# **Identifying Genetic Biomarkers for Bladder Cancer – DGE, Enrichment Analysis and CiberSortX**

1. Instructions and Problem Statement

### Overall Objectives of the Project

Exploring and analyzing molecular changes at different stages of bladder cancer in comparison to baseline tissue.

### Group 4 Specific Tasks:

Using Stage-1 Non-Invasive bladder cancer as the baseline, perform the following steps:

1. Gene expression comparisons to generate a list of significantly differentially expressed genes as follows:
   * Compare Stage-2 Invasive samples against Stage-1 Non-Invasive samples.
   * Compare Stage-1 Non-Invasive samples against Precancerous Stage samples.
2. Downstream Enrichment Analysis to:
   * Generate a list of significantly enriched pathways for the comparison between Stage 2 Invasive and Stage 1 Non Invasive samples
   * Generate a list of significantly enriched pathways for the comparison between Stage 1 Non Invasive and Precancerous samples
3. CIBERSORT-powered Immuno-Oncology Analyses:
   * Determine the top 5 types of Immune Cells in Pre Cancer vs Stage 1 group of samples based on fraction averages.
   * Determine the top 5 types of Immune Cells in Stage 1 vs Stage 2 group of samples based on fraction averages.
4. Final Analysis and Visualizations
   * Create graphs based on cibersort results
   * Comparison of results of pathway analysis from gene expression data and cibersort results
   * Generate a table with selected pathways and selected gene ontology categories
   * Explore relevance of findings to bladder cancer with focus on mechanisms of recurrence.

## Comparative Analysis and Visualization of Results

**We analyzed Stage 1 Non-Invasive bladder cancer against Stage 2 Invasive Bladder Cancer.**

### 2.1 Visualizations Based on CiberSort Results for Bladder Cancer Stage 1 Non-Invasive and Stage 2 Invasive Tumor

The team used R to create the following graphs:

* Average Bar Graph
* Stacked Bar Graph comparing stage 1 tumor with stage 2
* Line Graph comparing stage 1 tumor with stage 2

### 2.2 Comparison of Significant Pathways against Significant Immune Cells in Bladder Cancer Stage 1 Non-Invasive and Stage 2 Invasive Tumor

Began by identifying the main pathways of interest in the Reactome and Gene Ontology databases. We shortlisted the following pathways:

* Assembly Of Collagen Fibrils And Other Multimeric Structures R-HSA-2022090
* Extracellular Matrix Organization R-HSA-1474244
* Positive Regulation Of Apoptotic Process (GO:0043065)
* Positive Regulation Of Programmed Cell Death (GO:0043068)

On the other end, we identified the top immune cells highly expressed from the Cibersort analysis based on their fraction averages. These included:

* Regulatory T cells
* Macrophages
* Resting Memory T cells
* Mast Cells

Once main pathways were selected, and the immune cells of interest were identified, we found research articles relating the pathway to bladder cancer. Further, we investigated the connection between selected immune cells and the biological pathway. Our main findings are as follows, which are explained in greater detail in the excel file attached:

* **Collagen Fibrils Pathway and their connection with T Cells:**
  + We found that the collagen network becomes denser and more disorganized in invasive cancer, contributing to a stiffer extracellular matrix, which facilitates tumor cell invasion and metastasis. This altered microenvironment supports cancer progression by promoting cell motility and enhancing invasive behavior. [[1]](https://pmc.ncbi.nlm.nih.gov/articles/PMC5347718/)
  + Relevant Immune Cells that interact with this pathway: T-cells
  + The hypothesized connection between T cells and Collagen function: Research indicates that higher collagen density suppresses T cell infiltration and function by creating a physical barrier and altering mechanical signaling. This impairs immune-mediated tumor eradication, emphasizing the role of extracellular matrix remodeling in modulating anti-tumor immunity. This could potentially contribute to the development of tumor from non-invasive form to invasive. [[2]](https://jitc.bmj.com/content/7/1/68.citation-tools)
* **Extracellular Matrix Organization and its relevance to the function of Neutrophils/ Macrophages:**
  + It is studied that the extracellular matrix is made up of collagen, laminins, and a variety of other proteins. In bladder cancer, the tumor is trying to invade and therefore attacks the ECM. As the cancer progresses and the tumor attempts to invade, the composition of the ECM can change. [[3]](https://pmc.ncbi.nlm.nih.gov/articles/PMC2717820/)
  + Relevant immune cells that may interact with this pathway: Macrophages M2 or Neutrophils.
  + Hypothesis on the connection between Macrophages/Neutrophils and Extracellular Matrix Organization: When the ECM is damaged (potentially by a tumor), neutrophils work and can pull parts of undamaged ECM to the wound site. Macrophages can produce collagen to rebuild ECM scaffolding. They work through receptor-mediated uptake and degradation of collagen. [[4]](https://www.science.org/doi/10.1126/science.abp8964)
* **Positive Regulation of Apoptotic Process and Connection with Mast Cells/Macrophages**
  + There is evidence that certain apoptosis genes can be related to developing bladder cancer. Defects in apoptosis can be part of many disease processes including autoimmune diseases, neurodegenerative diseases, cancer, heart disease, and more. Positive regulation of apoptosis means cells are dying at a higher rate than maybe they should. This increase in apoptosis makes sense as bladder cancer invasion occurs. From stage 1 to stage 2. [[5]](https://pmc.ncbi.nlm.nih.gov/articles/PMC10577908/)
  + Relevant Immune Cells: Mast Cells and Macrophages
  + Research suggests that Mast cells may release a chemical when activated that promotes apoptosis. When functioning correctly, the mast cells kill the tumor cells, but if not working properly just promotes further inflammation. This allows the tumor to suppress other immune cells.
* **Programmed Cell Death and Relation with Mast Cells and Macrophages:**
  + This biological process is essentially the same as the one above it and the same articles will apply. Defects in the positive regulation of this process can become factors in accelerated aging and death of cells. This increases the risk of invasive forms of tumor. [[6]](https://pmc.ncbi.nlm.nih.gov/articles/PMC10577908/) [[7]](https://pmc.ncbi.nlm.nih.gov/articles/PMC8445627/)

