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## Review Article

# B cells and their mediators as targets for therapy in solid tumors

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## ABSTRACT

B cells have recently been appreciated as paracrine mediators of solid tumor development. Their ability to influence various hallmarks of cancer development, aside from antigen presentation, can be attributed to the diversity of soluble mediators they express, including cytokines and immunoglobulins, that can act directly and indirectly on the diversity of leukocyte subsets that infiltrate developing tumors, evolving neoplastic cells, as well as select T cell populations in secondary lymphoid organs and within tumor stroma. Herein, we review the literature supporting these interactions and discuss novel approaches to ameliorate protumoral B cell effects for anti-cancer therapy.

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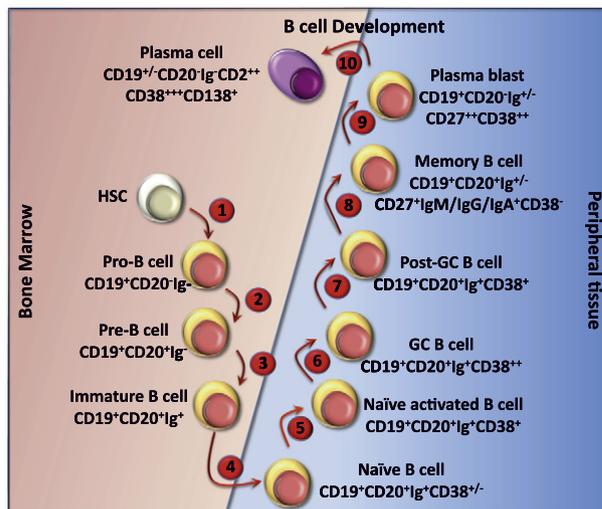
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## Introduction

B lymphocytes, appropriately named for their original discovery in the Bursa of Fabricius, develop and mature in bone marrow, but are activated in peripheral lymphoid tissues in response to antigen (Ag) exposure, similar to T lymphocytes. However, unlike T cells whose primary immunological role is to “help” programming of other leukocytes or to exert selective killing (cytotoxicity), stimulation of naïve B cells culminates in production of secreted versions of B cell receptors (BCR) in the form of immunoglobulins (Ig), commonly referred to as antibodies. In addition, B cells secrete a variety of cytokines, e.g., interleukin (IL)-6, IL-10, tumor necrosis factor (TNF)- $\alpha$ , granulocyte monocyte-colony stimulating factor (GM-CSF) and lymphotoxin (LT), etc., that in turn regulate diverse activities of other leukocytes and non-hematopoietic cells. Moreover, their cell surface expression of major histocompatibility complex class (MHC)-II enables secondary antigen presentation to helper T ( $T_H$ ) cells. In homeostatic tissues, these activities are critical for maintaining tissue/organ health, but in pathological disease processes such as autoimmunity and cancer development, these same activities are diverted [1,2]. Reviewed herein are the experimental and clinical studies supporting the paradoxical activities of B cells and their products in fostering carcinogenesis, examination of the paracrine mechanisms involved, and a discussion of a potential role for inhibitory B cell therapies in the clinic as either monotherapy, or in combination with chemotherapy, for patients with B cell-regulated solid tumors.

## B cell subtypes in health and disease

The developmental progression from hematopoietic progenitor to pro- to pre- to finally immature, antigenically naïve B cell occurs consistently and predictably in every B cell subset prior to their exit from the fetal liver or adult bone marrow (in mammals) and chemotaxis to the periphery (Fig. 1). This process is directed by recombination activating genes 1 and 2 (*RAG-1/2*), DNA recombinases expressed in T and B cells that mediate T cell and B cell receptor gene rearrangement, culminating in cell surface expression, a requisite for peripheral survival [3]. Once maturation and peripheral residence is established, stochastic paracrine signals determine acquisition of specified functions allowing categorization into individual subsets. These include but are not limited to: B-1 cells that produce natural antibodies, further subdivided in the peritoneum into  $CD5^+$  B-1a (marginal zone in the spleen) and  $CD5^-$  B-1b, transitional-2 marginal zone precursor B subsets, canonical B-2 cells, B10/B regulatory cells (Bregs) that secrete the anti-inflammatory cytokine IL-10, plasma cells that hypersecrete Igs and follicular  $CD1d^loCD5^-$  splenic B cells [4] (for a review on B cell development and differentiation, please see [3]). Most, but not all B cells (e.g., plasma cells), express the CD20 antigen at varying levels, a cell surface receptor with a currently unidentified ligand, that serves as a  $Ca^{2+}$  ion channel [5,6] critical for T cell-independent antibody responses [7]. Importantly, CD20 has been successfully exploited for targeted monoclonal antibody (mAb) therapy enabling efficient B cell depletion [8]. These various B cell subsets possess numerous bioactivities deriving from their functional plasticity—a topic reviewed in greater detail elsewhere [9].

