ANALYSIS OF IMMUNOLOGICAL ENVIRONMENT IN BLADDER CANCER USING MACHINE LEARNING ALGORITHM

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Abstract

Recent studies have demonstrated varying analyses of the presence of immune infiltrating cells in bladder cancer. CIBERSORT, a deconvolution machine learning (ML) algorithm, quantifies the fractions of cells from bulk tissue gene expression profiles (GEP). Comparing the different progressions of bladder cancer (i.e.: normal bladder, non-invasive bladder cancer, invasive bladder cancer, and normal-looking bladder mucosa surrounding cancer), the immune behavior across different stages can be evaluated. The behavior of the tumor infiltrating immune cells (TIICs) were largely illustrated through the enrichment of Macrophages M0, Dendritic Cells (DCs), and T cells regulatory (Tregs) in invasive bladder cancer. The behavior of such immune cells demonstrates potential for research into immunotherapy treatments (Geissman, 2004).

Key Words: Machine Learning, bladder cancer, immunotherapy, immune system

Introduction

Bladder cancer is the 10th most common form of cancer worldwide, with an estimated 549,000 new cases and 200,000 deaths (Xue, et. Al., 2019). The importance of evaluating the tumor microenvironment is increasingly prevalent as there are emerging innovations in immunotherapy. The TME consists of a variety of different cells, each that contributes to the growth or lack thereof of a tumor in bladder cancer. The main actors of focus in the process of the development of a tumor in invasive bladder cancer are macrophages, mast cells, Tregs, and dendritic cells (DCs).

The action of Tumor Infiltrating Immune Cells (TIICs) in the TME can largely be categorized into two branches: anti-tumor and pro-tumor. Anti-tumor components are capable of recognizing the tumor and working to destroy it, while-pro-tumor components can evolve to escape the natural defense that the immune system invokes. Evaluating the presence of each of these cells can provide information for immunotherapy targets. Because bladder cancer has a high mutational burden, immune checkpoint inhibitors can be investigated to look for a significant response in bladder cancer patients. (Patel et. Al, 2020)

MCs are activated after ligand binding via the Fcγ, complement and/or pathogen-associated molecular patterns (PAMP) receptors, releasing bioactive molecules such as histamine, proteases, lipid mediators, cytokines, and chemokines. These molecules are required for direct pathogen killing, recruitment of immune cells, increased angiogenesis, vascular permeability, and degradation of the injured tissue.[1] (Wu et. Al, 2020) Mast cells are typically part of the second line of defense in the immune system, wherein the release of histamines allows for the widening

of blood vessels in an inflammatory response.

DCs are constitutive residents of skin and mucous membranes that rapidly respond to microenvironmental signals, turning into mature DCs capable of antigen capture and cross-priming to naïve B and T lymphocytes. [2] (Wu et.al, 2020) DCs are key in the specific aspect of the immune system, wherein they are primed by tumor antigens for B-lymphocyte and T-lymphocyte reactions.

Classically activated (M1) macrophages are activated in response to a microenvironment enriched with Th1 cytokines (IFN-γ, GM-CSF, IL-12, ROI, RNI, iNOS, and CXCL10). Alternatively activated (M2) macrophages are formed in response to Th2 cytokines (IL-4, IL-10, IL-13, M-CSF, CCL2, CCL5, CCL22, and HIF-1α) and are characterized by the expression of JMJD3, arginase-1, YM, and FIZZ1 genes and secretion of IL-4, IL-10, and IL-13 upon activation, an expression/secretion profile more in tune with tissue remodeling activities. [3] (Wu et.al, 2020) Macrophages act as phagosomes, effectively absorbing pathogens after they are detected.

Tregs turn off inflammatory and humoral responses after the trigger signal has been eliminated, thus preventing chronic immune stimulation and autoimmunity (induce arrest in cell cycle of cytotoxic T cells and block DCs maturation, among many other functions ([4] [5] Wu et.al, 2020)

The history of the development of the treatment of bladder cancer is lacking due to its insufficient funding. The concept of immunotherapy dates back to the 19th and early 20th century wherein treatments such as vaccines, non-specific cytokines, and adoptive cell therapies were introduced by Wilhelm Busch, William B. Coley, and Paul Ehrlich. (Munhoz et.al, 2016).

In the early 2000s, bladder cancer was the most underfunded among the common cancers (Patel et.al, 2020). Because of such limited research, there were long periods in which little advancements were made in the treatment of bladder cancer. The development of immunotherapy targets dates back to the 1970s and 1980s wherein the initial testing for cisplatin, a form of chemotherapy, in a perioperative (pre-surgery) setting. Cisplatin continued to be used as a treatment for bladder cancer, with different variations of the treatment throughout time. For example, in the 1980s, cisplatin-based chemotherapy was first tested as a neoadjuvant therapy, a therapy that would shrink the tumor before the main treatment;

