# B cells and cancer: To B or not to B?

Wolf Herman Fridman, Florent Petitprez, Maxime Meylan, Tom Wei-Wu Chen, Cheng-Ming Sun, Lubka T Roumenina, Catherine Sautès-Fridman

# B cells and cancer : to B or not to B?

Wolf H. Fridman*[1], Florent Petitprez[2#], Maxime Meylan[1], Tom Wei-Wu Chen[3], Cheng-Ming Sun[1], Lubka T. Roumenina[1] and Catherine Sautès-Fridman[1]

[1]Centre de Recherche des Cordeliers, Sorbonne Université, Inserm, Université de Paris, F-75006, Paris, France.

[2]Programme Cartes d'Identité des Tumeurs, Ligue Nationale contre le Cancer, F-75013, Paris, France.

[3]Department of Oncology, National Taiwan University Hospital, Taipei, Taiwan

*corresponding author :

Wolf H. Fridman, Centre de Recherche des Cordeliers, 15 rue de l'Ecole de Médecine, 75006 Paris, France ; +33144279192 ; herve.fridman@crc.jussieu.fr

#Present address : MRC Centre for Reproductive Health, The University of Edinburgh The Queen's Medical Research Institute, Edinburgh, UK

Words : 2944

Summary statement:
Tertiary Lymphoid Structures, adjacent to tumor nests, are sites where anti-tumor adaptive T and B cell immune responses are generated. Fridman et al highlight the impact of B cells and the antibodies they produce in tumor immunity and patient's response to immunotherapy.

Abstract

Whereas T cells have been considered as the major immune cells of the Tumor Microenvironment able to induce tumor regression and control cancer clinical outcome, a burst of recent publications pointed to the fact that B cells may also play a prominent role. Activated in germinal centers of Tertiary Lymphoid Structures, B cells can directly present tumor-associated antigens to T cells or produce antibodies that increase antigen presentation to T cells or kill tumor cells, resulting in a beneficial clinical impact. Immune complexes can also increase inflammation, angiogenesis and immunosuppression via macrophage and complement activation resulting in deleterious impact.

## INTRODUCTION

At the beginning of the millennium, it was demonstrated that the immune Tumor Microenvironment (TME), particularly the density, localization and functional orientation of T cells, is of paramount importance for controlling tumor growth and spread (Galon et al., 2006; Fridman et al., 2012). It established the rationale for paradigm changing immunotherapies utilizing antibodies against immune check-point inhibitors (ICI) such as CTLA-4, PD-1 and PD-L1, that have revolutionized the treatment of cancers (Sharma and Allison, 2015). Since these therapies aim to reinvigorate exhausted T cells, it is not surprising that they are mostly effective in highly mutated tumors, which are more likely to express more tumor-specific neo-antigens, resulting in a strong T cell infiltrate, such as Non Small Cell Lung Cancer (NSCLC), melanoma and Micro Satellite Instable (MSI) tumors (Rizvi et al., 2015). However, although being very efficient and inducing long term responses in many cancer types, most patients are resistant to ICI therapies (Hirsch et al., 2019), urging for searching other components of the immune TME that are involved in tumor control and may provide novel therapeutic approaches. In this respect, the role of B cells has, until recently, been underestimated. In fact, it had been reported in some murine models that B cells, and the antibodies they produce, may favor cancer occurrence and spread. In the recent years, however, studies in human cancers showed that the density of B cells, particularly in Tertiary Lymphoid Structures (TLS) in the tumor-adjacent TME correlates with favorable prognosis and predict therapeutic response to ICI even in tumors with low Tumor Mutational Burden (TMB). We will put in perspective the different roles of B cells in cancer immunity, with emphasis on TLS, discuss the mechanisms underlying their effects and their use as prognostic biomarkers and therapeutic targets.

## B CELLS IN TUMOR IMMUNOLOGY: LESSONS FROM MURINE MODELS

The question of the impact of B lymphocytes in the immune control of tumors has been addressed in several murine models, unraveling potential mechanisms of action of B cells in cancer immunity. In a transgenic mouse model of multistage epithelial carcinogenesis in genetically invalidated RAG1mice, the group of LM Coussens (de Visser et al., 2005) observed a high reduction of innate cells infiltrate in premalignant skin and of carcinoma incidence. Both were restored upon transfer of B cells or serum from tumor-bearing immunocompetent mice. The mechanism underlying these effects is that B lymphocytes are activated in tumor-developing mice and produce antibodies that deposit in the precancerous lesions, fueling chronic inflammation through FcγReceptor (FcγR) activation of innate cells migrating into the pre-neoplastic and neoplastic TME. Whereas immune complexes did not activate complement in this model (Medler et al., 2018), complement was found to contribute to pro-tumoral effects through antibody driven chronic inflammation in CMT and TC1 lung cancer models (Roumenina et al., 2019a; Kwak et al., 2018). Immune complexes may also increase angiogenesis via the induction of VEGF production by activated macrophages (Tan and Coussens, 2007). In addition, B cells may inhibit T cell responses in particular via the production of immunosuppressive cytokines (DeNardo et al., 2010). In models of fibrosarcoma and breast cancer, B cell depletion with anti-IgM antibodies highly reduced the incidence of metastases as compared to control mice (Brodt and Gordon, 1978; Barbera-Guillem et al., 2000). In genetically invalidated deficient B cell mice, the growth of several types of tumors including B16 melanoma, EL4 thymoma and MC38 colon carcinoma was also reduced (Qin et al., 1998; Shah et al., 2005). This effect was attributed to the lack of inhibition of the anti-tumor T cell response by B cell produced cytokines such as IL10 (Inoue et al., 2006). Finally, in a model of inflammation-driven Hepatocellular Carcinoma (HCC), B cells rich TLS were found to serve as a niche protecting tumor progenitor cells and favoring the growth of malignant cells via the production of lymphotoxin β (LT-β) (Finkin et al., 2015).

Altogether these observations led to a "bad reputation" for B cells in cancer and, following several decades of unsuccessful search for protective spontaneously produced anti-tumor antibodies, reinforced the paradigm that T cells, particularly $CD8^+$/cytotoxic T cells, were the major, if not the only, component of beneficial anti-tumor immunity. A few reports challenged this statement such as the observation that treatment of tumor bearing mice with a B cell depleting anti-CD20 antibody resulted in a large, more than 2 fold, increase in B16 melanoma volume and number of lung metastases. In this model, B cell depletion impaired induction of Interferon γ (IFNγ) producing Th1

cells (DiLillo et al., 2010) supporting the concept that B cells may be crucial for generating efficient T cell immunity. In conclusion, as in human where each patient is unique, in mice each model is different and speaks for itself.

## B CELLS IN HUMAN CANCERS

The impact of B cells in cancer has been largely unraveled in studies performed in human tumors. The observation that CXCL13, a chemoattractant for B lymphocytes, is a major determinant of favorable prognosis suggests that the presence of B lymphocytes may be crucial. Thus, in Colorectal Cancer (CRC), an unsupervised screening for the impact of chemokine genes expression on cancer outcome revealed that tumors lacking CXCL13 had less intratumoral B cells and a worse prognosis than tumors expressing it (Bindea et al., 2013). The CXCL13 signature was also associated with favorable prognosis in melanoma (Helmink et al., 2020). In Soft Tissue Sarcoma (STS), a high B cell signature significantly correlated with longer OS, independently of the histology of the tumors, whereas there was no correlation between T cell signatures and OS. The B cell signature was the best predictor for OS even when combined with CD8, PD-1 or CTLA-4 signatures (Petitprez et al., 2020)..The fact that Tfh cells, which play a major role in B cell activation, correlate with favorable prognosis in breast (Gu-Trantien et al., 2017) and in head and neck (Cillo et al., 2020) carcinoma also militates for a role of B cells in tumor immunity.

Direct evidence for an impact of B cells came from studies showing that their intra-tumoral density is associated with good prognosis in breast cancer (Mahmoud et al., 2012), CRC (Edin et al., 2019; Berntsson et al., 2018), NSCLC (Germain et al., 2014), head and neck cancer (van Herpen et al., 2008), ovarian cancer (Nielsen et al., 2012; Milne et al., 2009; Santoiemma et al., 2016), biliary tract cancer (Goeppert et al., 2013), primary cutaneous melanoma (Garg et al., 2016), metastatic melanoma (Cabrita et al., 2020), HCC (Shi et al., 2013; Garnelo et al., 2017). In CRC, B cells were found associated with good outcome (Edin et al., 2019; Berntsson et al., 2018). Analysis of clonal diversity of B-cell infiltrates showed that decreased diversity was associated with improved survival in primary cutaneous melanoma but with diminished OS in renal cell carcinoma (Iglesia et al., 2014, 2016; Selitsky et al., 2019). Such clonal expansion is suggestive, but not demonstrative, of the presence of anti-cancer B cells and does not inform about their specificity. The presence of plasma cells in breast cancer (Yeong et al., 2018), at the vicinity of CD8T cells in ovarian cancer, and the expression of their signature in breast (Seow et al., 2020) and ovarian cancer (Nielsen et al., 2012) were associated with favorable outcome (Table 1 and Figure 1). However, an analysis of 54 cohorts of encompassing 25 cancer types, revealed that, although the pronostic impact of tumor infiltrating B cells was positive in 50% of the studies, it was deleterious or neutral in 9% and 41%, respectively (Wouters and Nelson, 2018). A few studies addressed the question of the role of B regulatory cells in human cancers. The frequencies of $CD19^{+}$-IL-$10^{+}$ Bregs correlated with shorter OS in bladder cancer (Zirakzadeh et al., 2020) and in BC (Murakami et al., 2019), the coexistence of Bregs with Treg correlates with shorter metastasis free survival in BC (Ishigami et al., 2019).

