# Team 1 Summary Document

### **Compare results of pathway analysis from gene expression data and CIBERSORT analysis.**

We compared the EnrichR results from the Reactome database with our Cibersort findings to identify both overlapping and non-overlapping pathways. The overlapping pathways were linked to immuno-oncology, reflecting changes in immune cell types observed in our Cibersort results. In contrast, the non-overlapping pathways were associated with cancer biology.

**Key Findings:**

* **Overlapping Pathways:**
  + **RHO GTPase Signaling:** Supports immune cell migration; aligns with decreased macrophages M2 and CD4 memory T cells in pre-cancer samples.
  + **Adaptive Immune System:** Reduced CD4 memory T cells and CD8 T cells reflect immune suppression, linked to bladder cancer progression.
  + **TP53 Pathways:** Dysregulated cell cycle and apoptosis associated with tumor progression and immune evasion.
* **Non-Overlapping Pathways:**
  + **Mitotic Cell Cycle:** Highlights cancer-specific mechanisms like unchecked proliferation.
  + **STING-Mediated Response:** Suggests unique innate immune activation changes.

**Immune Cell Shifts:**Pre-cancer samples show decreased resting immune cells (e.g., CD4 T cells, macrophages M2) and increased regulatory/activated cells (e.g., Tregs, gamma delta T cells), suggesting immune evasion.

### **Find pathways from Step 2 that are relevant to CIBERSORT results**

**How did we pick pathways?** We first looked at Cibersort results, trying to understand what changed the most or stayed the same when comparing normal with precancerous. Then we each picked a pathway in Reactome (that was a top hit) and is related to that specific immune cell type.

| Pathway / GO category | Analysis Method | Biological Relevance | Links to Literature |
| --- | --- | --- | --- |
| **MHC Class II Antigen Presentation (R-HSA-2132295)** | I was interested in finding a pathway associated with T cells CD4 memory resting because we determined that the average fraction of this cell type changed the most (decrease of **0.08137** or about a **39%** reduction) from normal to precancerous. This pathway is essential to CD4+ memory T cell responses and was a top hit in our Reactome results (combined score of 67.19). | MHC (Major histocompatibility complex molecules) Class II molecules play a critical role in immune response; they bind and present antigenic peptides to CD4+ T cells, which recognize the antigen, thereby initiating an immune response. With regards to immunoncology, some tumors express these molecules, which allows for the immune system to recognize and identify the presence of the tumor and initiate an immune response. | https://aacrjournals.org/clincancerres/article/25/8/2392/82426/Biological-Consequences-of-MHC-II-Expression-by;[**https://www.frontiersin.org/journals/cell-and-developmental-biology/articles/10.3389/fcell.2022.757137/full**](https://www.frontiersin.org/journals/cell-and-developmental-biology/articles/10.3389/fcell.2022.757137/full)**;**<https://pmc.ncbi.nlm.nih.gov/articles/PMC6314495/#:~:text=MHC%20class%20II%20binds%20antigenic,cells%20and%20T%20helper%20cells> |
| **Cell Cycle, Mitotic R-HSA-69278** | This pathway is related to cell division, and problems with cell division can result in cancer or recurrence. This pathway has association with cancers, and could be a link to our immune cells. It was the first in our Reactome list (combined score of 171.39). | Mitosis is a part of the function of most cells, including immune cells. Cyclins and Cyclin-dependent kinases have important roles in the cell cycle, and problems with these proteins can lead to tumor suppressing functions not working. Cancer cells may then perform mitosis out of control. This pathway relates to the regulation of these proteins and properly maintaining the mitosis of the cell cycle. | 1. [Relation between cell cycle mitosis and cancer](https://www.ncbi.nlm.nih.gov/books/NBK563158/) 2. [Pathway Description](https://pubchem.ncbi.nlm.nih.gov/pathway/Reactome:R-HSA-69278) 3. [Relation between mitosis and reccurence of bladder cancer](https://www.sciencedirect.com/science/article/pii/S0046817718303952?via%3Dihub) |
| **RHO GTPase Effectors R-HSA-195258** | This pathway is associated with both macrophages and T cells, whose average fractions decreased from normal to precancerous stages. It ranked among the top pathways in the Reactome analysis, with a high combined score of 79.12. | Rho GTPases play a central role in various cellular processes, including cell proliferation, survival, and migration, and their overexpression is frequently observed in cancers. RHOA, a key member of this family, is crucial in innate immune cells such as macrophages, neutrophils, and dendritic cells, where it facilitates migration and pathogen internalization in response to infection. In adaptive immunity, RHOA regulates T-cell development in the thymus and T-cell migration, highlighting its broad functional significance in both cancer and immune responses. | 1. [Rho GTPase signaling in cancer progression and dissemination | Physiological Reviews](https://journals.physiology.org/doi/full/10.1152/physrev.00045.2020) 2. [High expression of spindle assembly checkpoint proteins CDC20 and MAD2 is associated with poor prognosis in urothelial bladder cancer | Virchows Archiv](https://link.springer.com/article/10.1007/s00428-013-1473-6) |
| **Toll-like Receptor Cascades (R-HSA-168898)** | I observed a significant increase in the mean proportion of activated dendritic cells among immune cells when comparing normal cell types (0.014) to tumor cell types (0.050), reflecting a +0.036 increase. According to bladder cancer literature, the tumor microenvironment is characterized by immune suppression and evasion. Since dendritic cells (DCs) serve as a critical link between the innate and adaptive immune responses, playing a key upstream role in immune activation, I explored relevant Reactome pathways from my EnrichR analysis. This led me to the ‘Toll-like Receptor Cascades’ pathway, which directly involves dendritic cell activation. | Dendritic cells (DCs) play a central role in the Toll-like Receptor (TLR) Cascades, but some tumors take advantage of DC’s by persistently stimulating their TLR and driving them to a tolerogenic DC (tDC) phenotype.  Tolerogenic dendritic cells disrupt the TLR Cascades by inhibiting pro-inflammatory responses, suppressing antigen presentation, and promoting regulatory T cells. This can enable tumor progression and immune evasion. | 1. [Dendritic Cells in the Cancer Microenvironment](https://pmc.ncbi.nlm.nih.gov/articles/PMC3564245/) 2. [Accumulation of plasmacytoid DC: Roles in disease pathogenesis and targets for immunotherapy](https://onlinelibrary.wiley.com/doi/full/10.1002/eji.201040602) 3. [ICOS-Ligand Expression on Plasmacytoid Dendritic Cells Supports Breast Cancer Progression by Promoting the Accumulation of Immunosuppressive CD4+ T Cells](https://aacrjournals.org/cancerres/article/72/23/6130/577542/ICOS-Ligand-Expression-on-Plasmacytoid-Dendritic) |