"Scher et al treated 50 patients with MIBC using 1 to 5 cycles of methotrexate, vinblastine, doxorubicin, and cisplatin (MVAC), and 30 subsequently underwent RC (radiation cystectomy). Among the patients who underwent RC, 33% achieved a pathologic complete response (pCR), and an additional 17% had downstaging of disease to less than a pathologic T2 (pT2) tumor classification with negative lymph node status (<pT2N0)" (11). Other combinations of radiation therapy and chemotherapeutic agents, such as cisplatin, paclitaxel, 5-FU, and mitomycin, have been explored. Treatment for bladder cancer has also advanced to involve dose-dense treatment, as opposed to standard-dose. However, over the last decade treatment for bladder cancer has shifted towards understanding the "genomic complexity" of bladder cancer and it's "responsiveness to current therapies." (Patel et.al, 2020) Of recent significance, the U.S. Department Of Human and Health Services released a National Cancer Plan on April 3, 2023. The plan, dubbed the Cancer Moonshot, develops a framework to "end cancer", as presented by President Joe Biden and First Lady Jill Biden. The plan is a continuation of the National Cancer Act of 1971, which determined the necessity of a national commitment to make progress in treatment for cancer. The plan involves eight goals to reduce the cancer death rate by at least half

in the next 25 years. The goals include preventing cancer, detecting cancers early, developing effective treatments, eliminating inequities, delivering optimal care, engaging every person, maximizing data utility, and optimizing the workforce. The goal for maximizing data utility is of particular significance to the CIBERSORT and genomic-based therapies. It enforces the secure maintenance of patient data as well as the collaboration across institutions with already available data. The plan notes that it will support the development of data visualization and analysis tools as well as the infrastructure to make them accessible to researchers. It continues in this vein to mention the utility of machine learning algorithms in identifying patterns in large, complex data sets, and the importance of training the ML algorithms on accurate, reliable data.

Large-sequencing and genomic analysis, such as CIBERSORT, allows for the further exploration of treatments for bladder cancer. The effect of CIBERSORT's calculation of fractional presence in the bladder cancer TME is twofold; firstly, the interaction between cells (positive/negative correlation between various cell types) can provide estimates for cell presence as a whole. Secondly, those TIICs demonstrating significantly high presences in the TME may be considered targets for immunotherapy (Chimal et.al, 2013). Ultimately, CIBERSORT serves as the initial computational analysis to jumpstart potential immunotherapies and research into the tumor infiltrating leukocytes.

Methodology

The CIBERSORT ML algorithm was used to quantify the relative fractional presence of TIICs in the aforementioned stages of bladder cancer. The Gene Expression Profiles (GEPs) used were from a public data set conducted by Korea Research Institute of Bioscience & Biotechnology. The study used 165 primary bladder cancer samples, 23 recurrent non-muscle invasive tumor

tissues, 58 normal looking bladder mucosa surrounding cancer, and 10 normal bladder mucosa for microarray analysis (17). For each gene expression profile (GEP) data in this study, a microarray analysis was performed. The GEP data set was downloaded and 500 genes were selected for analysis for each patient, with 1 row representing a patient, and the 500 columns representing each of those various genes. The data was uploaded and were compared against the CIBERSORT signature matrix with 1,000 permutations. The data was then compiled into XLS for analysis of linear regression trends, as well as comparison between the aforementioned stages of bladder cancer. Comparisons were determined between each stage of bladder cancer wherein the cells with the greatest magnitude in relative change were identified for further analysis. The absolute difference between the two values were evaluated and compiled to create bar graphs.

Results

In terms of macrophages, the transition from normal to pre-cancerous bladder tissue, the presence of macrophages (M0, M1, M2) remained relatively unchanged. Macrophages increased by a fraction of 0.012 and Macrophages M1 and M2 decreased by -0.01 and -0.02 respectively. In the comparison between NIMBC and a normal bladder, the specific macrophages began to split in their negative/positive trending nature. Macrophages M0 showed a significant positive change, with a 0.0259 increase (0.029 to 0.055). However, Macrophages M1 and M2, demonstrated a significant decrease with a 0.038 decrease (0.054 to 0.016) and 0.032 decrease (0.121 to 0.089), respectively. In the comparison between MIBC and normal bladder tissue, the patterns remained relatively the same. Macrophages M1 and Macrophages M2 demonstrated significant decreases of 0.027 (0.054 to 0.027) and 0.053 (0.121 to 0.068), respectively. Similar

to NMIBC, Macrophages M0 had a significant positive increase of 0.055 (from 0.029 to 0.084).

In terms of dendritic cells (DCs), the transition from normal to pre-cancerous bladder tissue, the DCs showed significant positive growth (top 50% of cells in the TME demonstrating an increase). The DCs began with a fraction of 0.014 and increased to 0.05, a 0.036 increase. In the comparison between normal and non-invasive bladder cancer, the dendritic cells demonstrated a significant increase, from 0.014 to 0.0125, a 0.111 increase. In the comparison between MIBC and normal bladder tissue, the increase was significant. In the transition from normal to pre-cancerous bladder tissue, the dendritic cells showed significant positive growth (top 50% of cells in the TME demonstrating an increase). ant and positive; the quantity of the increase is almost exactly that of the increase calculated by the comparison of NMIBC and normal bladder tissue. The DCs increased 0.116 (from 0.014 to 0.13). DCs showed the most significant positive change amongst all TIICS in the TME.

