### Title

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# Role of B cells in Responses to Checkpoint Blockade Immunotherapy and Overall Survival of Cancer Patients

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## Abstract

The role of B-cells in the tumor microenvironment and B-cell mediated anti-tumor immune responses remains relatively understudied. Recent seminal studies have discovered that B-cells and associated tertiary lymphoid structures (TLS) correlate with responses to checkpoint blockade immunotherapy and are prognostic for overall survival of cancer patients. B-cell subsets have remarkable functional diversity and include professional antigen presenting cells, regulatory cells, memory populations, and antibody-producing plasma cells. Importantly secreted antibodies can independently activate innate immune responses and induce the cancer immunity cycle. Thus, B-cells and B-cell mediated antibody responses comprise the largely underappreciated second arm of the adaptive immune system and certainly deserve further attention in the field of oncology. Here, we review the known functions of B-cells in the tumor microenvironment, the contribution of B-cells to the anti-tumor activity of immunotherapies, and the role of B-cells in the overall survival of cancer patients.

## Introduction

B cells and the antibodies they produce constitute the humoral arm of the adaptive immune system. With the widespread adoption of immune-modulating cancer therapies, our understanding of the role immune cells play in combating cancer has increased dramatically. However, significantly more attention has been focused on T-cells and myeloid cells when compared to B-cells. B cells have remarkable functional diversity and a wide range of subsets. For example, B1 B cells, which have a distinct developmental pathway and largely function independent of T cells, are outside of the scope of this review and are reviewed elsewhere.[1] B2 B cells, which are often referred to as “conventional” B cells, will be the focus of this review and will be referred to as B cells.

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In this review, we will first provide a brief overview of B cell development, differentiation, and key functions that form the basis of understanding B cell biology. We will then discuss B cells in the context of tumor immunology, outlining critical B cell subsets present in the tumor microenvironment, with a focus on tertiary lymphoid structures (TLS), plasma cells, and regulatory B cells. We will then review the role of B cells in the response to checkpoint blockade immunotherapy, in light of recently published seminal studies identifying a role for TLS and B cells in patient outcomes. Overall, this review will summarize our existing understanding of B cell biology integrating fundamental immunologic principals with application to immuno-oncology, as it is becoming clear that B-cells play a critical role in tumor biology (Figure 1).

## Introduction to B cell development

B cells recognize antigens through the B cell receptor (BCR), which is composed of immunoglobulin segments, specifically a light chain and heavy chain pair.[2] The BCR is structurally identical to an antibody, and this unique feature allows B cells to recognize native three-dimensional antigen structures, which is very different from the processed epitopes presented on MHC that T-cells recognize. Under normal physiology, there are two central goals of B cell development: 1) eliminate or alter B cells that recognize self-antigens, and 2) generate a diverse repertoire of B cells capable of recognizing a wide range of foreign antigens.

B cells are derived from a common lymphoid progenitor (CLP) residing in the bone marrow, hence the name B cells (Figure 2). Under the influence of bone marrow stromal cells, maturation of the B cell begins with random rearrangements of the D and J segments of the heavy chain[3]. By default, the heavy chain is of the mu isoform, which encodes for an IgM immunoglobulin subtype. The cell next undergoes V-DJ rearrangement to form a pre-BCR, which is critical for survival and selection of large pre-B cells. Importantly, during both D-J and V-DJ arrangement, non-templated nucleotides are added at the junctions, further increasing diversity.[4] Upon subsequent V-J rearrangement of a kappa light chain to pair with the heavy chain, the cell is now an immature B cell expressing a IgM BCR. After an immature B-cell undergoes alternative splicing of the BCR transcript to express surface IgD, it is then termed a mature B cell, which can migrate to secondary lymphoid organs (SLOs), such as lymph nodes, spleen, and tonsils, amongst others (Figure 2).[5]

## B cell function, antibody production, and antigen presentation

The most well-known function of B cells is to produce antibodies which are secreted into blood, lymph, saliva, cerebrospinal fluid, and other bodily fluids or humors, hence the term humoral immunity (Figure 1). Antibodies are predominantly generated by plasma cells and can be produced during the primary as well as secondary immune responses. During a primary immune response, IgM antibodies are the first subtype that is produced; IgM-expressing B cells can undergo class-switch recombination (CSR) to form different antibody subtypes (IgG, IgE, IgA). Also unique to B-cells is the process of somatic hypermutation (SHM), during which nucleotides of the V regions of heavy and light chain genes are mutated at a remarkably high frequency in order to significantly increase the affinity of an antibody.

