

Building on our discussion about how Mitoxantrone induces senescence in lung cancer cells and enhances LUNX-targeting therapy, I will now focus on another key point of this drug: how it regulates gene expression to impact the immune system and inflammation.

#### Slide 1: MA Plot

This is the MA plot, which gives us a global view of gene expression patterns between DMSO and Mitoxantrone conditions.

- The X-axis shows the average expression levels (logCPM), while the Y-axis represents fold changes (logFC).
- Each dot represents a gene:
  - Black dots indicate no significant changes.
  - Red dots show genes with significant upregulation or downregulation.

To better assess their significance, we turn to the Volcano plot.

#### Slide 2: Volcano Plot

The Volcano helps us to identify genes that play significant roles in Mitoxantrone's effects.

- The X-axis shows fold changes (log<sub>2</sub>FC), while the Y-axis represents significance (-log<sub>10</sub> p-value).
- Gray dots are non-significant genes, while:
  - Red dots are significantly upregulated genes.
  - Blue dots are significantly downregulated genes.

This plot helps us to identify genes that play significant roles in Mitoxantrone's effects. With these genes identified, we moved on to analyze their biological functions.

#### Slide 3: GOrilla Analysis

We used the GOrilla tool to analyze under-expressed genes. This diagram shows the relationships between different molecular functions, starting from broad categories and narrowing down to specific ones.

1. At the top level, we see Molecular Function, which covers all possible gene functions.

2. Moving down, Binding refers to the interaction between molecules. This is a fundamental function for gene products like proteins. It includes two more specific subcategories: protein binding (where the gene product binds to a protein, such as signaling proteins to initiate a response), and signaling receptor binding (it refers to binding to a receptor, which is crucial for signal transmission between cells).
3. Another branch: Molecular Function Regulator: this category shows that these genes may regulate molecular activities. As we go deeper into this branch, we see receptor regulatory activity (these genes might regulate the activity of receptors, such as turning signaling pathways on or off) and receptor ligand activity (these genes might encode ligands, which bind to cell surface receptors and activate or inhibit specific signaling pathways).
4. Following the arrows, this leads us to Cytokine activity. Cytokine activity is the most specific function in this pathway. It refers to the ability of certain genes to produce cytokines, small proteins that are critical for cell communication in the immune system.

#### **Slide 4: Linking Mitoxantrone to Cytokine Activity**

Many of the downregulated genes in our analysis are linked to cytokine activity.

Here is a paper which aligns with our findings, according to Dr. Edward Fox's 2004 paper in *Neurology*:

- “Mitoxantrone suppresses the proliferation of T cells, B cells, and macrophages. It impairs antigen presentation and decreases the secretion of pro-inflammatory cytokines.”

#### **Slide 5: A Dual Role for Mitoxantrone**

Mitoxantrone demonstrates a dual role:

1. Inducing Senescence, as discussed in earlier sections.
2. Regulating Immunity by altering gene expression, reducing inflammation, and enhancing therapy.

These findings highlight its potential as a comprehensive strategy for treating cancer.