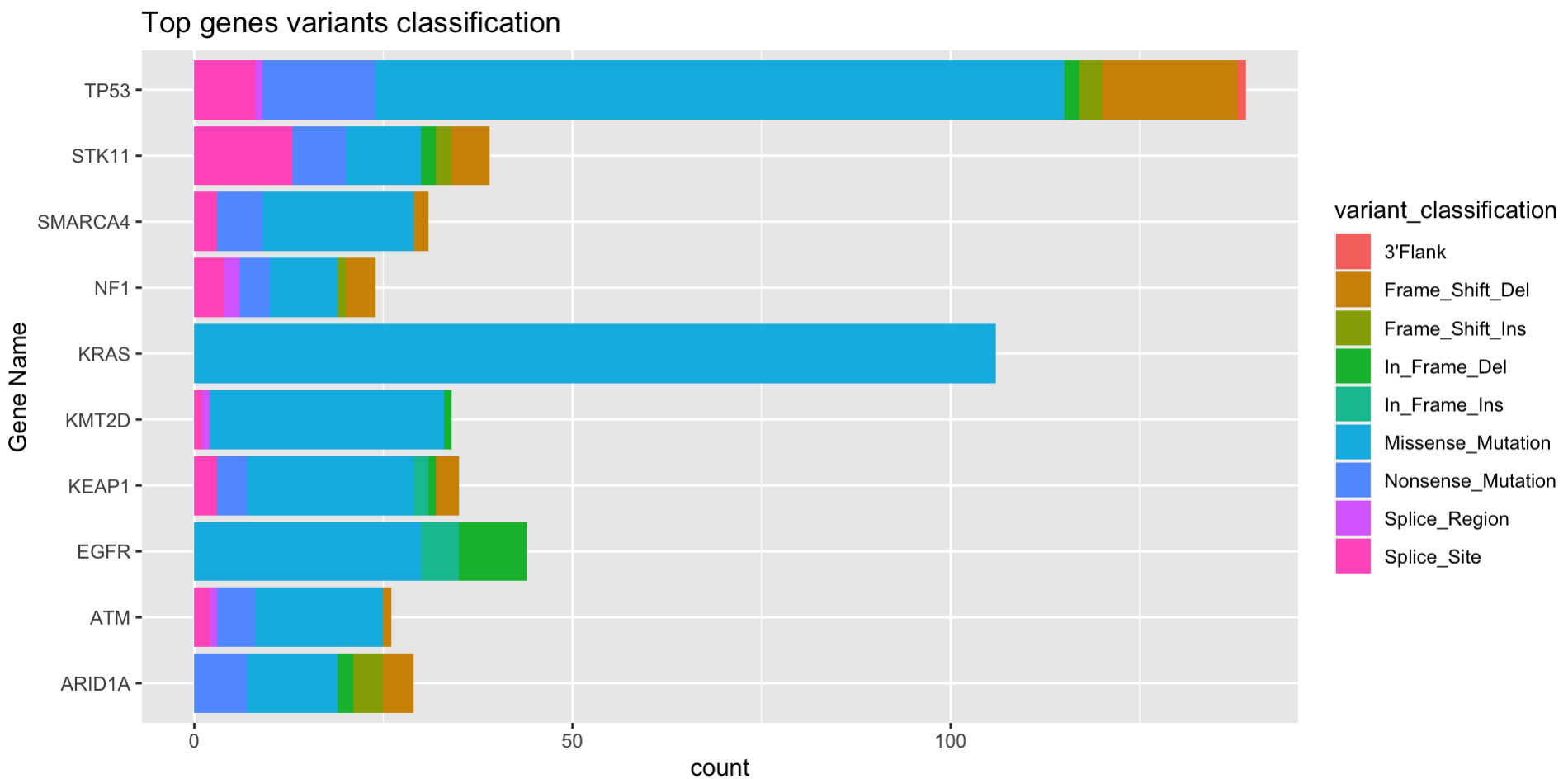
Visualize Data

```
1  gnomeR::ggcomut(nsclc_cohort$cohort_mutations_extended)
```



Visualize Data

```
1  gnomeR::ggtopgenes(nsclc_cohort$cohort_mutations_extended)
```



Additional Items & Next Steps

- Additional visualizations and color palettes useful for genomic data are available in {gnomeR} package
- Some data may require additional data checks. See {gnomeR vignette} for helpful tips on data QA.
- It may be appropriate to **oncoKB annotate** your data and only analyze oncogenic mutations (see oncoKB.org for more information)
- Some projects may utilize CNA Segmentation data. See {gnomeR documentation} for more information on available tools.

Thank You!