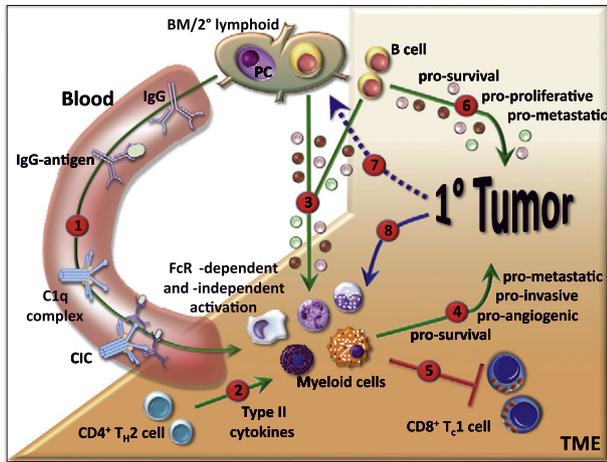


**Fig. 1 – B cell development and subset differentiation.** Differentiation of hematopoietic stem cell (HSC) progenitors into naïve B cells occurs in the bone marrow of adult mammals and subsequently continues in peripheral lymphoid tissue for further phenotypical differentiation and potential antigen experience (numerical order indicates differentiation progression). Not only do these specific subsets express a unique gene repertoire, but they also are harbored in peripheral tissue sites at relatively higher frequencies to other B cell subsets. For instance,  $CD5^+/CD1d^+$  B regulatory cells (Bregs) maintain their largest populations in the peritoneal cavity, where they serve an anti-inflammatory function attributed to their high expression of IL-10. GC, germinal center.

As indicated above, B cells are the sole producers of Igs and thus serve a critical role as initiators and modulators of humoral immunity. Hence, through their regulated production of antigen-specific Igs, B cells are capable of shaping an immune response through microbial neutralization, promotion of opsonization, chemotaxis, activation, and de-activation of pro-inflammatory myeloid cells; all necessary mechanisms of pathogen elimination. Given that developing solid tumors display similar characteristics to tissues damaged by autoimmune dysfunction, e.g., chronic immune cell infiltration, tissue remodeling, angiogenesis, and altered cell survival pathways, it is not surprising that individuals with some forms of autoimmunity (RA or systemic sclerosis) also harbor an increased relative risk for cancer [10,11]. To that end, recent studies have begun to shed light on the role of peripheral and tumor-infiltrating B cells as potentiators of solid tumor development (Fig. 2).

## B cell cooperation with complement factors

A notable, yet underappreciated role for B cells in tumor development is the proteolytic induction of complement proteins that partner with Igs to form circulating immune complexes (CICs), and subsequently signal through complement receptors that are variably expressed on all leukocytes [12]. Induction of the complement cascade occurs in blood plasma and tissue, and is coordinated by a series of proteolytic zymogens, triggered on the surface of a pathogen and during activation of diverse inflammatory events.