In terms of T cells regulatory (Tregs), the transition from normal to pre-cancerous bladder tissue, Tregs showed significant positive growth (top 50% of cells in the TME demonstrating an increase). The Tregs began with a presence of 0.03 and increased to 0.071 and an increase of approximately 0.04. In the comparison between NMIBC and normal bladder tissue, the proportion of Tregs was also enriched. The presence increased by 0.104 (from 0.031 to 0.135). In the comparison between MIBC and normal bladder tissue, Tregs also increased significantly, from 0.031 to 0.092 (a 0.061 increase).

In terms of mast cells, the transition from normal to pre-cancerous bladder tissue, mast cells began to show a negative trend. The initial fraction determined was 0.141, which decreased to 0.098, an approximate decrease of 0.043. Mast cells continued in their negative trend in their

comparison between NMIBC and normal bladder tissue, with a 0.082 decrease (0.141 to 0.059). In the comparison between MIBC and normal bladder tissue, mast cells resting, similar to NMIBC and pre-cancerous tissue, demonstrated significant negative change. From an initial value of 0.141, the presence of mast cells decreased to 0.08, a 0.061 decrease.

Discussion

Observing and comparing the presence of the DCs, MCs, Macrophages, and Tregs allows for a greater understanding of the behavior in the TME, particularly as clustered through the different stages of progressions of bladder cancer.