### **Describe how you worked together as a team and the contributions of each member.**

Our team worked together by holding a meeting to review the results from EnrichR and CIBERSORTx. During the meeting, we agreed on a clear plan for selecting pathways that matched the assignment instructions. We divided the tasks among team members and set priorities to ensure everything was covered. To stay organized, we created a shared folder to store all relevant documents. We kept open communication throughout the process, regularly sharing progress updates and offering support to one another. This collaborative approach ensured that all perspectives were considered, and each member contributed their strengths, leading to a well-coordinated effort to efficiently complete Step 4 of the project.

**Individual Contributions:**

* **Natalie:** Created the **Average Bar Graph** and provided analysis for the **“MHC Class II Antigen Presentation (R-HSA-2132295)”** pathway.
* **Stephen:** Created the **Line Graph** and provided analysis for the **“Cell Cycle, Mitotic (R-HSA-69278)”** pathway.
* **Austin:** Created the **Stacked Bar Graph** and provided analysis for the **“Toll-like Receptor Cascades (R-HSA-168898)”** pathway.
* **Fadwa:** Provided analysis for the **“RHO GTPase Effectors (R-HSA-195258)”** pathway, compared the pathway analysis from gene expression data and CIBERSORT analysis and organized the **summary document**.

### **Describe what challenges you faced and how you overcame them**

One of the main challenges we faced was determining whether to focus on immune cells with the greatest change in proportion from normal to tumor cell type, or to focus on immune cells with the highest proportions in the tumor cell type. As a compromise, we divided tasks among the group: half of the team focused on immune cells with the greatest change in proportion, while the other half focused on those with the highest overall proportion in the tumor cell type.