The intratumoral B cells undergo isotypic switch and produce IgG or IgA antibodies directed against tumor antigens upon in vitro culture, as shown in NSCLC (Germain et al., 2014) and in ovarian cancer (Montfort et al., 2017). The impact of the isotypes of antibodies produced by intratumoral B cells is critical for their Fc-dependent activities which can be mediated through Fc receptors or through complement activation. Indeed by activating NK cells or macrophages via Antibody Dependent Cellular Cytotoxicity (ADCC) (Clynes and Ravetch, 1995) and macrophages via Antibody Dependent Cellular Phagocytosis (ADCP) (Gul, van Egmond 2015), IgG antibodies may participate in anti-tumor activity, locally and systematically as illustrated by the favorable prognostic impact of circulating IgG anti-MUC1 antibodies in breast, pancreatic and gastric cancers (Hamanaka et al., 2003; Kurtenkov et al., 2007; Fremd et al., 2016). Analysis of RNASeq data from cutaneous melanoma tumors from TCGA also showed an association between high levels of IgG1/IGH transcripts and favorable prognosis (Bolotin et al., 2017). In contrast, in clear cell Renal Cell Cancer (ccRCC), tumor bound IgG participate to the activation of the classical complement pathway in situ, fueling chronic inflammation and yielding poor prognosis. This occurs through the binding of complement component

C1q, originating from macrophages, on tumor-associated IgG antibodies. Recruitment of tumor-cell produced C1r, C1s, C4, C2, C3 and C5 allows complement activation and chronic inflammation (Roumenina et al., 2019a). A protumoral role of B cells has been also been found in squamous cell carcinoma (SCC) (Affara et al., 2014) occurring by deposition of IgG-containing immune complexes that foster Fcγ receptor-dependent activation of myeloid cells and fuel inflammation. In NSCLC the classical pathway is activated in part in an IgM-dependent manner (Kwak et al., 2018), conferring poor prognosis (Ajona et al., 2013). The impact of anti-tumor IgA antibodies on patient's prognosis seems deleterious as suggested by analysis of IgA/IgGH transcripts in melanoma, and by in situ analyses in melanoma and bladder cancer (Bosisio et al., 2016; Welinder et al., 2016). In HCC, $IgA^+$B cells inhibit cytotoxic T cells responses that prevent hepatocarcinogenesis in the inflamed liver (Shalapour et al., 2017). The impact of B cells, although undoubtful, may therefore be a tumor-contexture dependent double edged sword.

**B CELLS AND TERTIARY LYMPHOID STRUCTURES (TLS)**

Comprehensive analyses of the TME in different human cancers led to the observation that in human tumors, B cells were mostly located in TLS. TLS are ectopic lymphoid organs that develop in inflamed tissues in the context of chronic antigen stimulation. In cancers, pro-immunogenic inflammation can also be induced or increased by treatments such as chemo- (Lu et al., 2020; Kuwabara et al., 2019) or radiotherapy (Boivin et al., 2018).TLS are formed in inflamed sites, starting upon contact of IL-7-secreting stromal cells with tissue resident monocytic cells, Th17 or B cells in a CXCL13 rich milieu (Buckley et al., 2015; Nayar et al., 2016; Barone et al., 2016; Jones et al., 2016). CCL21 and CXCL12 participate in lymphocytes recruitment, while CXCL13 and CCL19, together with adhesion molecules, govern the structural organization of the forming TLS (Pitzalis et al., 2014). TLS are sites of generation of immune responses to locally produced antigens, developing even in the absence of lymph nodes in LT-α invalidated mice (Moyron-Quiroz et al., 2006). Mature TLS contain a T cell zone where mature Dendritic Cells (DC) present antigen to T cells and a prominent B cell zone organized in a Germinal Center (GC) with Follicular Dendritic Cells (FDC) and proliferating B cells, expression of Activation Induced Deaminase (AID) and BCL6 allowing class switch and maturation towards plasma cells (Sautès-Fridman et al., 2019). They are surrounded by High Endothelial Venules (HEV) (Ager, 2017).

In 2008, it was reported that TLS were present in NSCLC tumors and that their density correlated with favorable prognosis (Dieu-Nosjean et al., 2008). Subsequently, it was shown that T cells in $TLS^+$ tumors presented a Th1/cytotoxic functional orientation (Goc et al., 2014) and that B cells were also activated in TLS GCs to proliferate and differentiate into plasma cells producing antibodies to tumor-associated antigens. High density of B cell follicles correlated with longer PFS and OS in NSCLC (Germain et al., 2014). Presence of TLS was reported in many cancer types including melanoma (Ladányi et al., 2007), bladder (Zirakzadeh et al., 2020), colorectal (Di Caro et al., 2014; Posch et al., 2017), gastric (Yamakoshi et al., 2020), gastrointestinal stromal tumors (GIST) (Lin et al., 2020), HCC (Li et al., 2020a; Calderaro et al., 2019) ovarian (Kroeger et al., 2016), oral (Li et al., 2020b), squamous lung (Siliņa et al., 2018), and pancreatic carcinomas (Hiraoka et al., 2015)(Table 1 and Figure 1),(reviewed in (Sautès-Fridman et al., 2019)). In general, the presence of TLS inside or adjacent to tumor nests correlated with favorable prognosis (Sautès-Fridman et al., 2019). The degree of TLS maturation seems to be important since GC-containing TLS were the best predictors of lack of cancer recurrence in stage II/III CRC (Posch et al., 2017), SCC (Siliņa et al., 2018) and HCC (Calderaro et al., 2019) whereas pre-cancerous lesions with immature TLS progressed toward malignancy (Meylan et al., 2020). Indeed, in pre-malignant early stages of HCC, TLS found in high grade dysplastic and early HCC nodules were immature, in the form of lymphoid aggregates without a fully matured GC. Their presence correlated with markers of inflammation, immunosuppression and immune exhaustion which may favor transition to HCC (Meylan et al., 2020). This finding is reminiscent of the work of Finkin et al (Finkin et al., 2015) showing that TLS may serve as niches protecting tumor cells in a murine model of HCC. Together with the reports in murine models of premalignant stages (de Visser et al., 2005), they support a different impact of TLS and B cells according to the stage of cancer. Altogether, these works indicate that TLS may shape cancer controlling immunity and that B cells inside GCs are important in this process and influence clinical outcome.

## B CELLS PREDICT THERAPEUTIC RESPONSE TO IMMUNOTHERAPY BY ICI

Whereas immunotherapy using antibodies to check-point inhibitors (ICI) aims to to reinvigorate effector T lymphocytes, several publications pointed to the fact that B cells may be major players of therapeutic efficacy.

In STS, B cell and plasma cell signatures were pathognomonic of an immune rich Sarcoma Immune Class (SIC) also characterized by the presence of TLS. Patients with this SIC highly responded (50%) to Pembrolizumab, an anti-PD-1 antibody whereas none of the patients from immune desert classes responded (Petitprez et al., 2020). This finding may change the medical care of STS patients who were considered as poor responders to ICI. It has generated a prospective clinical trial in which patients to be treated are selected on the basis of B cell rich TLS (ClinicalTrials.gov Identifier: NCT02406781). In melanoma, TLS and B cell signatures, and not T cell signatures, predicted therapeutic responses to Pembrolizumab and Ipilimumab, an anti-CTLA-4 antibody. B cells in tumors of responding patients exhibited oligoclonal repertoires of the Ig genes as compared to the polyclonal B cell repertoires of non-responding patients. Moreover, B cells and TLS densities increased during treatment in responding, but not in non-responding, patients (Helmink et al., 2020) and a TLS gene signature synergized with a T effector signature to predict responses to ICI with anti-PD-1 and anti-CTLA-4 antibodies (Cabrita et al., 2020). In contrast, Breg signature was associated with lack of response to anti-CTLA-4 in cutaneous melanoma (Selitsky et al., 2019).