**Fig. 2 – B cells contribute to solid tumorigenesis.** As discussed in the text, B lymphocytes exhibit numerous tumor-promoting mechanisms systemically (1) through antibody production (IgG) and complement conjugation (C1q) to form immune complexes (CIC), that in turn are deposited into the tumor microenvironment (TME) and subsequently ligate to either Fc $\gamma$ R or C5aR expressed on tissue-resident or tumor-infiltrating myeloid cells. CIC/Fc $\gamma$ R interactions cooperate with T<sub>H</sub>2 type cytokines (IL-4, IL-13, IL-10) produced by CD4<sup>+</sup> T cells (2), as well as B cell-produced polypeptide growth factors and cytokines (GM-CSF, IL-6, IL-10) (3) to initiate and sustain T<sub>H</sub>2-dominant programming of myeloid cells. These in turn provide pro-survival and pro-angiogenic factors, matrix remodeling enzymes, and pro-metastatic molecules to evolving neoplastic cells (4), as well as factors that suppress CD8<sup>+</sup> cytotoxic T cell proliferation/activity (5). Several B cell-derived factors also directly regulate pro-survival and chemoresistance pathways in malignant tumor cells, including LT $\beta$ , WNT16 and TNF $\alpha$  (6). Experimental data indicate that Ig-production by B cells is likely antigen-dependent (7) thus involving trafficking of antigen-presenting cells to secondary (2°) lymphoid organs. Myeloid cell presence in TMEs in part derives from factors expressed by evolving neoplastic cells including toll-like receptor-regulated pathways, GM-CSF, CSF1, the chemokine CCL2, TNF $\alpha$ , etc., (8). PC, plasma cell.

Three unique pathways exist [classical, mannose-binding lectin (MBL), and alternative] and are orchestrated by specific complement proteins (C1q, MBL, and C3, respectively) that are initiated by distinct stimuli (Ag:Ab complexes, MBL:mannose ligation and pathogen surfaces, respectively), but ultimately converge on the C3 convertase, a complement protein necessary for T cell-dependent antibody responses [13]. Subsequently, these pathways diverge into three ultimate fates: phagocytic recruitment, opsonization and/or innate cytotoxicity of microbes. C5a, the dominant pro-inflammatory complement factor, decreases the activation threshold of myeloid cells by increasing the ratio of activating:inhibitory Ig receptor ratio providing feed-forward synergism for B cell activity [14,15]. In part because of this, preferential binding of IgG1 to inhibitory Ig receptors is hypothesized to be a preventative mechanism for immune complex activation by circulating myeloid cells [16]. Additional anti-inflammatory properties for IgG1 appear to involve blockade of neutrophil C5a signaling through physical interaction of dectin-1 and downstream SHIP

(SH2 domain-containing inositol 5'-phosphatase 1) and Syk (spleen tyrosine kinase) phosphorylation [17], adding complexity to the rationale design of myeloid signaling inhibitors dependent on tumor-promoting or tumor-regressing phenotypes. Finally, signaling downstream of the receptor for C5a (C5aR) has been implicated as a novel mechanism for accumulation of immunosuppressive Gr-1<sup>+</sup>CD11b<sup>+</sup> monocytes in tumors and their T cell-inhibitory properties, again supporting the dynamic functionality B cells exhibit in the tumor microenvironment (TME) [18].

### Role of immunoglobulins in tumor-associated chronic inflammation

While little is known on the functional significance of circulating immune complexes (CICs; Igs in biochemical complex with complement proteins) in tumor development, the role of CICs in inflammatory and autoimmune diseases has been examined extensively. CIC deposition into tissue parenchyma is a consequence of leaky vasculature, in both tumor [19–21] and pathologic [22] angiogenesis. CIC (and Ig) deposition initiates multiple inflammatory cascades by mechanisms involving activation of complement pathways and/or engagement of the receptors for the crystallizable region (Fc) receptor common  $\gamma$  chain (Fc $\gamma$ R) for IgG broadly expressed on myeloid cells [23]. As such, Fc $\gamma$ Rs represent a functional link between adaptive and innate immunity by coupling interactions between circulating (auto) antibodies and innate immune cells [16]. In humans, four Fc $\gamma$ R variants (Fc $\gamma$ RI/CD64, Fc $\gamma$ RII/CD32, Fc $\gamma$ RIII/CD16, and Fc $\gamma$ RIV) are constitutively expressed unequally on myeloid cells. Activating types of Fc $\gamma$ Rs form multimeric complexes with Fc $\gamma$  that contain an intracellular tyrosine-based activating motif (ITAM), whose activation triggers oxidative bursts, cytokine release, phagocytosis, antibody-dependent cell-mediated cytotoxicity, and degranulation [23]. Induction of these processes is the functional downstream consequence of Syk kinase activity, and to some degree Bruton's tyrosine kinase (Btk) activity. The phosphorylative capabilities of these two enzymes is prompted by initial IgG/Fc $\gamma$ R ligation events that initiate a classical intracellular kinase cascade resulting in transcriptional activities of JNK, p38 and ERK1/2, as well as critical Ca<sup>2+</sup> secondary messenger signaling [24]. In contrast, engagement of Fc $\gamma$ RII (or Fc $\gamma$ RIIb in mice), an inhibitory low-affinity receptor for IgG, terminates activation signals initiated by antigen crosslinking due to its immune tyrosine-based inhibitory motif (ITIM) [23]. Activation of the ITIM motif in this context instead utilizes SHIP, a phosphatase that blunts Btk signaling through its counteracting dephosphorylating catalytic behavior, thus tuning or even abrogating Fc $\gamma$ R/ITAM signaling cascades [25].