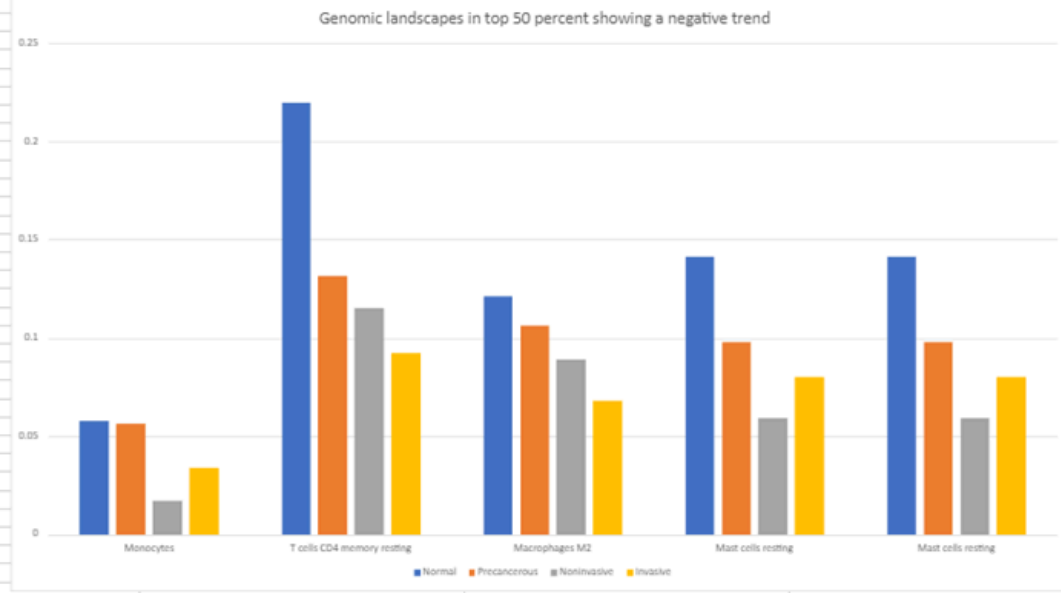

Figure 1: Genomic landscapes in top 50 percent showing a negative trend

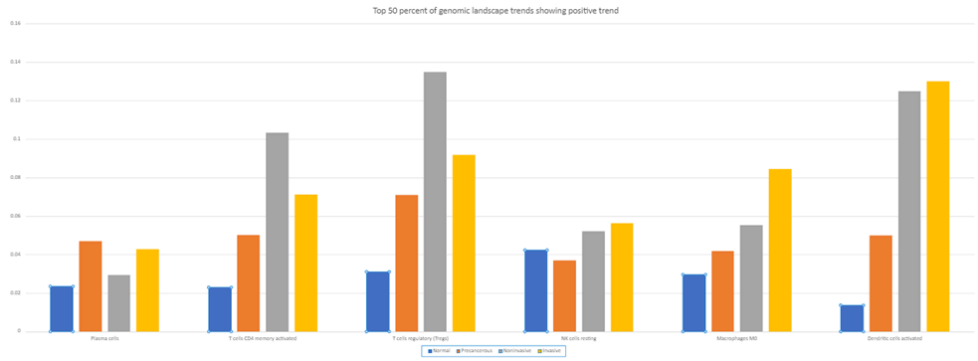

Figure 2: Genomic landscapes in top 50 percent showing a positive trend

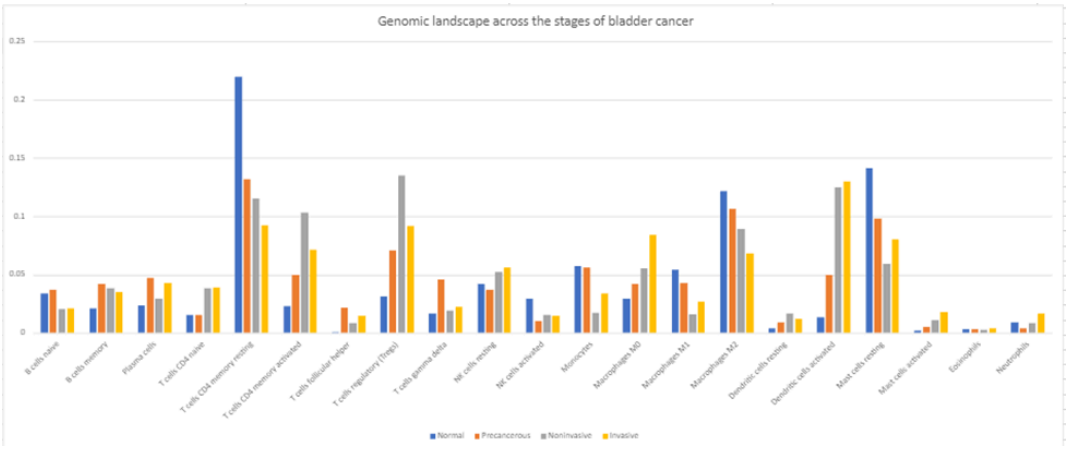

Figure 3: Genomic landscapes across all stages of bladder cancer

Macrophages M2, for example, meet the infections, and as a phagosome is supposed to facilitate the removal of the pathogen. M0 macrophages, alternatively, are the precursors for early-stage macrophages and are later differentiated into M1s and M2s. However, the Macrophages M0 evolved to become anti-tumor components. They are programmed by the tumor to induce cytokines that help the tumor to grow and inhibit other immune cells. As illustrated by Figure 2, the Macrophages M0 showed a significant positive change. In fact, the coefficient of determination when analyzing the trends of Macrophages M0 and Macrophages M2 was nearly perfect (-0.95 and 0.99) respectively. These strong linear trends seem to suggest correlation that

should be studied further. Microscopically, this is occurring because bladder cancer releases and delivers miRNAs which obstruct M1 phenotype polarization, drive M2 phenotype polarization, or switch phenotypes from M1 to M2 by binding to downstream targets or macrophages. In this same vein, bladder cancer with certain gene alterations may further allow for this recruitment and polarization of M2 macrophages. (Xue, 2019). It is therefore of the utmost importance to analyze data such as that of CIBERSORT to further understand the precise recruitment and polarization of M2 macrophages as illustrated through the data.

The increase in Tregs further demonstrated the cell behavior in the TME. Tregs inhibit T cell proliferation and cytokine production to prevent autoimmunity (Kondelkova et.al, 2010). In other words, Tregs work to inhibit the antitumor behavior that the Macrophages M0 proliferate. T cells are a relatively highly researched TIIC due to its high proportion (Peng, 2021). While some variety of T cells can have pro-tumor effects – such as CD8+ T cells and natural killer (NK) cells that mediate antitumor responses (allowing for a better OS) – Tregs secrete inhibitory cytokines (i.e.: TGF-Beta and IL-10) that perpetuate the inhibition of the anti-tumor effect. This behavior is particularly reflected in the data as the increase in the fractional presence of Tregs is associated with the progression of bladder cancer. With the presence of M2 macrophages, the immune system calls upon Tregs to release cytokines to inhibit the anti-tumor effect that the macrophages have initiated. In recent years, there has been an increase in immunotherapy targeting this specific anti-tumor immune pathway. For example, the 5-year OS rate from immunotherapy now surpasses 25% for patients with high programmed cell death protein ligand-1 (PD-l1) expression (tumor proportion score > 50%) (Peng et.al, 2021). T cells CD4+ memory also showed significant negative decrease – as a result of the inhibition pathways from Tregs. Studies in prognostic significance of various TIICs demonstrated that patients with higher infiltrating levels

of activated memory CD4+ T cells (as well as naïve B cells, gamma delta T cells, etc.) had longer OS with 95% confidence intervals. This prognostic evaluation is clearly reflected in the data. As bladder cancer progresses (indicating that the OS decreased), CD4+ T cells showed a decrease (Peng et.al, 2021).

Separate studies involving the analysis of bladder cancer data via CIBERSORT continue their research to include the association between the presence of TIICs and various genes. Two hub genes (CXCL12 and CD3E) were explored in terms of their correlation with TIICs. Hub genes are genes that have many interactions with other genes (Yu et. al, 2017). It was determined that naive B cells, resting mast cells, M2 macrophages were positively associated with CXCL12, while follicular helper T cells, resting dendritic cells, and activated dendritic cells were negatively associated with CXCL12 (Liu, et. al, 2021). Based on the data accumulated by CIBERSORT, those cells demonstrating a positive association with CXCL12 demonstrate an overall decrease in fractional presence. Alternatively, those cells that had a negative association with CXCL12 demonstrated an increase as the stages of bladder cancer progressed. The two correlations may indicate that CXCL12 is a potential gene of interest in future bladder cancer research.

Conclusion

Further analysis of the data can include the quantification of the presence of TIICs based upon the newfound “immunological shift” in cancer; that is that the process of cancer comprises at least five phases: immunosurveillance, immunoselection, immunoescape, oncotraining, and oncopromotion (Ramirez et.al, 2013). The first phase represents a functional immune system, requiring one to quantify what a “normal” immune system would look like. The second phase is

characterized by an equilibrium reached between tumor cells and immune cells. This analysis would require a specific differentiation between the tumor and immune cells analyzed by CIBERSORT. Oncotraining is more difficult to analyze simply by looking at the quantification of the presence of the TIICs. The phase is defined by a shift of immune cells from anti- to pro-tumoral activities, allowing for tumor growth, or oncopromoting.