Not all B cell subtypes are likely to participate in response to ICI, and recent data showed that plasmablasts are in particular more frequent in responder patients (Griss et al., 2019). Indeed multi-omics predictions are not absolute but provide significant predictive correlations which a open a new field of investigations integrating B cells as major players and potential targets of novel immunotherapeutic approaches. Single-cell transcriptomics analysis of tumor-infiltrating B cells allowed to find transcriptomic programs that are specifically expressed in B cell infiltrating ICI-responsive tumors (Helmink et al., 2020). Such analyses may pave the way to better understanding how each B cell subtype contributes to patient survival and response to ICI.

## B CELLS AND CANCER: MECHANISMS OF ACTION

The functions of B cells are multiple and, although a truly mechanistic aspect of their impact is still missing, Figure 2 illustrates potential mechanisms that may operate in the participation of B cell to tumor immunity.

**Antigen presentation**:

B cells recognize and internalize native proteins and glycoproteins via the BCR. They internalize the proteins thanks to the Immuno Tyrosine Activation Motif (ITAM) of the CD79$\alpha$ and $\beta$ signaling chains associated with the antigen-recognizing Ig within the BCR. Subsequent cytoplasmic processing of the proteins allows antigenic peptide association with MHCII molecules and presentation of the complex to CD4 T helper cells and with MHCI associated peptides to CD8T cells. This mechanism of T cell activation occurs in lymph nodes but also in TLS (Bruno et al., 2017; Garaud et al., 2019; Wouters and Nelson, 2018). Close contact between BCR-bound antigen and DCs favors antigen transfer to the latter and supports efficient antigen presentation to T cells (Harvey et al., 2014). In addition, B cells can capture immune complexes via complement receptors and transfer these antigens to FDCs (Phan et al., 2007), increasing the germinal center response. In tumors, B cells may therefore enhance tumor-associated antigen presentation to proximal T cells resulting in a stronger T cell response.

In TLS GCs, plasma cells are generated and produce IgG antibodies to tumor-associated antigens, the immune complexes formed being internalized by DCs which process the antigens and present them in a very efficient way to CD4 and CD8 T cells. It is known that the quantity of antigen necessary to induce a T cell response is much lower (1000-10 000 times) when internalized via an immune complex than its native counterpart (Kalergis and Ravetch, 2002). This mechanism may explain why B cells are crucial to obtain efficient T cell responses in poorly mutated tumors in which the antigenic load is low and therefore not sufficient to directly activate T cells. It may be the case in STS, ovarian

cancer or RCC. It may also amplify T cell responses in tumors with high TMB such as melanoma or NSCLC.

Another important modulator of B cell activation is complement which can be activated on dying tumor cells, as recently shown in breast cancer tumors in response to chemotherapy. Through binding to CR2 expressed by B cells, complement cleavage product C3b induces the generation of an ICOS-$L^+$ B cell subset which boosts T cell immunity by enhancing tumor specific CD8T cells and the Th1/Treg ratio. Emergence of this B cell subset is associated with improved therapeutic efficacy of neo-adjuvant chemotherapy in BC, particularly in triple negative tumors, and with prolonged survival of the patients (Lu et al., 2020; Sautès-Fridman and Roumenina, 2020).

**Antibody production :** In addition to amplifying T cell immunity via antigen presentation, antibodies produced by plasma cells in TLS GCs exert effector functions. Antibodies directed against tumor -associated antigens such as LAGE-1, MAGE antigens and NY-ESO-1 were detected in supernatants of tumor infiltrating B cells from half of the patients in NSCLC (Germain et al., 2014). Antibodies against the tumor-associated antigen MUC1 overexpressed in tumors under an unglycosylated form and against ganglioside GD3, CEA, MUC1 and FN1 have been detected in BC (Montfort et al., 2017; Coronella et al., 2002; Garaud et al., 2019; Pavoni et al., 2007). These are tumor -associated rather than cancer-specific antibodies (such as mutated RAS) but they may be efficient in anti-tumor responses since they recognize and bind to membrane antigens expressed by tumor cells. One of the interests of B cells for responses to immunotherapy is, that although effector T cells almost always recognize patient's selective private neo-antigens (Tran et al., 2017), B cells and the antibodies they produce may recognize shared tumor-associated antigens (Heesters et al., 2016). IgG antibodies bind to Fcγ Receptors on NK cells and macrophages, activating them to destroy tumor cells via ADCC.

ADCC is very efficient to kill target cells even when they have a low antigen load. It may however be only poorly operating in solid tumors through NK cells are scarce and often anergic (Platonova et al., 2011). Macrophages, which are often the most prominent tumor infiltrating hematopietic cells, may act as effector cells, inducing the killing of tumor cells via ADCC (Clynes and Ravetch, 1995) or their phagocytosis, via ADCP (Gül and van Egmond, 2015). Following ADCP, macrophages may also upregulate PD-L1 and IDO and support local immunosuppression (Su et al., 2018). In addition, macrophages may be immunosuppressive cells through the production of pro-angiogenic (VEGF) or immunosuppressive (TGFβ) cytokines (Campa et al., 2015; Amornsiripanitch et al., 2010). By activating macrophages, IgG immune complexes may also support chronic inflammation (Clynes and Ravetch, 1995; Sylvestre et al., 1996), angiogenesis and immunosuppression, thus favoring tumor growth. A similar mechanism may operate when tumor cell bound IgG activate locally produced complement with the production of the anaphylatoxins, pro-angiogenic and pro-inflammatory complement components C3a and C5a (Roumenina et al., 2019b). Complement activation could also lead to tumor cell killing but it seems unlikely since malignant tumor cells in solid tumors are generally equipped with complement inhibiting molecules (CD46, CD55, CD59) (Roumenina et al., 2019b). Interestingly, antibodies against the complement regulator Factor H (FH) were found in patients with NSCLC and were shown to protect against tumor progression (Campa et al., 2015; Amornsiripanitch et al., 2010). These antibodies recognize a FH neoantigen, produced by the tumor cells. B cells from anti-FH positive patients were sorted and used to select an antibody with therapeutic properties in mouse models (Bushey et al., 2016). Although it was suggested that the anti-FH antibodies stimulate complement-mediated cancer cells killing, the classical pathway is deleterious in NSCLC (Ajona et al., 2013; Kwak et al., 2018), indicating that further studies are needed to unravel the complex mechanisms by which the B-cells and produced immunoglobulins affect tumor progression.

In general, the IgA isotype is often characteristic of the Breg/Treg circuit that regulate mucosal inflammation, Treg cells producing Transforming Growth Factor-β (TGFβ), which mediates isotype class switching to IgA (Stavnezer and Kang, 2009). In HCC, $IgA^+$ B cells inhibit cytotoxic T cells responses that prevent hepatocarcinogenesis in the inflamed liver (Shalapour et al., 2017).

In addition, a few studies suggest that activated B cells may directly induce tumor cell apoptosis (Garnelo et al., 2017; Jahrsdörfer et al., 2006).

**Regulatory role**

Tumor B cell produce anti-tumor antibodies of IgA isotype as shown in NSCLC (Germain). These IgA producing B cells were shown to modulate CD8T cells cytotoxicity in HCC mouse model

(Shalapour). Through production of immunosuppressive cytokines such as IL-10 (Shen and Fillatreau, 2015), Breg may locally inhibit T cell activation toward tumor-associated antigens .

## CONCLUDING REMARKS

In conclusion, B cells may be a double edge sword, resulting either in tumor cell destruction by increasing T cell responses and via ADCC or in tumor growth by fueling chronic inflammation, angiogenesis or immunosuppression via immune complex formation or complement activation. However, we still poorly understand the heterogeneity and diversity of B cell subsets in tumors, which posts a major obstacle and dilemma to target B cells as oncological treatments. In addition, as for T cells, most intratumoral B cells may be bystanders and not anti-tumoral. The respective importance of bystander and cancer-specific B cells needs to be further evaluated in this emerging field.

The exploding investigations in this new field will not only provide a better understanding of anti-tumor immunity but also propose novel prognostic and predictive markers as well as novel therapeutic targets. Thus, the analysis of intratumoral B cells by single cell technology, the identification of their Ig repertoire and the characterization of the antibodies they produce in long survivors immunotherapy responder patients will permit to design new therapeutic mononoclonal antibodies. We are at the very beginning of a newly open and very promising avenue.

## REFERENCES


Affara, N.I., B. Ruffell, T.R. Medler, A.J. Gunderson, M. Johansson, S. Bornstein, E. Bergsland, M. Steinhoff, Y. Li, Q. Gong, Y. Ma, J.F. Wiesen, M.H. Wong, M. Kulesz-Martin, B. Irving, and L.M. Coussens. 2014. B cells regulate macrophage phenotype and response to chemotherapy in squamous carcinomas. *Cancer Cell*. 25:809–821. doi:10.1016/j.ccr.2014.04.026.