Fc $\gamma$ R expression is necessary for assembly and cell-surface localization of Fc $\gamma$ RI, Fc $\gamma$ RIII, and Fc $\gamma$ RIV; thus, Fc $\gamma$ R-deficient mice [26] lack all activating Fc $\gamma$ Rs, whereas Fc $\gamma$ RII expression is unaltered. Since Fc $\gamma$ R-deficient mice are resistant to CIC-mediated hypersensitive reactions (e.g., alveolitis, glomerulonephritis, skin Arthus reaction), whereas mice deficient in Fc $\gamma$ RII exhibit enhanced CIC-mediated inflammatory responses [23] it is clear that distinct downstream signaling networks are regulated by ITAM versus ITIM motifs and differentially relay humoral immune-mediated signals to myeloid cells.

Regarding solid tumors, while it is well known that cancer patients develop antibodies to tumor-associated antigens (evidence exist for c-myc, HER-2/neu, and p53 [27]) these rarely are sufficient to eradicate tumorigenic cells. Instead, there is a reciprocal relationship between levels of CICs (or presence of CD20<sup>+</sup> B cells) within tumor parenchyma for some cancer types where increased presence correlates with increased tumor burden and poor prognosis (reviewed in [1]), while in other cancer types, no correlation has been identified [27]. Formation of ectopic tertiary lymphoid structures with organized clusters of CD20<sup>+</sup> B cells in these circumstances may instead be indicative of immune activation [28–30].

Experimental evidence indicating a protumoral role for humoral immunity derives from studies utilizing mouse models of solid tumor development where absence of B cells, FcR $\gamma$  or other B cell-selective effectors is associated with deficient tumorigenesis. Using the K14-HPV16 transgenic mouse model of squamous cell carcinoma (SCC) development [31], we reported that genetic deletion of B and T lymphocytes attenuated recruitment of innate immune cells (mast cells, monocytes, macrophages, granulocytes) into premalignant skin [20]. As a consequence, tissue remodeling, angiogenesis and epithelial hyperproliferation were significantly abated, culminating in reduced carcinoma incidence [20]. Importantly, transfer of B220<sup>+</sup>CD19<sup>+</sup> B cells or serum from K14-HPV16 mice into T and B lymphocyte-deficient/HPV16 mice resulted in restored characteristics of premalignancy, e.g., Ig deposition in neoplastic skin, chronic myeloid cell infiltration, activation of angiogenic vasculature and keratinocyte hyperproliferation [20]. Similar findings have been reported in murine models of colorectal cancer [32]. Together, these data indicate that peripheral B cell activation is an essential step for early epithelial neoplasia and that B cell-derived soluble mediators are necessary for establishing chronic inflammatory states that potentiate malignant progression.

More recently, the B cell-dependent mechanisms involved in regulating squamous carcinogenesis have been identified, that being activation of Fc $\gamma$  receptor-dependent pathways in infiltrating myeloid cells, specifically mast cells and macrophages, following their ligation by Ig-containing CIC deposited in parenchyma of premalignant tissue [20,33]. Notably, this protumorigenic paracrine pathway can be reprogrammed to instead resist tumor progression by either B cell- or Fc $\gamma$ -genetic deletion, wherein infiltrating mast cells and macrophages lose their protumorigenic properties (angiogenic, CD8<sup>+</sup> T cell-suppressive), and instead secrete angiostatic (CXCL10) [33] and CD8<sup>+</sup> T cell-chemoattractant chemokines functionally significant for instilling tumor repression (Coussens lab, unpublished observations). Similarly, pharmacological inhibition of Syk in K14-HPV16 mice halts pre-malignant progression, analogous to genetic B cell or FcR $\gamma$ -deficiency (Coussens lab, unpublished observations). Whether this represents biology unique to myeloid cell reprogramming or also inhibition/reprogramming of other Syk-expressing cells (such as required for BCR signaling) remains to be elucidated.