Importantly, the different antibody subtypes have different effector functions, analogous to an archer using different types of arrows.[6] IgM is a pentamer which allows for binding of multiple antigens, fixation, and initiation of the complement system. IgG is the most common subtype, with multiple subclasses termed IgG1, IgG2, IgG3 and IgG4, vary primarily in the Fc region and ability to activate Fc-receptor mediated antibody phagocytosis and antibody dependent cellular cytotoxicity (ADCC). IgE is predominant in hypersensitivity reactions and is unique in its high affinity binding to (Fcε-RI) on basophils and mast cells. IgA is actively transported and secreted into mucosal bodily fluids and plays a key role in mucosal immunity. Using this modular approach and class switching, a B cell specific for a given epitope can keep the same Fab fragment and specificity while swapping out the Fc region to best neutralize a given antigen. Careful attention should be paid to these distinct subclasses and the Fc region during antibody drug development, as this can have profound effects on the immunological effects as well as clinical activity of monoclonal antibody drugs.[7]

During a primary response, a subset of activated B cells will follow a pathway in which they enter a B cell follicle, rapidly proliferate, and form a germinal center (GC), in order to undergo SHM and CSR, and ultimately differentiate into long-lived plasma cells and memory B cells. Germinal center formation is the hallmark of an adaptive B-cell immune response and GC development can be seen in SLOs and more recently in the tumor itself as a tertiary lymphoid structures (TLS). Thus it is not surprising that TLS were recently correlated with responses to checkpoint blockade immunotherapy as they represent a distinct hallmark of an adaptive immune response.[8–10]

There is a fair amount known regarding the complex dynamics involved in GC formation.[11] Of particular importance are T follicular-helper (T-FH) cells, which provide key signaling interactions with activated B cells, and follicular dendritic cells (FDCs).[12] Canonically, GCs have two distinct zones: a dark zone (DZ) containing a dense network of B cells and reticular cells, and a light zone (LZ) featuring T-FH cells, FDCs, macrophages, and B cells. In the DZ, B cells undergo rapid proliferation and SHM, while in the LZ, B cells possessing high-affinity BCRs are selected for and undergo CSR.[13] B cells containing higher affinity BCRs will receive more help from T-FH cells in the form of paracrine cytokines and growth factors. This competition for limited growth factors leads to a process termed affinity maturation during which the highest-affinity B cells are positively selected to re-enter the DZ to undergo successive rounds of proliferation, culminating in clonal selection of the B cell clone harboring the highest affinity BCR.[14] Ultimately, upon exiting the GC, B cells can differentiate into antibody-producing plasma cells or memory B cells, which remain quiescent state but can rapidly differentiate upon re-challenge.[15]

Overall, identification of unique markers corresponding to differential downstream activity of memory B cells and plasma cells will be crucial to understanding how these specific cell subsets respond to tumor antigens. Along these lines, our group recently reported in this journal that single B-cell markers, namely CD19 or IgJ, the latter being highly expressed in plasma cells, were highly prognostic for 3 year overall survival of patients with head and neck and cervical squamous cell carcinomas.[16] Interestingly we found that CD19 expression level in the tumor was a stronger predictor for overall survival than either CD8 or CD45

expression levels, supporting the independent prognostic capacity of B-cell biomarkers in survival of head and neck cancer patients and illustrating the powerful role that B-cells play in outcomes of cancer patients.[16]

## Role of B Cells in the tumor microenvironment and their effect of patient prognosis

In the tumor microenvironment, tumor-infiltrating B cells (TIL-B) can be widely identified by their expression of CD19 or CD20. A number of studies have now correlated the effect of TIL-B on patient outcomes and have demonstrated a heterogeneous effect based on specific tumor anatomic site, histology, and molecular subgroup – selected studies are highlighted in Table 1.[16–23] In many clinical and human studies, high expression of B-cell markers is correlated with significantly improved outcomes.[16–19,21] Yet, other clinical studies, and mouse models depleting B cells with anti-CD20 antibody or genetic approaches, have reported either pro-tumorogenic or non-significant roles of B cells.[24–26] Specifically, Damsky et al., found that B cell content was not associated with either overall survival or response to anti-PD-1 in patients with melanoma.[27] When subsequently evaluating murine models of colon carcinoma (MC38) and melanoma (YUMMER1.7), the investigators found that B cell depletion using anti-CD20 antibodies did not impair anti-tumor responses. In contrast, Griss et al., concluded that tumor-associated B cells are critical for maintaining Intratumoral inflammation in melanoma, as B cell depletion was associated with decreased CD4+ and CD8+ T cells.[28] The authors specifically identifying plasmablast-like cells as critical in dictating the immune microenvironment, and importantly, high expression of a gene signature associated with this cell subset was associated with improved survival.