CIBERSORT's ML algorithm is the foundational computational analysis for future biological research. It allows for the expansion into various elements of bladder cancer, such as the differentiation of Macrophages M0 into Macrophages M1 and M2 throughout the stages of bladder cancer. Equipped with the data from the computational analysis, researchers can look at the actual TME to determine the specific microscopic interactions within the cell. It is also of possible consideration to explore the association with certain genes and the presence of TIICs. For example, Caldesmon (CALD1), may become a promising biomarker of BCLA (YH Du, 2020). To continue in this line of genomics would allow for a greater variety of tests to serve as partial diagnoses, as well as a more specific line of immunotherapy treatment.

Abbreviations:

NIMBC: Non-Muscle Invasive Bladder Cancer; MIBC: Muscle Invasive Bladder Cancer; OS: Overall Survival; BLCA: Bladder cancer; MCs: Mast Cells; DCs: Dendritic Cells; T cells Regulatory: Tregs; ML: Machine Learning; AI: Artificial Intelligence;

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Presentation:

1. 18-20 min presentation for individual and mentor
2. Intro will include career path, job title, and what I do to work w/ the mentor
3. Senior advisor must be present on the 15th of May
4. Mentors don't have to be in-person
5. Reflection paper on the 31st

Gusev notes:

1. Look @ graphs, make them more specific
    a. Change the names of the graphs, design of graphs (font-size → increase font-size)
    b. Graphs should be readable
2. Title is good
3. Presented information about different types of cells
4. XLS = formatted file in excel → fix the abbreviation
5. Fix mechanism methodology → "absolute difference"
6. Unique property of bladder cancer is that there are always macrophages present in normal bladder → bladder tissue evolved to have first response army of macrophages for immediate response to pathogens
    a. Residual macrophages in bladder tissue → find another source
7. Add sections for different clusters of bladder cancer?
8. **Mast cells aren't often seen in the immunological landscape**
9. Group cells that show the same trend → make graphs more specific (ones that are on the same cell)
    a. Make font different color
    b. Fractional cell presence of tumor infiltrating immune cells in bladder cancer, sorted by magnitude of change
    c. 50 percent isn't statistically sound
    d. Choose the ones that are obvious (keep 4/6)
10. Weren't sure what was going on with the M2 and M0
11. "Genomic" to "immunological"
12. Make one big slide in powerpoint (add more figures, more visuals)
    a. Diagram of CIBERSORT

KEY WORDS/PROCESSES:

- *Cytotoxicity:* the toxicity caused due to the action of chemotherapeutic agents on living cells (quality of being toxic to cells)
- *Phagocytosis:* recognition and ingestion of particles larger than 0.5 micrometers into a plasma membrane derived vesicle known as phagosome (i.e.: macrophages)
- *Cytolysis (osmotic lysis):* pathologic dissolution or disintegration of cells, occurs when a cell bursts due to an osmotic imbalance that has caused excess water to diffuse into the cell
- *Cytokines:* proteins that are crucial in controlling the growth and activity of other immune system cells and blood cells
- *Chemokines:* stimulates the migration of cells (mainly leukocytes), help maintain the homeostasis of the immune system, attract leukocytes to areas of inflammation

NOTES:

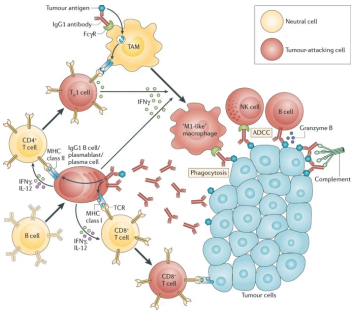
1. <u>Tumor-infiltrating M2 macrophages driven by specific genomic alterations are associated with prognosis in bladfder cancer:</u>
    a. "Additionally, M2 Macrophages accounted for the majority proportion of all subtypes of infiltrating macrophages in bladder cancer…which indicated that the infiltration of M2 Macrophages may provide a good estimate of the overall macrophage population in bladder cancer." (589)
    b. Also explored the different M2 macrophage infiltration levels among different gene mutation types → "specific mutations may influence M2 macrophage infiltration"
    c. Tumor infiltrating M2 Macrophages were most predominant in the "basal" subtype of cancer
    d. "Novel immunotherapies targeting M2 Macrophages or their combination with immune checkpoint inhibitors may present promising results in the treatment of bladder cancer and other types of cancer (43, 44)"
    e. M2 macrophages might stimulate angiogenesis
    f. "Bladder cancer also affects macrophages by releasing and delivering miRNAS, which may impede the M1 phenotype polarization, drive M2 phenotype

polarization of macrophages, or switch phenotypes from M1 to M2 by binding to downstream targets or macrophages (9, 52, 53)"

g. Bladder cancer with "specific gene alterations may promote the recruitment and polarization of M2 macrophages in the TME via exosomal miRNAs"
h. Exosomes are membrane vesicles released by cells into extracellular environment that regulate biological activities of target cells