### Tumorigenic roles for B cell-derived cytokines

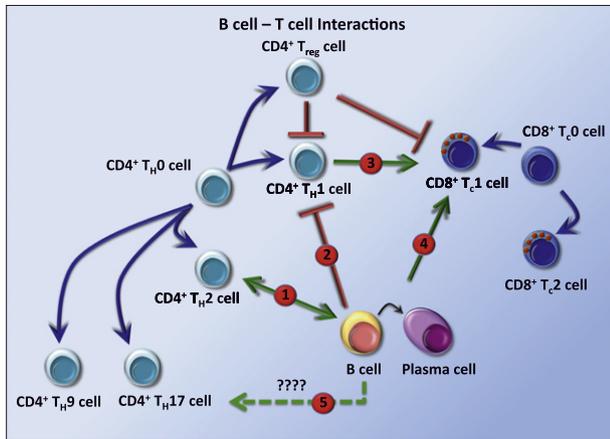
The data above indicate that B cells potentiate solid tumor development by mechanisms involving humoral immunity;

however, in other experimental tumor models, serum CIC/Ig fails to reinstate B cell-dependent tumor parameters thus implying a role for other B cell-derived mediators (Fig. 2). During prostate carcinogenesis, the wingless-type MMTV integration site family member 16B (WNT16B) is upregulated by nuclear factor of kappa light polypeptide gene enhancer (NF- $\kappa$ B) in B cells after DNA damage, and via a paracrine mechanism activates the canonical Wnt program in evolving tumor cells, the result of which is chemoresistance, in combination with enhanced tumor cell survival and disease progression [34]. In addition, B cell-derived lymphotoxin (LT)- $\beta$  promotes survival of metastatic prostate cancer cells in castration-resistant disease by stimulating I kappa B kinase (I $\kappa$ K)-alpha and STAT3 activity in malignant cells, thus provoking androgen-refractory regrowth and metastasis [35].

In contrast to HPV16-induced SCCs, squamous carcinomas possessing an activated *ras* oncogene, whereas B cells are required for progression to full malignancy, is dependent on B cell-derived TNF $\alpha$  [36]. In the absence of B cell-derived TNF $\alpha$ , neoplastic tissue instead contains increased levels of interferon (IFN)- $\gamma$  and CD8<sup>+</sup> T cells, and significant reductions in IL-10-producing B regulatory cells, thus indicating that tumor cell-intrinsic oncogenic signaling can also direct mechanisms of protumoral leukocyte programming. Because CD5<sup>+</sup> B cells in mice include well-defined populations of IL-10-expressing cells (Bregs/B10; CD19<sup>+</sup>CD24<sup>hi</sup>CD38<sup>hi</sup> B cells in humans [37]), it seems plausible to hypothesize that some of the Ig-independent pro-tumorigenic properties of B cells involve these regulatory populations. This perhaps represents B cell biology unique to conditions of “sterile” inflammation where an immune response would have no imperative to eliminate a pathogenic microorganism, and instead would favor resolution of acute inflammation to avoid harmful, chronic immune activation. These phenomena have been observed in several other cancer models where Breg cells residing in the peritoneum provide a reservoir of resistant B cells to anti-CD20 mAb therapy in mice [9]. B cells that resist depletion by anti-CD20 antibodies are predominantly of a CD5<sup>+</sup>/CD1d<sup>hi</sup> phenotype that encompasses the majority of IL-10-producing B cells; these cells greatly enhance implantable A20 lymphoma expansion in an IL-10-dependent manner [38]. Interestingly, macrophages co-cultured with B10 lymphoma cells display reduced major histocompatibility complex (MHC) class II and CD86 expression, and resist lipopolysaccharide-stimulated TNF $\alpha$  and nitric oxide production [38] thus indicating that IL-10 production by B cells directly favors protumorigenic type 2 programming of macrophages, while simultaneously inhibiting macrophage-mediated antibody-dependent cell-mediated cytotoxicity (ADCC) of anti-CD20-bound B cells[6]. Several other studies have circumstantially implicated IL-10 production by B cells in mediating the macrophage-regulated CD8<sup>+</sup> T cell anti-tumor response, the remainder of which will be discussed below.