Ager, A. 2017. High Endothelial Venules and Other Blood Vessels: Critical Regulators of Lymphoid Organ Development and Function. *Front Immunol*. 8:45. doi:10.3389/fimmu.2017.00045.

Ajona, D., M.J. Pajares, L. Corrales, J.L. Perez-Gracia, J. Agorreta, M.D. Lozano, W. Torre, P.P. Massion, J.P. de-Torres, E. Jantus-Lewintre, C. Camps, J.J. Zulueta, L.M. Montuenga, and R. Pio. 2013. Investigation of complement activation product c4d as a diagnostic and prognostic biomarker for lung cancer. *J. Natl. Cancer Inst.* 105:1385–1393. doi:10.1093/jnci/djt205.

Amornsiripanitch, N., S. Hong, M.J. Campa, M.M. Frank, E.B. Gottlin, and E.F. Patz. 2010. Complement factor H autoantibodies are associated with early stage NSCLC. *Clin. Cancer Res.* 16:3226–3231. doi:10.1158/1078-0432.CCR-10-0321.

Barbera-Guillem, E., M.B. Nelson, B. Barr, J.K. Nyhus, K.F. May, L. Feng, and J.W. Sampsel. 2000. B lymphocyte pathology in human colorectal cancer. Experimental and clinical therapeutic effects of partial B cell depletion. *Cancer Immunol. Immunother.* 48:541–549. doi:10.1007/pl00006672.

Barone, F., D.H. Gardner, S. Nayar, N. Steinthal, C.D. Buckley, and S.A. Luther. 2016. Stromal Fibroblasts in Tertiary Lymphoid Structures: A Novel Target in Chronic Inflammation. *Front Immunol*. 7:477. doi:10.3389/fimmu.2016.00477.

Berntsson, J., J. Eberhard, B. Nodin, K. Leandersson, A.H. Larsson, and K. Jirström. 2018. Expression of programmed cell death protein 1 (PD-1) and its ligand PD-L1 in colorectal cancer: Relationship with sidedness and prognosis. *Oncoimmunology*. 7:e1465165. doi:10.1080/2162402X.2018.1465165.

Bindea, G., B. Mlecnik, M. Tosolini, A. Kirilovsky, M. Waldner, A.C. Obenauf, H. Angell, T. Fredriksen, L. Lafontaine, A. Berger, P. Bruneval, W.H. Fridman, C. Becker, F. Pagès, M.R. Speicher, Z. Trajanoski, and J. Galon. 2013. Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. *Immunity*. 39:782–795.

doi:10.1016/j.immuni.2013.10.003.
Boivin, G., P. Kalambaden, J. Faget, S. Rusakiewicz, P. Montay-Gruel, E. Meylan, J. Bourhis, G. Lesec, and M.-C. Vozenin. 2018. Cellular Composition and Contribution of Tertiary Lymphoid Structures to Tumor Immune Infiltration and Modulation by Radiation Therapy. *Front. Oncol.* 8. doi:10.3389/fonc.2018.00256.
Bolotin, D.A., S. Poslavsky, A.N. Davydov, F.E. Frenkel, L. Fanchi, O.I. Zolotareva, S. Hemmers, E.V. Putintseva, A.S. Obraztsova, M. Shugay, R.I. Ataullakhanov, A.Y. Rudensky, T.N. Schumacher, and D.M. Chudakov. 2017. Antigen receptor repertoire profiling from RNA-seq data. *Nature Biotechnology*. 35:908–911. doi:10.1038/nbt.3979.
Bosisio, F.M., J.S. Wilmott, N. Volders, M. Mercier, J. Wouters, M. Stas, W.A. Blokx, D. Massi, J.F. Thompson, R.A. Scolyer, N. van Baren, and J.J. van den Oord. 2016. Plasma cells in primary melanoma. Prognostic significance and possible role of IgA. *Mod. Pathol.* 29:347–358. doi:10.1038/modpathol.2016.28.
Brodt, P., and J. Gordon. 1978. Anti-tumor immunity in B lymphocyte-deprived mice. I. Immunity to a chemically induced tumor. *J. Immunol.* 121:359–362.
Bruno, T.C., P.J. Ebner, B.L. Moore, O.G. Squalls, K.A. Waugh, E.B. Eruslanov, S. Singhal, J.D. Mitchell, W.A. Franklin, D.T. Merrick, M.D. McCarter, B.E. Palmer, J.A. Kern, and J.E. Slansky. 2017. Antigen-Presenting Intratumoral B Cells Affect CD4+ TIL Phenotypes in Non-Small Cell Lung Cancer Patients. *Cancer Immunol Res*. 5:898–907. doi:10.1158/2326-6066.CIR-17-0075.
Buckley, C.D., F. Barone, S. Nayar, C. Bénézech, and J. Caamaño. 2015. Stromal cells in chronic inflammation and tertiary lymphoid organ formation. *Annu. Rev. Immunol.* 33:715–745. doi:10.1146/annurev-immunol-032713-120252.
Bushey, R.T., M.A. Moody, N.L. Nicely, B.F. Haynes, S.M. Alam, S.T. Keir, R.C. Bentley, K. Roy Choudhury, E.B. Gottlin, M.J. Campa, H.-X. Liao, and E.F. Patz. 2016. A Therapeutic Antibody for Cancer, Derived from Single Human B Cells. *Cell Rep*. 15:1505–1513. doi:10.1016/j.celrep.2016.04.038.
Cabrita, R., M. Lauss, A. Sanna, M. Donia, M.S. Larsen, S. Mitra, I. Johansson, B. Phung, K. Harbst, J. Vallon-Christersson, A. van Schoiack, K. Lövgren, S. Warren, K. Jirström, H. Olsson, K. Pietras, C. Ingvar, K. Isaksson, D. Schadendorf, H. Schmidt, L. Bastholt, A. Carneiro, J.A. Wargo, I.M. Svane, and G. Jönsson. 2020. Tertiary lymphoid structures improve immunotherapy and survival in melanoma. *Nature*. 577:561–565. doi:10.1038/s41586-019-1914-8.
Calderaro, J., F. Petitprez, E. Becht, A. Laurent, T.Z. Hirsch, B. Rousseau, A. Luciani, G. Amaddeo, J. Derman, C. Charpy, J. Zucman-Rossi, W.H. Fridman, and C. Sautès-Fridman. 2019. Intra-tumoral tertiary lymphoid structures are associated with a low risk of early recurrence of hepatocellular carcinoma. *Journal of Hepatology*. 70:58–65. doi:10.1016/j.jhep.2018.09.003.
Campa, M.J., E.B. Gottlin, R.T. Bushey, and E.F. Patz. 2015. Complement Factor H Antibodies from Lung Cancer Patients Induce Complement-Dependent Lysis of Tumor Cells, Suggesting a Novel Immunotherapeutic Strategy. *Cancer Immunol Res*. 3:1325–1332. doi:10.1158/2326-6066.CIR-15-0122.
Cillo, A.R., C.H.L. Kürten, T. Tabib, Z. Qi, S. Onkar, T. Wang, A. Liu, U. Duvvuri, S. Kim, R.J. Soose, S. Oesterreich, W. Chen, R. Lafyatis, T.C. Bruno, R.L. Ferris, and D.A.A. Vignali. 2020. Immune Landscape of Viral- and Carcinogen-Driven Head and Neck Cancer. *Immunity*. 52:183-199.e9. doi:10.1016/j.immuni.2019.11.014.
Clynes, R., and J.V. Ravetch. 1995. Cytotoxic antibodies trigger inflammation through Fc receptors. *Immunity*. 3:21–26. doi:10.1016/1074-7613(95)90155-8.
Coronella, J.A., C. Spier, M. Welch, K.T. Trevor, A.T. Stopeck, H. Villar, and E.M. Hersh. 2002. Antigen-Driven Oligoclonal Expansion of Tumor-Infiltrating B Cells in Infiltrating