This holistic analysis will give us a good foundation of what to include in our presentation, but further research must be done. In particular, we would need to explore the relevance of these pathways, and immuno-oncology functions to explore and understand the **recurrence** of bladder cancer particularly, even after surgical removal.

## References

[[1]](https://pmc.ncbi.nlm.nih.gov/articles/PMC5347718/) Brooks, M., Mo, Q., Krasnow, R., Ho, P. L., Lee, Y. C., Xiao, J., Kurtova, A., Lerner, S., Godoy, G., Jian, W., Castro, P., Chen, F., Rowley, D., Ittmann, M., & Chan, K. S. (2016). Positive association of collagen type I with non-muscle invasive bladder cancer progression. *Oncotarget*, *7*(50), 82609–82619. <https://doi.org/10.18632/oncotarget.12089>

[[2]](https://jitc.bmj.com/content/7/1/68.citation-tools) Kuczek DE, Larsen AMH, Thorseth M*, et al*Collagen density regulates the activity of tumor-infiltrating T cells*Journal for ImmunoTherapy of Cancer* 2019;**7:**68. doi: 10.1186/s40425-019-0556-6

[[3]](https://pmc.ncbi.nlm.nih.gov/articles/PMC2717820/) Brunner, A., & Tzankov, A. (2007). The role of structural extracellular matrix proteins in urothelial bladder cancer (review). *Biomarker insights*, *2*, 418–427. <https://doi.org/10.4137/bmi.s294>

[[4]](https://www.science.org/doi/10.1126/science.abp8964) Tara E. Sutherland *et al.* ,The extracellular matrix and the immune system: A mutually dependent relationship.*Science***379**,eabp8964(2023).DOI:[10.1126/science.abp8964](https://doi.org/10.1126/science.abp8964)

[[5]](https://pmc.ncbi.nlm.nih.gov/articles/PMC10577908/) Zou, Z., Li, Z., Sun, W., Gao, W., Liu, B., Liu, J., & Guo, Y. (2023). Establishment of prognostic model of bladder cancer based on apoptosis-related genes, in which P4HB promotes BLCA progression. *BMC urology*, *23*(1), 167. <https://doi.org/10.1186/s12894-023-01331-5>

[[6]](https://pmc.ncbi.nlm.nih.gov/articles/PMC10577908/) Zou, Z., Li, Z., Sun, W., Gao, W., Liu, B., Liu, J., & Guo, Y. (2023). Establishment of prognostic model of bladder cancer based on apoptosis-related genes, in which P4HB promotes BLCA progression. *BMC urology*, *23*(1), 167. <https://doi.org/10.1186/s12894-023-01331-5>

[[7]](https://pmc.ncbi.nlm.nih.gov/articles/PMC8445627/) Yang, Z., Xu, Y., Bi, Y., Zhang, N., Wang, H., Xing, T., Bai, S., Shen, Z., Naz, F., Zhang, Z., Yin, L., Shi, M., Wang, L., Wang, L., Wang, S., Xu, L., Su, X., Wu, S., & Yu, C. (2021). Immune escape mechanisms and immunotherapy of urothelial bladder cancer. *Journal of clinical and translational research*, *7*(4), 485–500.