2. Identification of gene expression profiles and immune cell infiltration signatures between low and high tumor mutation burden groups in bladder cancer:
    a. "We obtained a list of TMB related genes which may influence the infiltrations of immune cells. We also found a higher proportion of CD8 T cells, CD4 T cells and NK cells in the high TMB group."
    b. "The results shown that tumors with high TMB were significantly associated with high fractions of CD8 T cells, CD4 memory T cells, follicular helper T cells and resting NK cells. In low TMB group there is a higher fraction of mast cell"
    c. "Chemokines and cytokines are well known to guide macrophages, T-cells and other immune cells to the tumor microenvironment and influence the outcome of the patients 32. CXCL10 may play an important role in regulating immune cell migration, differentiation, and activation in bladder cancer."
    d. "These data indicated that TMB can affect the immune cell infiltration signatures and high TMB attracted effector cells of the immune system. Tissue resident memory T cells are a key factor in making tumors dormant; hence, it is essential to establish a cancer-immune system balance (34)."
3. Profiles of Immune Infiltration in Bladder Cancer and its Clinical Significance: an Integrative Genomic Analysis:
    a. The fractions of M0 and M1 macrophages were higher in the tumor tissues than in the normal tissues, whereas the fractions of naive B cells and resting mast cells were significantly lower in the tumor tissues (Figure IB)
4. The cancer‐associated fibroblasts related gene CALD1 is a prognostic biomarker and correlated with immune infiltration in bladder cancer:
    a. Moreover, BLCA represents a growing number of cancers characterized by the infiltration of a significant number of immune cells in the tumor microenvironment (TME) [4, 5], making it suitable for immunotherapy.
    b. " Therefore, through these bioinformatics means, the present study uncovered and validated that Caldesmon 1 (CALD1), a key gene associated with CAFs, was crucial in regulating both the stromal and immune microenvironment of BLCA. Consequently, it may become a promising biomarker of BLCA progression."
5. Prognostic significance of tumor-infiltrating immune cells in muscle-invasive bladder cancer - PMC
    a. Furthermore, patients with higher infiltrating levels of naive B cells, gamma delta T cells, follicular helper T cells, CD8+ T cells, activated memory CD4+ T cells,

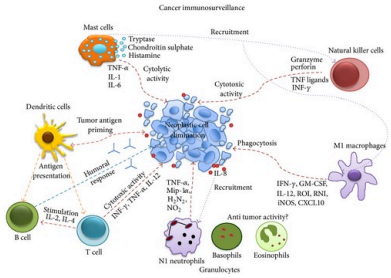
and plasma cells had longer OS (the HRs of 22 subtypes of TIICs for OS are shown with 95% confidence intervals [CIs] in Figure S2).

6. B cells, plasma cells and antibody repertoires in the tumour microenvironment | Nature Reviews Immunology
    a. Recent data show that B cells and plasma cells located in tumors or in tumor-draining lymph nodes can have important roles in shaping antitumor immune responses.
    b. T cells and B cells interact and undergo cooperative selection, specialization and clonal expansion.
    c. Plasma cells are present in tumor infiltrates, and even low counts of these cells are able to produce large amounts of cytokines (20)
    d. T cells and thereby shape antigen-specific immune responses within the tumor microenvironment (24,25,26)
    e. 

7. Recent advances in understanding antitumor immunity - PMC
    a. The term “antitumor immunity” refers to innate and adaptive immune responses which lead to tumor control.
    b. Despite the recent advances with immune checkpoint-directed approaches, the concept of “immunotherapy” dates back to the 19 th and early 20 th century with Wilhelm Busch, William B. Coley, and Paul Ehrlich and comprises distinct strategies, including vaccines, non-specific cytokines, and adoptive cell therapies (1)
8. Protumor Activities of the Immune Response: Insights in the Mechanisms of Immunological Shift, Oncotraining, and Oncopromotion - PMC
    a. Experimental and clinical studies indicate that cells of the innate and adaptive immune system have both anti- and pro-tumor activities.
    b. The tumor microenvironment shifts immune cells to perform functions more in tune with the tumor needs (oncotraining); these functions are related to chronic inflammation and tissue remodeling activities. Among them are increased proliferation and survival, increased angiogenesis and vessel permeability, protease secretion, acquisition of migratory mesenchymal characteristics, and self-renewal properties that altogether promote tumor growth and metastasis (oncopromotion).

c. This duality displayed by the immune system has led to the concept of an "immunological shift" in cancer, in which immune and transformed cells interact in a dynamic process comprising at least five phases: immunosurveillance, immunoselection, immunoescape, oncotraining, and oncopromotion.
    i. The first phase represents a functional immune system engaging in protective functions that successfully eliminates aberrant/malignant cells
    ii. In the second phase, an equilibrium is reached between tumor cells and immune cells; this phase depends on the mutational rate of the transforming cell, which creates rapidly proliferating clones, resistant to death and/or self-renewal capacities; thus, the immunosurveillance function is incapable of eliminating all aberrant cells and instead selects clones with increasingly tumorigenic properties.
    iii. The immunologically shaped tumor clones take advantage of some immune functions to create a microenvironment in which immune cells are switched from anti- to pro-tumoral activities, in a collective mechanism referred to as oncotraining.
    iv. Together, these processes result in an oncopromoting phase favoring tumor growth, local invasion, and metastasis.