Ductal Carcinoma of the Breast. *The Journal of Immunology*. 169:1829–1836. doi:10.4049/jimmunol.169.4.1829.
DeNardo, D.G., P. Andreu, and L.M. Coussens. 2010. Interactions between lymphocytes and myeloid cells regulate pro- versus anti-tumor immunity. *Cancer Metastasis Rev.* 29:309–316. doi:10.1007/s10555-010-9223-6.
Di Caro, G., F. Bergomas, F. Grizzi, A. Doni, P. Bianchi, A. Malesci, L. Laghi, P. Allavena, A. Mantovani, and F. Marchesi. 2014. Occurrence of tertiary lymphoid tissue is associated with T-cell infiltration and predicts better prognosis in early-stage colorectal cancers. *Clin. Cancer Res.* 20:2147–2158. doi:10.1158/1078-0432.CCR-13-2590.
Dieu-Nosjean, M.-C., M. Antoine, C. Danel, D. Heudes, M. Wislez, V. Poulot, N. Rabbe, L. Laurans, E. Tartour, L. de Chaisemartin, S. Lebecque, W.-H. Fridman, and J. Cadranel. 2008. Long-term survival for patients with non-small-cell lung cancer with intratumoral lymphoid structures. *J. Clin. Oncol.* 26:4410–4417. doi:10.1200/JCO.2007.15.0284.
DiLillo, D.J., K. Yanaba, and T.F. Tedder. 2010. B cells are required for optimal CD4+ and CD8+ T cell tumor immunity: therapeutic B cell depletion enhances B16 melanoma growth in mice. *J. Immunol.* 184:4006–4016. doi:10.4049/jimmunol.0903009.
Edin, S., T. Kaprio, J. Hagström, P. Larsson, H. Mustonen, C. Böckelman, K. Strigård, U. Gunnarsson, C. Haglund, and R. Palmqvist. 2019. The Prognostic Importance of CD20+ B lymphocytes in Colorectal Cancer and the Relation to Other Immune Cell subsets. *Sci Rep*. 9:19997. doi:10.1038/s41598-019-56441-8.
Finkin, S., D. Yuan, I. Stein, K. Taniguchi, A. Weber, K. Unger, J.L. Browning, N. Goossens, S. Nakagawa, G. Gunasekaran, M.E. Schwartz, M. Kobayashi, H. Kumada, M. Berger, O. Pappo, K. Rajewsky, Y. Hoshida, M. Karin, M. Heikenwalder, Y. Ben-Neriah, and E. Pikarsky. 2015. Ectopic lymphoid structures function as microniches for tumor progenitor cells in hepatocellular carcinoma. *Nature Immunology*. 16:1235–1244. doi:10.1038/ni.3290.
Fremd, C., S. Stefanovic, P. Beckhove, M. Pritsch, H. Lim, M. Wallwiener, J. Heil, M. Golatta, J. Rom, C. Sohn, A. Schneeweiss, F. Schuetz, and C. Domschke. 2016. Mucin 1-specific B cell immune responses and their impact on overall survival in breast cancer patients. *Oncoimmunology*. 5:e1057387. doi:10.1080/2162402X.2015.1057387.
Fridman, W.H., F. Pagès, C. Sautès-Fridman, and J. Galon. 2012. The immune contexture in human tumours: impact on clinical outcome. *Nat. Rev. Cancer*. 12:298–306. doi:10.1038/nrc3245.
Fridman, W.H., L. Zitvogel, C. Sautès-Fridman, and G. Kroemer. 2017. The immune contexture in cancer prognosis and treatment. *Nat Rev Clin Oncol*. 14:717–734. doi:10.1038/nrclinonc.2017.101.
Galon, J., A. Costes, F. Sanchez-Cabo, A. Kirilovsky, B. Mlecnik, C. Lagorce-Pagès, M. Tosolini, M. Camus, A. Berger, P. Wind, F. Zinzindohoué, P. Bruneval, P.-H. Cugnenc, Z. Trajanoski, W.-H. Fridman, and F. Pagès. 2006. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science*. 313:1960–1964. doi:10.1126/science.1129139.
Garaud, S., L. Buisseret, C. Solinas, C. Gu-Trantien, A. de Wind, G. Van den Eynden, C. Naveaux, J.-N. Lodewyckx, A. Boisson, H. Duvillier, L. Craciun, L. Ameye, I. Veys, M. Paesmans, D. Larsimont, M. Piccart-Gebhart, and K. Willard-Gallo. 2019. Tumor infiltrating B-cells signal functional humoral immune responses in breast cancer. *JCI Insight*. 5. doi:10.1172/jci.insight.129641.
Garg, K., M. Maurer, J. Griss, M.-C. Brüggen, I.H. Wolf, C. Wagner, N. Willi, K.D. Mertz, and S.N. Wagner. 2016. Tumor-associated B cells in cutaneous primary melanoma and improved clinical outcome. *Hum. Pathol.* 54:157–164. doi:10.1016/j.humpath.2016.03.022.
Garnelo, M., A. Tan, Z. Her, J. Yeong, C.J. Lim, J. Chen, K.H. Lim, A. Weber, P. Chow, A. Chung, L.L.P. Ooi, H.C. Toh, M. Heikenwalder, I.O.L. Ng, A. Nardin, Q. Chen, J.-P.

Abastado, and V. Chew. 2017. Interaction between tumour-infiltrating B cells and T cells controls the progression of hepatocellular carcinoma. *Gut*. 66:342–351. doi:10.1136/gutjnl-2015-310814.
Germain, C., S. Gnjatic, F. Tamzalit, S. Knockaert, R. Remark, J. Goc, A. Lepelley, E. Becht, S. Katsahian, G. Bizouard, P. Validire, D. Damotte, M. Alifano, P. Magdeleinat, I. Cremer, J.-L. Teillaud, W.-H. Fridman, C. Sautès-Fridman, and M.-C. Dieu-Nosjean. 2014. Presence of B cells in tertiary lymphoid structures is associated with a protective immunity in patients with lung cancer. *Am. J. Respir. Crit. Care Med.* 189:832–844. doi:10.1164/rccm.201309-1611OC.
Goc, J., C. Germain, T.K.D. Vo-Bourgais, A. Lupo, C. Klein, S. Knockaert, L. de Chaisemartin, H. Ouakrim, E. Becht, M. Alifano, P. Validire, R. Remark, S.A. Hammond, I. Cremer, D. Damotte, W.-H. Fridman, C. Sautès-Fridman, and M.-C. Dieu-Nosjean. 2014. Dendritic cells in tumor-associated tertiary lymphoid structures signal a Th1 cytotoxic immune contexture and license the positive prognostic value of infiltrating CD8+ T cells. *Cancer Res.* 74:705–715. doi:10.1158/0008-5472.CAN-13-1342.
Goeppert, B., L. Frauenschuh, M. Zucknick, A. Stenzinger, M. Andrulis, F. Klauschen, K. Joehrens, A. Warth, M. Renner, A. Mehrabi, M. Hafezi, A. Thelen, P. Schirmacher, and W. Weichert. 2013. Prognostic impact of tumour-infiltrating immune cells on biliary tract cancer. *Br. J. Cancer*. 109:2665–2674. doi:10.1038/bjc.2013.610.
Griss, J., W. Bauer, C. Wagner, M. Simon, M. Chen, K. Grabmeier-Pfistershammer, M. Maurer-Granofszky, F. Roka, T. Penz, C. Bock, G. Zhang, M. Herlyn, K. Glatz, H. Läubli, K.D. Mertz, P. Petzelbauer, T. Wiesner, M. Hartl, W.F. Pickl, R. Somasundaram, P. Steinberger, and S.N. Wagner. 2019. B cells sustain inflammation and predict response to immune checkpoint blockade in human melanoma. *Nat Commun*. 10:4186. doi:10.1038/s41467-019-12160-2.
Gül, N., and M. van Egmond. 2015. Antibody-Dependent Phagocytosis of Tumor Cells by Macrophages: A Potent Effector Mechanism of Monoclonal Antibody Therapy of Cancer. *Cancer Res.* 75:5008–5013. doi:10.1158/0008-5472.CAN-15-1330.
Gu-Trantien, C., E. Migliori, L. Buisseret, A. de Wind, S. Brohée, S. Garaud, G. Noël, L.D. C.V., J.-N. Lodewyckx, C. Naveaux, H. Duvillier, S. Goriely, D. Larsimont, and K. Willard-Gallo. 2017. CXCL13-producing TFH cells link immune suppression and adaptive memory in human breast cancer. *JCI Insight*. 2. doi:10.1172/jci.insight.91487.
Hamanaka, Y., Y. Suehiro, M. Fukui, K. Shikichi, K. Imai, and Y. Hinoda. 2003. Circulating anti-MUC1 IgG antibodies as a favorable prognostic factor for pancreatic cancer. *Int. J. Cancer*. 103:97–100. doi:10.1002/ijc.10801.
Harvey, B.P., M.T. Raycroft, T.E. Quan, B.J. Rudenga, R.M. Roman, J. Craft, and M.J. Mamula. 2014. Transfer of antigen from human B cells to dendritic cells. *Mol. Immunol.* 58:56–65. doi:10.1016/j.molimm.2013.10.013.
Heesters, B.A., C.E. van der Poel, A. Das, and M.C. Carroll. 2016. Antigen Presentation to B Cells. *Trends in Immunology*. 37:844–854. doi:10.1016/j.it.2016.10.003.
Helmink, B.A., S.M. Reddy, J. Gao, S. Zhang, R. Basar, R. Thakur, K. Yizhak, M. Sade-Feldman, J. Blando, G. Han, V. Gopalakrishnan, Y. Xi, H. Zhao, R.N. Amaria, H.A. Tawbi, A.P. Cogdill, W. Liu, V.S. LeBleu, F.G. Kugeratski, S. Patel, M.A. Davies, P. Hwu, J.E. Lee, J.E. Gershenwald, A. Lucci, R. Arora, S. Woodman, E.Z. Keung, P.-O. Gaudreau, A. Reuben, C.N. Spencer, E.M. Burton, L.E. Haydu, A.J. Lazar, R. Zapassodi, C.W. Hudgens, D.A. Ledesma, S. Ong, M. Bailey, S. Warren, D. Rao, O. Krijgsman, E.A. Rozeman, D. Peeper, C.U. Blank, T.N. Schumacher, L.H. Butterfield, M.A. Zelazowska, K.M. McBride, R. Kalluri, J. Allison, F. Petitprez, W.H. Fridman, C. Sautès-Fridman, N. Hacohen, K. Rezvani, P. Sharma, M.T. Tetzlaff, L. Wang, and J.A. Wargo. 2020. B cells and tertiary lymphoid structures promote immunotherapy response. *Nature*. 577:549–555. doi:10.1038/s41586-019-