Our interpretation is that these seemingly discrepant results reflect the marked heterogeneity of TIL-B present in the TME and the oversimplification when characterizing or depleting “B cells” as a single population. The analogy would be the reporting of discrepant results in different models based upon depleting “T cells,” but failing to account for differential activity of T cells subsets such as CD8 effector T-cells or CD4 T-regulatory cells. Additionally, some tumors may be dominated by regulatory B cells which may inhibit anti-tumor immunity, while other tumors may be dominated by effector B cells or plasma cells secreting anti-tumor antibodies. Furthermore, one major limitation of mice genetically engineered to be constitutively depleted of B-cells is skewed homeostatic proliferation of T-cell compartments, so that these B-cell deficient mice are more akin to studying T-cell expanded mice. In recognition of these complexities, three subsets of B cells have been the focus of significant research and will be discussed in more detail: Germinal center B cells associated with tertiary lymphoid structures (TLS), plasma cells, and regulatory B cells (Bregs).

Tertiary lymphoid structures refer to ectopic lymphoid structures that can form in the presence of chronic antigen exposure and can occur in many pathophysiologic conditions.[29] TLSs are characterized as discrete aggregations of lymphoid and myeloid cells, and in their most mature form, result in formation of GCs that mirror those found in SLOs as discussed above (Figure 1). TLSs can be identified immunohistologically, primarily by identifying colocalization of CD3+ and CD20+ cells; additional markers such as DC-LAMP, CD21, CD23, and MECA79 can be added to identify mature dendritic cells and high endothelial

venules to gauge maturation of these TLSs.[8,9] TLSs can additionally be inferred using a gene signature as discussed further below. Focusing on B cells, GC B cells in TLSs adopt a highly proliferative phenotype undergoing SHM and CSR in response to antigens in the TME, with successive differentiation into plasma cells and memory B cells.[30] This tumor-antigen-specific B cell response is exemplified by the presence of highly clonal B cell populations - a result of the GC reaction.[31] In addition, close proximity of B cells and T cells allow for reciprocal B cell help as they can adopt an APC phenotype to aid CD8+ T cells. In fact, a meta-analysis of the prognostic effect of T cells on outcomes has shown more dramatic effects when B cells or plasma cells are also present.[32]

An excellent study analyzed the expression of a 12-chemokine gene signature, which included chemokines vital for GC functioning, across multiple cancer types and demonstrated significant differences both across different tumor histologies and within the same subtype, that can improve patient outcomes; this review also demonstrated how there is a propensity for, but not guarantee of, TLS formation depending on tumor biology.[30] This also raises the question of what triggers TLS formation and crystalizes the need for further investigation into TLS and TLS-mediated modulation of the TME.

One of the critical pathways in which plasma cells are generated is following GC maturation. As antibodies are the principal product of a B-cell mediated response, understanding the effect of endogenous anti-tumor antibody production is of paramount importance. However, it is critical to stress that antibodies do not only have the potential to disrupt their binding target but serve as a scaffold for activation of the complement system, as well as flagging a cell or antigen for further interrogation. Thus, even if the first antibody binding to a tumor cell is not inherently neutralizing or cytotoxic, it can lead to development of a broader immune response against other epitopes expressed by that tumor cell, in a process termed epitope spreading.[33] As a key illustrative example, patients with metastatic castration-resistant prostate cancer were treated with sipuleucel-T, which targets prostatic acid phosphatase, and serum IgG titers against multiple diverse antigens were measured. Remarkably, patients with diverse humoral IgG reponses measured by upregulation of three secondary antigens had significantly improved overall survival.[34] Therefore, epitope spreading may be crucial for tumor eradication because a tumor has less chance of developing resistance to a diverse, multifaceted immune response.

Given stringent selection against self-antigens during B cell development, it is thought that the majority of tumor-specific antibodies recognize mutated self-antigens, representing neo-antigens, or abnormally expressed ‘cancer’ antigens. As such, high IgG1 expression in the TME is associated with non-silent mutation burden in lung cancer.[35] Additionally, antibodies against known cancer antigens can be detected in many patient’s serum; for example, MUC1 is a well-characterized cancer antigen, and antibodies against MUC1 can be detected in the early stages of other cancers, and is generally associated with an improved prognosis.[36] Interestingly, antibody isotype can differentially impact prognosis; in both breast cancer, IgG, but not IgM, recognizing MUC1 was predictive of survival.[36]