d. Among the anti-tumor activities found in the tumor microenvironment are cytotoxicity mediated by CD8+ T and NK cells, phagocytosis by M1 macrophages, cytolysis induced by mast cells, and humoral responses by B cells. Dendritic cells are primed by tumor antigens, which are then presented to T and B cells for adaptive responses.



e.

f. Dendritic cells (DCs), macrophages, and mast cells (MCs) constitutively reside in physiologically normal tissues acting as sentinels that monitor the microenvironment in search of stress signals; when tissue homeostasis is compromised they release cytokines, chemokines, reactive oxygen species (ROS), and bioactive mediators, which among many other functions induce mobilization and infiltration of other leukocytes to the injured site in the process of inflammation.
    i. In an inflamed tissue, innate immune cells perform diverse and redundant tasks when activated; for instance, both MCs and granulocytes release

their preformed granules to kill or inactivate invasive agents, while macrophages, neutrophils, and DCs carry out phagocytosis.

g. Mast cells: MCs are activated after ligand binding via the Fcγ, complement and/or pathogen-associated molecular patterns (PAMP) receptors, releasing bioactive molecules such as histamine, proteases, lipid mediators, cytokines, and chemokines. These molecules are required for direct pathogen killing, recruitment of immune cells, increased angiogenesis, vascular permeability, and degradation of the injured tissue.
h. Neutrophils: Although neutrophils' half-lives are only of a few hours, they survive much longer in an inflammatory microenvironment. Like MCs, this lineage protects against invading microorganisms and assists in wound healing through releasing of a wide variety of effector molecules stored in cytoplasmic granule
i. Macrophages:
    i. Classically activated (M1) macrophages are activated in response to a microenvironment enriched with Th1 cytokines (IFN-γ, GM-CSF, IL-12, ROI, RNI, iNOS, and CXCL10).
    ii. Alternatively activated (M2) macrophages are formed in response to Th2 cytokines (IL-4, IL-10, IL-13, M-CSF, CCL2, CCL5, CCL22, and HIF-1α) and are characterized by the expression of JMJD3, arginase-1, YM, and FIZZ1 genes and secretion of IL-4, IL-10, and IL-13 upon activation, an expression/secretion profile more in tune with tissue remodeling activities.
j. Dendritic cells: constitutive residents of skin and mucous membranes where they rapidly respond to microenvironmental signals, turning into mature DCs capable of antigen capture and cross-priming to naïve B and T lymphocytes.
    i. DCs collect tumor antigens either from phagocytized tumor cells or through a direct mechanism of capture from living tumor cells; hence they are essential initiators of anti-tumor adaptive immune responses.
    ii. Upon activation, CD8 cytotoxic T cells (CTLs) directly eliminate tumor cells while CD4 T helper cells (Th) stimulate B cells supporting both humoral and cytotoxic responses.
k. Tregs: turn off inflammatory and humoral responses after the trigger signal has been eliminated, thus preventing chronic immune stimulation and autoimmunity (induce arrest in cell cycle of cytotoxic T cells and block DCs maturation, among many other functions

9. Development of monocytes, macrophages and dendritic cells - PMC
    a. Dendritic cells initiate and regulate the highly pathogen-specific adaptive immune responses, and are central to the development of immunologic memory and tolerance.
    b. The mononuclear phagocyte system represents a subgroup of leucocytes originally described as a population of bone marrow-derived myeloid cells that

circulate in the blood as monocytes and populate tissues as macrophages in the steady state and during inflammation (1).

i. The discovery of dendritic cells (DCs) as a distinct lineage of mononuclear phagocytes, specialized in antigen presentation to T cells and the initiation and control of immunity (2), revealed additional roles of these cells in shaping the immune response to pathogens, vaccines and tumors, as well as additional heterogeneity.

c. Monocytes: Monocytes represent immune effector cells, equipped with chemokine receptors and pathogen recognition receptors that mediate migration from blood to tissues during infection.

i. They can also differentiate into inflammatory DCs or macrophages during inflammation, and possibly, less efficiently, in the steady state.

d. The development of the mononuclear phagocyte system is controlled by cytokines - small secreted proteins that promote cell-cell communication and can act as growth and differentiation factors.

10. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries
    a. Bladder cancer is the 10th most common form of cancer worldwide, with an estimated 549,000 new cases and 200,000 deaths (Table 1).
11. Treatment of muscle‐invasive and advanced bladder cancer in 2020 - Patel
    a. On the basis of its high mutational burden, immune checkpoint inhibitors were investigated in advanced bladder cancer, revealing durable responses in a subset of patients.
    b. In the early 2000s, bladder cancer was the most underfunded among the most common cancers in terms of National Institutes of Health funding
    c. Long periods of limited funding stifled research, contributed to a limited understanding of bladder tumor biology, and ultimately led to insufficient progress in treatment (Fig. 1).
    d. Ongoing activities have focused on improved application of therapies through the development of predictive biomarkers and rational combination regimens.
    e. Initial testing of cisplatin in the perioperative setting was based on the pioneering work in the 1970s and 1980s that established its activity in metastatic UC (mUC). [24-28]
    f. Neoadjuvant cisplatin-based chemotherapy was first tested in the 1980s as a potential treatment strategy for MIBC. Scher et al treated 50 patients with MIBC using 1 to 5 cycles of methotrexate, vinblastine, doxorubicin, and cisplatin (MVAC), and 30 subsequently underwent RC. Among the patients who underwent RC, 33% achieved a pathologic complete response (pCR), and an additional 17% had downstaging of disease to less than a pathologic T2 (pT2) tumor classification with negative lymph node status (<pT2N0).