1922-8.
van Herpen, C.M.L., R. van der Voort, J.A.W.M. van der Laak, I.S. Klasen, A.O. de Graaf, L.C.L. van Kempen, I.J.M. de Vries, T.D. Boer, H. Dolstra, R. Torensma, J.H. van Krieken, G.J. Adema, and P.H.M. De Mulder. 2008. Intratumoral rhIL-12 administration in head and neck squamous cell carcinoma patients induces B cell activation. *Int. J. Cancer*. 123:2354–2361. doi:10.1002/ijc.23756.
Hiraoka, N., Y. Ino, R. Yamazaki-Itoh, Y. Kanai, T. Kosuge, and K. Shimada. 2015. Intratumoral tertiary lymphoid organ is a favourable prognosticator in patients with pancreatic cancer. *Br. J. Cancer*. 112:1782–1790. doi:10.1038/bjc.2015.145.
Hirsch, L., L. Zitvogel, A. Eggermont, and A. Marabelle. 2019. PD-Loma: a cancer entity with a shared sensitivity to the PD-1/PD-L1 pathway blockade. *British Journal of Cancer*. 120:3–5. doi:10.1038/s41416-018-0294-4.
Iglesia, M.D., J.S. Parker, K.A. Hoadley, J.S. Serody, C.M. Perou, and B.G. Vincent. 2016. Genomic Analysis of Immune Cell Infiltrates Across 11 Tumor Types. *J. Natl. Cancer Inst.* 108. doi:10.1093/jnci/djw144.
Iglesia, M.D., B.G. Vincent, J.S. Parker, K.A. Hoadley, L.A. Carey, C.M. Perou, and J.S. Serody. 2014. Prognostic B-cell signatures using mRNA-seq in patients with subtype-specific breast and ovarian cancer. *Clin. Cancer Res.* 20:3818–3829. doi:10.1158/1078-0432.CCR-13-3368.
Inoue, S., W.W. Leitner, B. Golding, and D. Scott. 2006. Inhibitory effects of B cells on antitumor immunity. *Cancer Res.* 66:7741–7747. doi:10.1158/0008-5472.CAN-05-3766.
Ishigami, E., M. Sakakibara, J. Sakakibara, T. Masuda, H. Fujimoto, S. Hayama, T. Nagashima, T. Sangai, A. Nakagawa, Y. Nakatani, and M. Otsuka. 2019. Coexistence of regulatory B cells and regulatory T cells in tumor-infiltrating lymphocyte aggregates is a prognostic factor in patients with breast cancer. *Breast Cancer*. 26:180–189. doi:10.1007/s12282-018-0910-4.
Jahrsdörfer, B., S.E. Blackwell, J.E. Wooldridge, J. Huang, M.W. Andreski, L.S. Jacobus, C.M. Taylor, and G.J. Weiner. 2006. B-chronic lymphocytic leukemia cells and other B cells can produce granzyme B and gain cytotoxic potential after interleukin-21-based activation. *Blood*. 108:2712–2719. doi:10.1182/blood-2006-03-014001.
Jones, G.W., D.G. Hill, and S.A. Jones. 2016. Understanding Immune Cells in Tertiary Lymphoid Organ Development: It Is All Starting to Come Together. *Front Immunol*. 7:401. doi:10.3389/fimmu.2016.00401.
Kalergis, A.M., and J.V. Ravetch. 2002. Inducing tumor immunity through the selective engagement of activating Fcgamma receptors on dendritic cells. *J. Exp. Med.* 195:1653–1659. doi:10.1084/jem.20020338.
Kroeger, D.R., K. Milne, and B.H. Nelson. 2016. Tumor-Infiltrating Plasma Cells Are Associated with Tertiary Lymphoid Structures, Cytolytic T-Cell Responses, and Superior Prognosis in Ovarian Cancer. *Clin. Cancer Res.* 22:3005–3015. doi:10.1158/1078-0432.CCR-15-2762.
Kurtenkov, O., K. Klaamas, S. Mensdorff-Pouilly, L. Miljukhina, L. Shljapnikova, and V. Chuzmarov. 2007. Humoral immune response to MUC1 and to the Thomsen-Friedenreich (TF) glycotope in patients with gastric cancer: relation to survival. *Acta Oncol*. 46:316–323. doi:10.1080/02841860601055441.
Kuwabara, S., T. Tsuchikawa, T. Nakamura, Y. Hatanaka, K.C. Hatanaka, K. Sasaki, M. Ono, K. Umemoto, T. Suzuki, O. Sato, Y. Hane, Y. Nakanishi, T. Asano, Y. Ebihara, Y. Kurashima, T. Noji, S. Murakami, K. Okamura, T. Shichinohe, and S. Hirano. 2019. Prognostic relevance of tertiary lymphoid organs following neoadjuvant chemoradiotherapy in pancreatic ductal adenocarcinoma. *Cancer Sci.* 110:1853–1862. doi:10.1111/cas.14023.
Kwak, J.W., J. Laskowski, H.Y. Li, M.V. McSharry, T.R. Sippel, B.L. Bullock, A.M.

Johnson, J.M. Poczobutt, A.J. Neuwelt, S.P. Malkoski, M.C. Weiser-Evans, J.D. Lambris, E.T. Clambey, J.M. Thurman, and R.A. Nemenoff. 2018. Complement Activation via a C3a Receptor Pathway Alters CD4+ T Lymphocytes and Mediates Lung Cancer Progression. *Cancer Res.* 78:143–156. doi:10.1158/0008-5472.CAN-17-0240.
Ladányi, A., J. Kiss, B. Somlai, K. Gilde, Z. Fejős, A. Mohos, I. Gaudi, and J. Tímár. 2007. Density of DC-LAMP+ mature dendritic cells in combination with activated T lymphocytes infiltrating primary cutaneous melanoma is a strong independent prognostic factor. *Cancer Immunol Immunother*. 56:1459–1469. doi:10.1007/s00262-007-0286-3.
Li, H., J. Wang, H. Liu, T. Lan, L. Xu, G. Wang, K. Yuan, and H. Wu. 2020a. Existence of intratumoral tertiary lymphoid structures is associated with immune cells infiltration and predicts better prognosis in early-stage hepatocellular carcinoma. *Aging (Albany NY)*. 12. doi:10.18632/aging.102821.
Li, K., Q. Guo, X. Zhang, X. Dong, W. Liu, A. Zhang, Y. Li, J. Yan, G. Jia, Z. Zheng, W. Tang, L. Pan, M. An, B. Zhang, S. Liu, and B. Fu. 2020b. Oral cancer-associated tertiary lymphoid structures: gene expression profile and prognostic value. *Clin. Exp. Immunol.* 199:172–181. doi:10.1111/cei.13389.
Lin, Q., P. Tao, J. Wang, L. Ma, Q. Jiang, J. Li, G. Zhang, J. Liu, Y. Zhang, Y. Hou, W. Lu, R. Xue, and H. Tong. 2020. Tumor-associated tertiary lymphoid structure predicts postoperative outcomes in patients with primary gastrointestinal stromal tumors. *Oncoimmunology*. 9:1747339. doi:10.1080/2162402X.2020.1747339.
Lu, Y., Q. Zhao, J.-Y. Liao, E. Song, Q. Xia, J. Pan, Y. Li, J. Li, B. Zhou, Y. Ye, C. Di, S. Yu, Y. Zeng, and S. Su. 2020. Complement Signals Determine Opposite Effects of B Cells in Chemotherapy-Induced Immunity. *Cell*. doi:10.1016/j.cell.2020.02.015.
Mahmoud, S.M.A., A.H.S. Lee, E.C. Paish, R.D. Macmillan, I.O. Ellis, and A.R. Green. 2012. The prognostic significance of B lymphocytes in invasive carcinoma of the breast. *Breast Cancer Res. Treat.* 132:545–553. doi:10.1007/s10549-011-1620-1.
Medler, T.R., D. Murugan, W. Horton, S. Kumar, T. Cotechini, A.M. Forsyth, P. Leyshock, J.J. Leitenberger, M. Kulesz-Martin, A.A. Margolin, Z. Werb, and L.M. Coussens. 2018. Complement C5a Fosters Squamous Carcinogenesis and Limits T Cell Response to Chemotherapy. *Cancer Cell*. 34:561-578.e6. doi:10.1016/j.ccell.2018.09.003.
Meylan, M., F. Petitprez, L. Lacroix, L. Di Tommaso, M. Roncalli, A. Bougoüin, A. Laurent, G. Amaddeo, D. Sommacale, H. Regnault, J. Derman, C. Charpy, F. Lafdil, J.-M. Pawlotsky, C. Sautes-Fridman, W.H. Fridman, and J. Calderaro. 2020. Early hepatic lesions display immature tertiary lymphoid structures and show elevated expression of immune inhibitory and immunosuppressive molecules. *Clin. Cancer Res.* doi:10.1158/1078-0432.CCR-19-2929.
Milne, K., M. Köbel, S.E. Kalloger, R.O. Barnes, D. Gao, C.B. Gilks, P.H. Watson, and B.H. Nelson. 2009. Systematic analysis of immune infiltrates in high-grade serous ovarian cancer reveals CD20, FoxP3 and TIA-1 as positive prognostic factors. *PLoS ONE*. 4:e6412. doi:10.1371/journal.pone.0006412.
Montfort, A., O. Pearce, E. Maniati, B.G. Vincent, L. Bixby, S. Böhm, T. Dowe, E.H. Wilkes, P. Chakravarty, R. Thompson, J. Topping, P.R. Cutillas, M. Lockley, J.S. Serody, M. Capasso, and F.R. Balkwill. 2017. A Strong B-cell Response Is Part of the Immune Landscape in Human High-Grade Serous Ovarian Metastases. *Clinical Cancer Research*. 23:250–262. doi:10.1158/1078-0432.CCR-16-0081.
Moyron-Quiroz, J.E., J. Rangel-Moreno, L. Hartson, K. Kusser, M.P. Tighe, K.D. Klonowski, L. Lefrançois, L.S. Cauley, A.G. Harmsen, F.E. Lund, and T.D. Randall. 2006. Persistence and responsiveness of immunologic memory in the absence of secondary lymphoid organs. *Immunity*. 25:643–654. doi:10.1016/j.immuni.2006.08.022.
Murakami, Y., H. Saito, S. Shimizu, Y. Kono, Y. Shishido, K. Miyatani, T. Matsunaga, Y. Fukumoto, K. Ashida, T. Sakabe, Y. Nakayama, and Y. Fujiwara. 2019. Increased regulatory