For viral-induced malignancies, B cells that recognize specific viral antigens can be identified; a recent excellent analysis by Wieland, et al., carefully analyzed B cells present

in the TME in HPV-related head and neck cancer.[37] This study defined four distinct clusters of B cells in the TME: 1) activated B cells, 2) antibody-secreting cells, 3) germinal center B cells, and 4) transitory cells, which adopted a phenotype intermediate of antibody-secreting cells and germinal center B cells. Importantly, this study demonstrated that antibody-secreting cells, when isolated, produced antibodies that bound E2 and E6, key HPV viral proteins, opening the avenue for selectively targeting antibody-secreting cells to amplify downstream effects of the antibodies in mounting an anti-tumor humoral response. However, the correlation between patient outcome and the presence of antigen-specific B cells is unknown, and therefore further investigation into their prognostic value in not only head and neck cancer, but also other viral-mediated malignancies, is certainly warranted.

More broadly, regulatory B cells are those B cell subsets that have an immunosuppressive activity, and themselves are comprised of a heterogeneous group of cells that are not limited to plasma cells. Many discrete subsets of regulatory B cells have been found to secrete IL-10, and these include CD24(hi)CD27+, CD19+CD24(hi)CD38(hi), CD19+CD27+IgM+, amongst others.[38] Additionally, IL-35, lymphotoxin, and granzyme B produced by B cells possess regulatory effects. In contrast to its tumoricidal ability from CD8+ T cells, granzyme B produced by B cells activated by IL-21-secreting regulatory T cells may be transferred to T cells, and degrade a portion of the TCR to ultimately suppress T cell activity.[26] Plasma cells can also have immunosuppressive abilities, exemplified by IgA+ plasma cells in the tumor microenvironent.[24] In castration-resistant prostate cancer, resident IgA+ plasma cells expressing PD-L1 and IL-10 were identified to mediate resistance to oxaliplatin by inhibiting CD8+ cytotoxic T cell activity.[24] It is unclear if IgA expression is necessarily involved in the regulatory capacity of these plasma cells, or if they are a product of high TGF-beta levels in the TME, as TGF-beta is not only a potent immunosuppressive cytokine highly secreted in the TME, but is also a mediator for CSR for IgA antibodies. Paradoxically, antibodies themselves have demonstrated the direct ability to promote tumor spread in rare cases. For example, in breast cancer, an antibody produced by tumor-infiltrating B cells recognizing HSPA4 was associated with poor prognosis and was shown to promote metastasis to regional lymph nodes by activating the NF-kB pathway.[39] Indirectly, antibodies can form immune complexes that activate myeloid cell populations to trigger chronic inflammation, tissue remodeling, and angiogenesis, ultimately leading to disease progression.[40] Taken together, these data highlight the powerful and independent roles that antibodies themselves can play after being produced by B-cells or when given as immuno-modulating drugs.

## Role of B-cells in responses to checkpoint blockade immunotherapy

Given that B cells express CD80/86 and PD-L1, it logically follows that they too would be affected by anti-CTLA-4 and anti-PD-1/PD-L1 CBI. However, the impact of CBI on B-cell populations remains understudied. Histologic examination of NSCLC demonstrated responders to neoadjuvant anti-PD-1 CBI had formation of TLS in association with tumor cell death and tissue repir.[41] The effects of CBI include modulation of tumor-draining lymph nodes and systemic immune populations. Tumor-draining lymph nodes serve as key niches in which immune cells can react to tumor antigens, and a murine model evaluating elective nodal irradiation outlined consequences for T-cell responses and trafficking when irradiating

local lymph nodes.[42] Our group has shown in a murine model of HPV-related head and neck cancer that CBI and tumor-directed radiation therapy can dramatically increase GC formation in the tumor-draining lymph node.[16] In addition, three recent, high-profile studies have now provided ample evidence that demonstrate that B cells present in TLS are critical in responding to CBI and can predict responses to CBI, and these are discussed individually below.

In the first of these seminal studies, Cabrita, et al., investigated the prognostic role of TLS in metastatic melanoma lesions and found a clear association between GC formation and overall survival.[10] In a multivariate analysis, the authors demonstrated that tumors that expressed high levels of both CD20 and CD8 had the highest survival. We additionally highlight three key findings which deserve further attention: 1) the presence of immature TLS – those without clear GC formation – is not associated with patient outcomes, 2) TLS(high) tumors were associated with increased survival after CTLA-4 blockade independent of driving genetic mutations, and 3) while pre-treatment TLS expression scores did not differ between responders and non-responders, early, on-treatment scores were significantly different and signify the ability of CBI to induce TLS formation.