g. Another strategy to overcome toxicities related to standard-dose MVAC is administration in a dose-dense fashion (ddMVAC).
h. Over the last decade, next-generation sequencing technologies have facilitated several large-scale analyses to uncover the genomic complexity of UC and understand its responsiveness to current therapies.
    i. Notably, cisplatin forms DNA crosslinks that interfere with DNA replication and gene transcription, and tumors with impairment in DNA repair mechanisms may be more vulnerable to cisplatin.46, 47
i. Several different chemotherapeutic agents, including cisplatin, paclitaxel, 5-FU, and mitomycin, have been explored in combination with RT (Radiation Therapy) for MIBC

	Overall function	Function in BCLA
Plasma cells	● Plasma is the "fourth state of matter, a partially ionized gas composed of ions, electrons, and neutral particles." ● Helps body recover from injury, distribute nutrients, remove waste, and prevent infection while moving through circulatory system	● Novel treatment called Cold atmospheric plasma ● The isotype and specificity of the antibodies produced by plasma cells can drive distinct immune responses. (6) ○ Antibodies are specific to the B-cell that has stimulated the plasma cells, antibodies travel through plasma ● B-lymphocytes are responsible for humoral immunity → plasma can confer immunity ● Plasma cells are present in tumor infiltrates (cancer that has spread beyond 1 layer of tissue), and are able to produce

		large amounts of cytokines (6)
T cells CD4 memory activated		
Tregs		• T-cell activation relies on the interaction of the T-cell receptor with antigens presented as peptides through the major histocompatibility complex (MHC) by the APC. (6)
Macrophages M0		
Dendritic cells activated	• Dendritic cells are primed by tumor antigens, which are then presented to T and B cells for adaptive responses	•
Monocytes		
Macrophages M2		
Mast cells resting		
T cells CD4 memory resting		• B cells can present antigens to CD4 and CD8 T cells, creating antigen-specific immune responses within TME (6)

Immune System:

- Skin, mucous membranes, stomach acid = **first line of defense** (innate, generally respond, non-specific)
- **Second line of defense - inflammatory response:**
    - Mast cell assists with allergic and inflammatory responses, releases histamine to widen blood vessels

    - Macrophages: consume pathogens
    - Bring fluid, blood, etc. to "fight"
    - Phagocytes: class of cell that can eat up pathogens, has receptors to respond to things that it knows are bad, wraps around pathogen → when fully engulfed, the system is called a "phagosome"
        - Phagocyte takes the peptide chain and attach them to other proteins, connecting them to Major Histocompatibility Complex (MHC II)
            - Neutrophils: fast and numerous responders
            - Macrophages: versatile, heavy listing
            - Dendritic: best activators of the specific immune system
- Complement system: complements action of the immune system (works w/ specific or nonspecific)
- **Specific line of defense: adaptive immunity**
    - Cell-mediated:
        - Cytotoxic T cells, ability to destroy cells through releasing signals
        - Releases protein called perforin, causing holes in the cell membrane
        - Cytotoxic T cells activated by pathogens latching on
        - When macrophages consumes pathogen, process pathogen and go to macrophage's surface, causing T helper cells to bind, stimulating cytotoxic T cells
        - Helper T cells: activate cytotoxic t-cells
    - Humoral
        - Helper T cells can stimulate B (comes from bone marrow) cells which can have membrane-bound antibodies (tend to be in a y-shape)
        - In the development of B-cells, there is a shuffling of their DNA that leads to the diversity in the antibodies on the membrane of B-cells
        - Antibodies are generally very specific to the B-cell, can also be activated by free antigens
        - Memory cells (store memory of pathogen/antigen they were exposed to), activate helper T cells
        - Antibodies bind to the epitope of an antigen
    - Main actors are lymphocytes (a form of white blood cells)
        - Divided into B lymphocytes and T lymphocytes (B cells and T cells)
            - B-cells have membrane bound antibodies each with its own district variable proportion
                - Needs to bind pathogen and usually need to be stimulated by the helper T cell
                - Presents on MHC II complex, activated helper T cell that is specific to B-cell will activate

    - Once activate, starts cloning → turns into effector cells (turn into antibody making machines → "plasma cells") or memory cells (protects against future)
- All T-cells have t-cell receptors and have CD4 proteins or CD8 proteins
- CD4 receptor is what wants to go to MHC II complexes (most are helper T-cells and bind to B cells)
    - Differentiate into effector and memory cells → effector activates B cells and releases cytokines
- CD8 receptors bind to MHC 1 complex (most of the time they are cytotoxic), cell needs to die
    - CD8 effector mode = kill cells