B cells are involved in immune evasion in patients with gastric cancer. *Sci Rep*. 9:13083. doi:10.1038/s41598-019-49581-4.
Nayar, S., J. Campos, M.M. Chung, L. Navarro-Núñez, M. Chachlani, N. Steinthal, D.H. Gardner, P. Rankin, T. Cloake, J.H. Caamaño, H.M. McGettrick, S.P. Watson, S. Luther, C.D. Buckley, and F. Barone. 2016. Bimodal Expansion of the Lymphatic Vessels Is Regulated by the Sequential Expression of IL-7 and Lymphotoxin α1β2 in Newly Formed Tertiary Lymphoid Structures. *J. Immunol.* 197:1957–1967. doi:10.4049/jimmunol.1500686.
Nielsen, J.S., R.A. Sahota, K. Milne, S.E. Kost, N.J. Nesslinger, P.H. Watson, and B.H. Nelson. 2012. CD20+ Tumor-Infiltrating Lymphocytes Have an Atypical CD27− Memory Phenotype and Together with CD8+ T Cells Promote Favorable Prognosis in Ovarian Cancer. *Clin Cancer Res*. 18:3281–3292. doi:10.1158/1078-0432.CCR-12-0234.
Pavoni, E., G. Monteriù, D. Santapaola, F. Petronzelli, A.M. Anastasi, A. Pelliccia, V. D'Alessio, R.D. Santis, and O. Minenkova. 2007. Tumor-infiltrating B lymphocytes as an efficient source of highly specific immunoglobulins recognizing tumor cells. *BMC Biotechnol*. 7:1–17. doi:10.1186/1472-6750-7-70.
Petitprez, F., A. de Reyniès, E.Z. Keung, T.W.-W. Chen, C.-M. Sun, J. Calderaro, Y.-M. Jeng, L.-P. Hsiao, L. Lacroix, A. Bougoüin, M. Moreira, G. Lacroix, I. Natario, J. Adam, C. Lucchesi, Y.H. Laizet, M. Toulmonde, M.A. Burgess, V. Bolejack, D. Reinke, K.M. Wani, W.-L. Wang, A.J. Lazar, C.L. Roland, J.A. Wargo, A. Italiano, C. Sautès-Fridman, H.A. Tawbi, and W.H. Fridman. 2020. B cells are associated with survival and immunotherapy response in sarcoma. *Nature*. 577:556–560. doi:10.1038/s41586-019-1906-8.
Phan, T.G., I. Grigorova, T. Okada, and J.G. Cyster. 2007. Subcapsular encounter and complement-dependent transport of immune complexes by lymph node B cells. *Nat. Immunol.* 8:992–1000. doi:10.1038/ni1494.
Pitzalis, C., G.W. Jones, M. Bombardieri, and S.A. Jones. 2014. Ectopic lymphoid-like structures in infection, cancer and autoimmunity. *Nat. Rev. Immunol.* 14:447–462. doi:10.1038/nri3700.
Platonova, S., J. Cherfils-Vicini, D. Damotte, L. Crozet, V. Vieillard, P. Validire, P. André, M.-C. Dieu-Nosjean, M. Alifano, J.-F. Régnard, W.-H. Fridman, C. Sautès-Fridman, and I. Cremer. 2011. Profound coordinated alterations of intratumoral NK cell phenotype and function in lung carcinoma. *Cancer Res.* 71:5412–5422. doi:10.1158/0008-5472.CAN-10-4179.
Posch, F., K. Silina, S. Leibl, A. Mündlein, H. Moch, A. Siebenhüner, P. Samaras, J. Riedl, M. Stotz, J. Szkandera, H. Stöger, M. Pichler, R. Stupp, M. van den Broek, P. Schraml, A. Gerger, U. Petrausch, and T. Winder. 2017. Maturation of tertiary lymphoid structures and recurrence of stage II and III colorectal cancer. *Oncoimmunology*. 7. doi:10.1080/2162402X.2017.1378844.
Qin, Z., G. Richter, T. Schüler, S. Ibe, X. Cao, and T. Blankenstein. 1998. B cells inhibit induction of T cell-dependent tumor immunity. *Nat. Med.* 4:627–630. doi:10.1038/nm0598-627.
Rizvi, N.A., M.D. Hellmann, A. Snyder, P. Kvistborg, V. Makarov, J.J. Havel, W. Lee, J. Yuan, P. Wong, T.S. Ho, M.L. Miller, N. Rekhtman, A.L. Moreira, F. Ibrahim, C. Bruggeman, B. Gasmi, R. Zappasodi, Y. Maeda, C. Sander, E.B. Garon, T. Merghoub, J.D. Wolchok, T.N. Schumacher, and T.A. Chan. 2015. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science*. 348:124–128. doi:10.1126/science.aaa1348.
Roumenina, L.T., M.V. Daugan, R. Noé, F. Petitprez, Y.A. Vano, R. Sanchez-Salas, E. Becht, J. Meilleroux, B.L. Clec'h, N.A. Giraldo, N.S. Merle, C.-M. Sun, V. Verkarre, P. Validire, J. Selves, L. Lacroix, O. Delfour, I. Vandenberghe, C. Thuilliez, S. Keddani, I.B. Sakhi, E. Barret, P. Ferré, N. Corvaïa, A. Passioukov, E. Chetaille, M. Botto, A. de Reynies, S.M.