Similar conclusions were independently arrived at by Helmink, et al., who studied the role of TLS formation in both metastatic melanoma and clear cell renal cell carcinoma.[9] The authors demonstrate a clear association between outcomes and clusters of patients with tumors expressing high levels of genes associated with B cell presence and function independent of genomic subtype of disease. On histologic examination, patients with melanoma who responded to CBI demonstrated higher TLS to tumor ratios in early on-treatment biopsies. Of note, the authors show that T cells within TLSs had higher expression of activation markers, such as CD24, CD44, and granzyme B, elegantly illustrating the role of a TLS in serving multiple cell subsets apart from solely B cells. The authors then quantify differences in two complementary downstream B cell characteristics in patients who respond to CBI and conclude: 1) responders had higher BCR and diversity measured in B cell transcripts, and 2) responders possessed high proportions of class-switched memory B cells, activated B cells, and GC-like B cells in the TME. B cell oligoclonality and differentiation into antigen-experienced subsets are key products of the GC and serve as functional measures of the activity of TLSs in responding to CBI.

Finally, Petitprez, et al., analyzed the effects of B cells in patients with soft-tissue sarcomas treated with CBI.[8] They delineate the presence of five “sarcoma immune clusters” (SIC) that reflect the transcriptional profile of the TME and are associated with overall survival. Importantly, there was no significant association between tumor histology or tumor mutational burden and SIC grouping. One such group – SIC E – was characterized by high expression of immune cell-related genes and was associated with the best clinical outcomes. On closer examination, SIC E tumor had the highest expression of the B cell-specific chemokine CXCL13 and the vast majority of tumor samples possessing TLSs belonged to SIC E. Intriguingly, tumors with TLSs had higher rates of tumor-infiltrating CD3+ T cells, CD8+ T cells, and CD20+ B cells, illustrating the viability of TLSs in facilitating a robust immune response. These SICs importantly demonstrated differential responses to pembrolizumab, with 0% objective response rate in SIC A and B, which were characterized

by low immune cell infiltration, and 50% ORR in SIC E, the latter in contrast to the notion of soft-tissue sarcomas being non-responsive to CBI.

Taken together these seminal studies have established that B-cells and TLS play a key role in responses to immunotherapies and outcomes of cancer patients. Furthermore, these findings have revealed B-cell biology as an entirely new avenue for research in immuno-oncology.

## Role of conventional therapies in modulating B-cell populations

While immunotherapies have established significant clinical efficacy, the majority of newly diagnosed patients with cancer are treated with conventional therapies, with combinations of chemotherapy, radiation therapy, and surgery. A recent study extrapolating from clinical trial data estimated that in 2019, at most, 38.5% of patients were eligible for CBI, although approval of CBI in upfront treatment paradigms is clearly growing.[43] Therefore, there is a need to understand how conventional therapies, namely chemotherapy and radiation therapy, can impact B-cell populations (Figure 1). We comprehensively searched PubMed for all articles published between 2015–2020 with the following terms: (1) “B cell” and “chemotherapy” and “cancer” (n = 2471), (2) “B cell” and “radiation” and “cancer” (n = 572), (3) “tertiary lymphoid structures” and “chemotherapy” (n = 12), and (4) “tertiary lymphoid structures” and “radiation” (n = 5). We manually screened all articles and highlight below a selection that specifically describe the effect of these therapies on B cell subpopulations, activity, or differentiation in the context of solid malignancies.

In patients treated with chemotherapy, sharp declines in peripheral CD20+ B cell counts have been noted following the initiation of therapy.[44] One study in particular analyzed peripheral blood samples from breast cancer patients and found that B cells were exquisitely sensitive, falling to a median of 5.4% of pre-chemotherapy levels and <2% in a majority of patients, with only partial recovery 9 months following chemotherapy.[44] Specific B cell subset analysis demonstrated that memory B cells were more susceptible, comprising 38% of total B cells prior to therapy, and only 14% at 3-month follow up. Unsurprisingly, the authors note differential effects based on specific chemotherapy regimens. However, the implications of this and the relative contributions of T-cell versus B-cell lymphopenias on patient outcomes is still unclear and merits further investigation.

A recent excellent study by Lu et al., analyzed tumor-infiltrating B cells from patients with breast cancer treated with neoadjuvant chemotherapy (NAC).[45] Single-cell RNA sequencing (scRNA-seq) demonstrated clusters of B cells from pre-treatment samples with high expression of IL-10 that dramatically fell following NAC. In post-treatment samples, the B cells assumed a more immunostimulatory phenotype with high expression of ICOSL and low expression of IL-10 that preferentially accumulated near TLS; ICOSL+ B cells accounted for 45.1% of total B-cells following NAC compared to <1% at baseline. Importantly, the presence of ICOSL+ B cells was associated with improved disease-free survival (DFS) and OS across most subsets of breast cancer and was particularly pronounced in triple-negative breast cancer. Mechanistically, the switch to a more immunostimulatory phenotype was dependent on complement signaling through CR2 and CD55, which can interrupt the switch of a ICOSL+ B-cell phenotype. Moreover, tumor CD55 expression is higher in patients resistant to NAC, and high expression of CD55 in pre-treatment