### B cells as inhibitors of T<sub>H</sub>1-mediated anti-tumor immunity

In addition to altering local and circulating levels of cytokines, a significant role for B cells as (indirect) promoters of tumorigenesis lies in their ability to inhibit T<sub>H</sub>1-mediated anti-tumor



**Fig. 3 – Interactions of B cells with T cells. B cells foster cancer development by also regulating diverse T lymphocyte responses, in particularly enhancing  $T_H2$ -type programming of  $CD4^+$  T helper cells ( $T_H$ ) (1) while simultaneously repressing  $CD4^+$   $T_H1$  cells (2) that in turn indirectly (3), or directly (4) influence  $CD8^+$  cytotoxic T cell ( $T_c$ ) bioactivity. A role for B cells in regulating IL-17-expressing  $T_H$  cells ( $T_H17$ ) or IL-9 expressing  $T_H$  cells ( $T_H9$ ) can only be inferred at this point in time based on documented roles for B cells following infection, and in some autoimmune and chronic inflammatory disorders (5), however a recent study has identified a direct role for  $CD19^+CD24^{hi}CD38^{hi}$  Breg cells in regulating  $T_H17$  cell differentiation in patients with rheumatoid arthritis [37].**

immunity (Fig. 3). Enhanced  $T_H1$  (IFN- $\gamma$  producing  $T_H$  cells) and  $T_c$  (cytotoxic  $CD8^+$  T cells) anti-tumor immunity in B cell deficient mice ( $IgM^{-/-}$ ) leads to rejection and/or slowed onset of multiple transplanted tumor grafts [39]. Accordingly, direct IgG ligation of Fc $\gamma$ RI/III on macrophages inhibits IL-12 and upregulates IL-10 expression, a hallmark trait for protumorigenic macrophages [40,41]. Moreover, co-culturing total splenocytes from B cell-deficient mice with irradiated tumor cells enhances IFN- $\gamma$  production from  $CD8^+$  T cells, in part mediated by CD40L/CD40 interaction and increased production of tumor cell-stimulated IL-10 production from B cells [42]. Given that macrophage-mediated cytotoxic mechanisms in pancreatic adenocarcinomas are agonistically provoked following therapeutic CD40 antibody therapy [43,44], it is tempting to speculate that some of the clinical efficacy of agonist CD40 therapy is due to functional reprogramming of tumor-promoting B cells in manners similar to Syk inhibition.

### Perspectives and therapeutic opportunities

From a classical point of view, it would seem likely that B cells contribute to tumorigenesis by impairing the process, and in deed they may under some circumstances. That the vast majority of humans do not develop cancer could in part be attributed to B cells, and other leukocytes, performing their intended vocations as they do when maintaining homeostatic tissue/organ health. However, as scientists begin to evaluate the fundamental molecular and cellular mechanisms contributing to cancer development using more sophisticated immune-competent in vivo models, similar to previously

unappreciated protumorigenic roles for select T cell and myeloid cell subsets recently revealed (reviewed in [45,46]), B cells now also emerge as possessing protumorigenic activities. Given the inherent plasticity embedded within all leukocyte subsets, these discoveries present interesting opportunities for therapeutic intervention.

Regarding specific inhibition of pro-tumoral B cells, adjuvant use of rituximab, a depleting, humanized anti-CD20 MAb, either as monotherapy or (more likely) in combination with chemotherapy would theoretically be of clinical benefit. Explored on a small scale, previous use of rituximab for solid tumor therapy demonstrated limited clinical success [47,48] likely owing to its use as monotherapy. That said, it is also clear that some B cell subsets, specifically Breg cells, are refractory to depletion via anti-CD20 mAb [38,49]; thus, other therapeutic strategies targeting Breg cells, in addition to other B cell subsets may need to be considered. For example, ibrutinib, a small molecule inhibitor of Btk has shown efficacy in B-CLL/SLL [50] patients as well as in mouse models of cMyc-induced insulinoma [51]; thus, Btk (as well as Syk) inhibitors could also prove tractable for other cancers where B cells (and Btk or Syk signaling) contribute necessary mechanisms for tumor growth. Further interrogation of the significant B cell-regulated mechanisms contributing to solid tumor development will certainly reveal the degree to which B cells and their downstream effectors can be targeted therapeutically to improve outcomes for patients with cancer.

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