Oudard, A. Mejean, X. Cathelineau, C. Sautès-Fridman, and W.H. Fridman. 2019a. Tumor Cells Hijack Macrophage-Produced Complement C1q to Promote Tumor Growth. *Cancer Immunol Res*. 7:1091–1105. doi:10.1158/2326-6066.CIR-18-0891.
Roumenina, L.T., M.V. Daugan, F. Petitprez, C. Sautès-Fridman, and W.H. Fridman. 2019b. Context-dependent roles of complement in cancer. *Nat. Rev. Cancer*. 19:698–715. doi:10.1038/s41568-019-0210-0.
Santoiemma, P.P., C. Reyes, L.-P. Wang, M.W. McLane, M.D. Feldman, J.L. Tanyi, and D.J. Powell. 2016. Systematic evaluation of multiple immune markers reveals prognostic factors in ovarian cancer. *Gynecol. Oncol.* 143:120–127. doi:10.1016/j.ygyno.2016.07.105.
Sautès-Fridman, C., F. Petitprez, J. Calderaro, and W.H. Fridman. 2019. Tertiary lymphoid structures in the era of cancer immunotherapy. *Nature Reviews Cancer*. 19:307–325. doi:10.1038/s41568-019-0144-6.
Sautès-Fridman, C., and L.T. Roumenina. 2020. B cells and complement at the forefront of chemotherapy. *Nat Rev Clin Oncol*. 17:393–394. doi:10.1038/s41571-020-0376-0.
Selitsky, S.R., L.E. Mose, C.C. Smith, S. Chai, K.A. Hoadley, D.P. Dittmer, S.J. Moschos, J.S. Parker, and B.G. Vincent. 2019. Prognostic value of B cells in cutaneous melanoma. *Genome Med*. 11:36. doi:10.1186/s13073-019-0647-5.
Seow, D.Y.B., J.P.S. Yeong, J.X. Lim, N. Chia, J.C.T. Lim, C.C.H. Ong, P.H. Tan, and J. Iqbal. 2020. Tertiary lymphoid structures and associated plasma cells play an important role in the biology of triple-negative breast cancers. *Breast Cancer Res Treat*. doi:10.1007/s10549-020-05548-y.
Shah, S., A.A. Divekar, S.P. Hilchey, H.-M. Cho, C.L. Newman, S.-U. Shin, H. Nechustan, P.M. Challita-Eid, B.M. Segal, K.H. Yi, and J.D. Rosenblatt. 2005. Increased rejection of primary tumors in mice lacking B cells: inhibition of anti-tumor CTL and TH1 cytokine responses by B cells. *Int. J. Cancer*. 117:574–586. doi:10.1002/ijc.21177.
Shalapour, S., X.-J. Lin, I.N. Bastian, J. Brain, A.D. Burt, A.A. Aksenov, A.F. Vrbanac, W. Li, A. Perkins, T. Matsutani, Z. Zhong, D. Dhar, J.A. Navas-Molina, J. Xu, R. Loomba, M. Downes, R.T. Yu, R.M. Evans, P.C. Dorrestein, R. Knight, C. Benner, Q.M. Anstee, and M. Karin. 2017. Inflammation-induced IgA + cells dismantle anti-liver cancer immunity. *Nature*. 551:340–345. doi:10.1038/nature24302.
Sharma, P., and J.P. Allison. 2015. The future of immune checkpoint therapy. *Science*. 348:56–61. doi:10.1126/science.aaa8172.
Shen, P., and S. Fillatreau. 2015. Antibody-independent functions of B cells: a focus on cytokines. *Nat. Rev. Immunol.* 15:441–451. doi:10.1038/nri3857.
Shi, J.-Y., Q. Gao, Z.-C. Wang, J. Zhou, X.-Y. Wang, Z.-H. Min, Y.-H. Shi, G.-M. Shi, Z.-B. Ding, A.-W. Ke, Z. Dai, S.-J. Qiu, K. Song, and J. Fan. 2013. Margin-infiltrating CD20(+) B cells display an atypical memory phenotype and correlate with favorable prognosis in hepatocellular carcinoma. *Clin. Cancer Res.* 19:5994–6005. doi:10.1158/1078-0432.CCR-12-3497.
Siliņa, K., A. Soltermann, F.M. Attar, R. Casanova, Z.M. Uckeley, H. Thut, M. Wandres, S. Isajevs, P. Cheng, A. Curioni-Fontecedro, P. Foukas, M.P. Levesque, H. Moch, A. Linē, and M. van den Broek. 2018. Germinal Centers Determine the Prognostic Relevance of Tertiary Lymphoid Structures and Are Impaired by Corticosteroids in Lung Squamous Cell Carcinoma. *Cancer Res*. 78:1308–1320. doi:10.1158/0008-5472.CAN-17-1987.
Stavnezer, J., and J. Kang. 2009. The surprising discovery that TGF beta specifically induces the IgA class switch. *J. Immunol.* 182:5–7. doi:10.4049/jimmunol.182.1.5.
Su, S., J. Zhao, Y. Xing, X. Zhang, J. Liu, Q. Ouyang, J. Chen, F. Su, Q. Liu, and E. Song. 2018. Immune Checkpoint Inhibition Overcomes ADCP-Induced Immunosuppression by Macrophages. *Cell*. 175:442-457.e23. doi:10.1016/j.cell.2018.09.007.
Sylvestre, D., R. Clynes, M. Ma, H. Warren, M.C. Carroll, and J.V. Ravetch. 1996.

Immunoglobulin G-mediated inflammatory responses develop normally in complement-deficient mice. *J. Exp. Med.* 184:2385–2392. doi:10.1084/jem.184.6.2385.
Tan, T.-T., and L.M. Coussens. 2007. Humoral immunity, inflammation and cancer. *Curr. Opin. Immunol.* 19:209–216. doi:10.1016/j.coi.2007.01.001.
Tran, E., P.F. Robbins, and S.A. Rosenberg. 2017. "Final common pathway" of human cancer immunotherapy: targeting random somatic mutations. *Nat. Immunol.* 18:255–262. doi:10.1038/ni.3682.
de Visser, K.E., L.V. Korets, and L.M. Coussens. 2005. De novo carcinogenesis promoted by chronic inflammation is B lymphocyte dependent. *Cancer Cell*. 7:411–423. doi:10.1016/j.ccr.2005.04.014.
Welinder, C., K. Jirström, S. Lehn, B. Nodin, G. Marko-Varga, O. Blixt, L. Danielsson, and B. Jansson. 2016. Intra-tumour IgA1 is common in cancer and is correlated with poor prognosis in bladder cancer. *Heliyon*. 2:e00143. doi:10.1016/j.heliyon.2016.e00143.
Wouters, M.C.A., and B.H. Nelson. 2018. Prognostic Significance of Tumor-Infiltrating B Cells and Plasma Cells in Human Cancer. *Clin Cancer Res*. 24:6125–6135. doi:10.1158/1078-0432.CCR-18-1481.
Yamakoshi, Y., H. Tanaka, C. Sakimura, S. Deguchi, T. Mori, T. Tamura, T. Toyokawa, K. Muguruma, K. Hirakawa, and M. Ohira. 2020. Immunological potential of tertiary lymphoid structures surrounding the primary tumor in gastric cancer. *Int. J. Oncol.* 57:171–182. doi:10.3892/ijo.2020.5042.
Yeong, J., J.C.T. Lim, B. Lee, H. Li, N. Chia, C.C.H. Ong, W.K. Lye, T.C. Putti, R. Dent, E. Lim, A.A. Thike, P.H. Tan, and J. Iqbal. 2018. High Densities of Tumor-Associated Plasma Cells Predict Improved Prognosis in Triple Negative Breast Cancer. *Front. Immunol.* 9. doi:10.3389/fimmu.2018.01209.
Zirakzadeh, A.A., A. Sherif, R. Rosenblatt, E. Ahlén Bergman, M. Winerdal, D. Yang, J. Cederwall, V. Jakobsson, M. Hyllienmark, O. Winqvist, and P. Marits. 2020. Tumour-associated B cells in urothelial urinary bladder cancer. *Scand. J. Immunol.* 91:e12830. doi:10.1111/sji.12830.
.

**FIGURE LEGENDS**

Figure 1: Impact of B cells and TLS on prognosis and response to ICI

Analysis of 15 different tumors. The surface of the spot is proportional to the number of patients for each cancer with favorable (green), unfavorable (red) or with no impact (white) on PFS or OS and for therapeutic response to ICI.

Figure 2: Mechanisms of action of B cells in cancer

In germinal centers of TLS, B cells are selected by antigen presented by FDC -associated IC. They are activated with the help of Tfh cells, proliferate and differentiate into memory B cells and plasma cells. B cells present antigens to CD4 and CD8 T cells. Plasma cells produce IgG antibodies which may increase antigen presentation to T cells after uptake of IC by DC, kill or engulf tumor cells via ADCC and ADCP respectively (right side). B cells can also transfer antigen, directly to DC or via complement receptor 2 to FDC, under the form of opsonized immune complexes. Memory B cells and antibodies circulate in the blood where they can help controlling potential metastatic cells (right side). In contrast, binding of immune complexes to macrophages results in their activation and the production of pro-inflammatory mediators which exert pro-tumor activities. Tumor cell-bound antibodies can also activate complement which fuels inflammation and activate endothelial cells promoting tumor growth and spread (left side). Breg cells may also inhibit immune responses via the production of IL-10.

**ACKNOWLEDGMENTS**

Funding This work was supported by INSERM, Sorbonne Université, Université de Paris, CARPEM (Cancer Research for Personnalized Medecine) programme of the Sites Integrés de Recherche sur le Cancer (SIRIC), LabeX Immunooncology.

The authors have no conflicting financial interest.