biopsies is associated with worse clinical outcomes. This opens the possibility of rationally combining CD55-directed therapies with chemotherapy to improve activity, especially given pre-clinical studies demonstrating that therapies targeting CD55 can modulate the activity of trastuzumab.[46] Overall, this study eloquently demonstrates how detailed immunophenotyping of B cell subpopulations in response to chemotherapy can reveal novel avenues for therapeutic intervention.

In contrast to chemotherapy, which circulates systemically, radiation therapy predominantly works locally, targeting cells within the field of radiation. Dynamics of cellular reconstitution following radiation therapy was recently explored in the context of TLS using KP mice, one of the murine models with functional intratumoral TLS formation.[47] This study demonstrated that as early as two hours following radiation, there was a decrease in the size of TLS, which were restored 14 days after radiation.

Our group has recently published the effects of radiation therapy on B cells in murine models of HPV-related head and neck cancer and melanoma.[16] We found that following tumor-directed radiation, B cells in the tumor-draining lymph node had increased expression of MHC II, representative of increased capacity to serve as an APC. Further studies are required to investigate the specific role of these activated B cells in mediating anti-tumor responses. Radiation additionally increased tumor-antigen-specific B cells and class-switched memory B cells (IgG+PDL2+CD80-), products of germinal center maturation. BCR sequencing demonstrated shorter CDR3 lengths in B cells from mice treated with radiation, and previous vaccine studies have shown a correlation between shorter CDR3 loops and affinity maturation.[48] Interestingly, radiation also increased the proportion of CD1d(hi)CD5+ regulatory B cells known to secrete IL-10 and inhibit T-cell responses, and this effect could be mitigated by the additional treatment with PD-1 blockade, which resembles radiation-induced proliferation of regulatory T cells.[49] Cumulatively, a deeper understanding of how B cell respond to both novel and conventional therapies may reveal unique pathways to target for combinatorial activity (Figure 1).

## Conclusion

The past decade has seen rapid advancements in our understanding of the tumor microenvironment and the role that immunotherapies have on T cells and myeloid cells; however, the functional consequences of tumor-infiltrating B cells and antibody responses have been historically understudied. The seminal studies highlighted in this review establish an integral role for B cell and TLSs in responses to immunotherapy and patient outcomes in multiple different tumor types. While biomarkers used to predict responses to CBI continue to be reported, there is a need for further refinement and validation incorporating markers representing both arms of the adaptive immune response. The use of mature TLS as a biomarker is of particular interest as it follows fundamental immunological principles in the mounting of an endogenous, antigen-specific adaptive immune response. By dissecting the complex functions of B cells and the intricate interactions they have with other immune cell subsets, additional diagnostics and combinatorial therapeutics focused on B cells will undoubtedly be developed to improve response rates to immunotherapy and outcomes of cancer patients.

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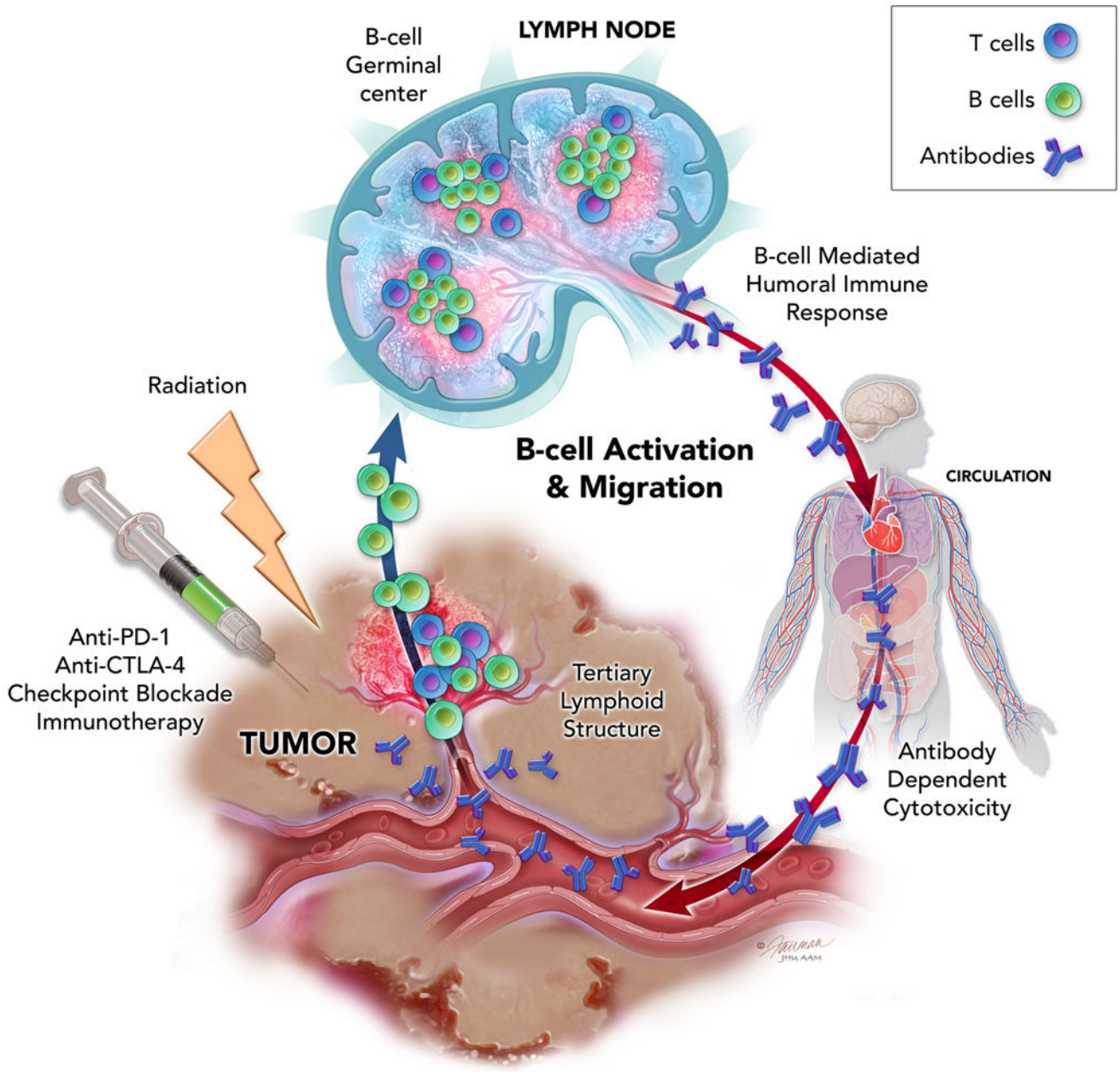


**Figure 1: Schematic diagram of B-cell activation, migration, and antibody responses.**

Intratumoral B-cells located in a Tertiary lymphoid structure (TLS) may impact immune responses. B-cells within secondary lymphoid organs can generate humoral antibody responses which travel systemically through circulatory system to tumor sites. Radiation and checkpoint blockade may potentially modulate processes of B-cell activation and/or migration. (Illustration by Jennifer E. Fairman, MA, MPS, CMI, FAMI Certified Medical Illustrator.)

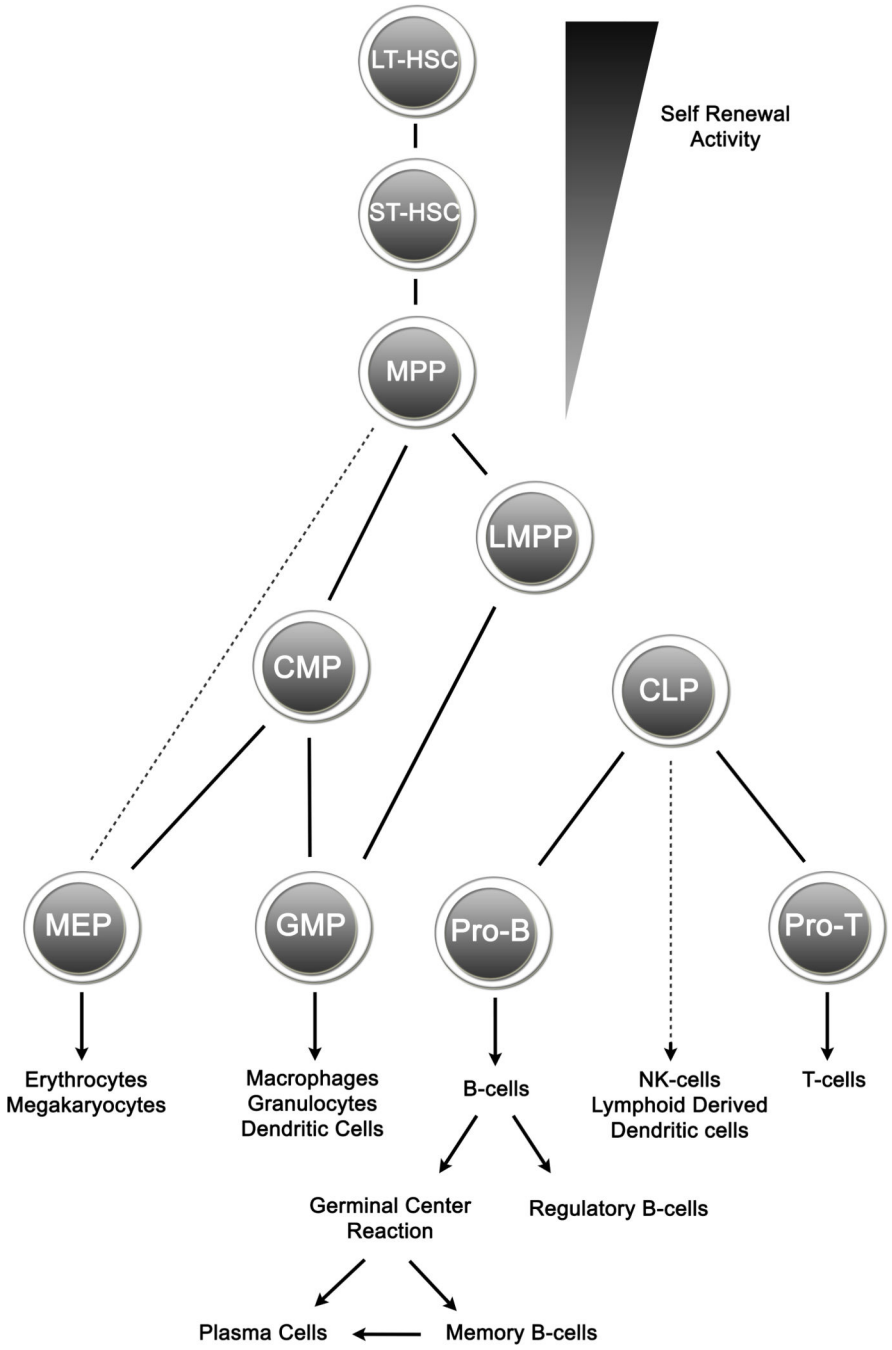


**Figure 2:**
Hematopoiesis demonstrating B-cell linage development from Hematopoietic Stem Cell and differentiated B-cell subpopulations.

**Table 1:**

Selected studies analyzed correlation between B cell presence and prognosis.

| Disease | Details on Association Between B Cell Markers and Prognosis | Statistical Conclusions | Ref |
|---|---|---|---|
| Breast Cancer | Improved metastasis-free survival (MFS) in highly proliferative, node-negative disease with high expression of B cell metagene | MFS HR 0.26–0.37 with high B cell metagene | 17 |
| Esophageal Adenocarcinoma | Improved overall survival with increased IGKC+ plasma cells | OS HR 0.10 with high IGKC expression on multivariate analysis | 21 |
| Gastric Adenocarcinoma | Improved overall survival with increased IGKC+ plasma cells | OS HR 0.46 with high IGKC expression on multivariate analysis | 21 |
| Head and Neck Squamous Cell Carcinoma | Improved overall survival with high expression of CD19 and IgJ; greater benefit in HPV+ disease compared to HPV-<br>Improved 5-year DFS with high peritumoral infiltration of CD20+ B cells | 3-year OS HR 0.17 and 0.22 with high CD19 and IgJ expression, respectively in HPV+ disease<br>5-year DFS of 100% vs 64% for high versus low peritumoral CD20+ B cell presence | 16<br>23 |
| Melanoma | Improved 5-year survival rates with high intratumoral CD20+ B cells | 5-year OS of 78% vs 59% for high vs low intra-tumoral B cell density | 18 |
| Non-Small Cell Lung Cancer | Improved survival rate and disease-specific survival with higher density of follicular B cells | 4-year survival rate of 97% vs 62% for high vs low follicular B cell in early-stage NSCLC<br>Median DSS of 56 vs 23 months for high vs low follicular B cells in advanced-stage NSCLC | 19 |
| Pancreatic Ductal Adenocarcinoma | Improved overall survival with increased B cells organized as tertiary lymphoid tissues; scattered tumor-infiltrating B cells did not significantly affect overall survival<br>Reduced overall survival with increased CD20+ B cells | Median survival of 16.9 vs 10.7 months for high vs low density of B cells organized in tertiary lymphoid tissue<br>OS HR of 2.03 for high CD20+ density on univariate analysis; OS HR of 1.48 on multivariate analysis | 20<br>22 |