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Review

The clinical significance of B-cell maturation antigen as a therapeutic target and biomarker

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Abstract

Introduction: B-cell maturation antigen (BCMA) is a cell membrane bound tumor necrosis factor receptor family member that is expressed exclusively on late stage normal and malignant B-cells and plasma cells. Addition of two of its ligands, B-cell activating factor and a proliferation inducting ligand, to normal B-cells cause B-cell proliferation and antibody production. Serum BCMA is elevated among patients with multiple myeloma (MM) and chronic lymphocytic leukemia (CLL), and is a prognostic and monitoring tool for these patients. The first anti-BCMA antibody (Ab) was developed in 2007. Recently, biotech and pharmaceutical companies have created various forms of BCMA-directed Abs (naked Abs, Ab drug conjugates, and bispecific Abs) and cellular therapies (chimeric antigen receptor T-cells) with promising clinical results.

Areas covered: This BCMA review encompasses full-text publications of original research articles and abstracts presented at hematology/oncology meetings.

Expert commentary: The limited preclinical and ongoing clinical studies published to date evaluating BCMA-directed therapies have shown great promise. It has also been demonstrated that BCMA is solubilized and elevated in the blood of MM, Waldenstrom’s macroglobulinemia and CLL patients, and is also responsible for the immune deficiency in MM. Reducing circulating levels may improve the efficacy of these treatments.

Keywords

B-cell activating factor; B-cell maturation antigen; chronic lymphocytic leukemia, immune deficiency; multiple myeloma; Waldenstrom’s macroglobulinemia
1. Introduction

B-cell maturation antigen (BCMA, also known as TNFRSF17, CD269) is a tumor necrosis factor receptor (TNFR) superfamily member initially identified on the cell membrane of normal and malignant B-lymphocytes and plasma cells [1-6]. It was also shown to be expressed on MM cell lines and primary MM cells [1-6]. A proliferation-inducing ligand (APRIL) and B-cell activating factor (BAFF, and also known as BLyS) are two agonist ligands of BCMA [7,8]. Exogenously added BAFF binds to the BCMA receptor on normal B-cells and results in their proliferation, differentiation and antibody (Ab) production [9-14]. The binding of APRIL to BCMA promotes human MM growth and immunosuppression in the bone marrow (BM) microenvironment [9-15]. *In vitro*, studies have shown that BAFF or APRIL protect interleukin-6 (IL-6) dependent MM cell lines from IL-6 deprivation-induced apoptosis, and from steroid-induced apoptosis [16]. Numerous *in vitro* studies have demonstrated the pro-survival role of membrane-bound BCMA on normal and malignant B- and plasma cells, when bound by its ligands BAFF or APRIL [7-16].

Given the importance of the BCMA receptor and its expression only on late-stage B- and plasma cells, it is a potentially ideal target for treating malignancies of this cell type. Thus, many BCMA-directed treatment approaches have been developed, ranging from anti-BCMA monoclonal (m) Abs as naked Ab and conjugated to drugs (ADC), to bispecific Abs which bind to both BCMA-expressing B/plasma-cells and CD3ε-expressing T-cells, to anti-BCMA chimeric antigen receptor (CAR)-T-cell-based approaches. Not only has the BCMA receptor been shown to be expressed on the membrane of MM cells, but it is also expressed on chronic lymphocytic leukemia (CLL) tumor cells. This TNFR is shed into the blood and solubilized levels are elevated among patients with both tumor types. These levels predict both progression-free survival (PFS) and overall survival (OS) as well as can be used to monitor changes in disease course more rapidly than currently used parameters to follow these patients. In addition, prevention of shedding of BCMA which has been shown to be possible with specific agents may improve the efficacy of BCMA-targeted approaches [17]. Among patients with primary immune deficient states who have impaired humoral immunity, levels of circulating BCMA are very low and may represent a new screening tool to diagnose these patients as well as follow their disease course.

1.1. Prognosis and monitoring of MM using sBCMA levels
It has been demonstrated that BCMA is elevated in the serum (s) of MM patients and plasma (p) from severe combined immunodeficient (SCID) mice bearing human MM [6]. Human MM xenograft model studies showed the presence of human BCMA released from the human MM cells into the plasma of SCID mice [6]. Mice bearing both secretory (defined as the presence of monoclonal immunoglobulin in the plasma of these mice) MM tumor LAGλ-1 or nonsecretory human MM tumor LAGκ-2, were dosed with anti-MM agents (previously demonstrated to be effective in these models), and when compared with untreated mice, a reduction in both tumor volumes and pBCMA as well as human IgG levels among mice bearing LAGλ-1 were observed [6]. The enzyme linked immunosorbent assay (ELISA) assay used to measure soluble human BCMA in the blood of xenograft models was purchased from a commercial vendor (R&D Systems, Minneapolis, MN). Notably, soluble BCMA levels are the same whether blood is collected in serum separator tubes (which yield serum) or heparinized tubes (which yield plasma); thus, when measuring soluble BCMA using an ELISA kit, the concentration/levels of sBCMA or pBCMA are not different. sBCMA levels also correlated with the percentage of plasma cells in the BM from MM patients [6]. Those with active MM had the highest sBCMA levels followed by smoldering MM and then monoclonal gammopathy of undetermined significance (MGUS). It was recently shown that sBCMA levels correlated with current clinical status of MM patients, when comparing those patients who were in complete remission (CR) versus those who achieved only a partial response (PR). Furthermore, sBCMA levels were also lower among responding patients when compared to those with progressive disease (PD) [6]. MM patients with sBCMA levels above the median had significantly shorter PFS (3.6 months) and OS (98 months) than patients whose levels were below the median (9.0 and 155 months, respectively). As expected, sBCMA levels positively correlated with M-protein levels. Given the much shorter half-life of soluble BCMA (24-36 hours) [18] compared with IgG (3-4 weeks) [19,20], clinical status changes in MM patients were observed much more rapidly with sBCMA levels compared to IgG [21,22]. This is an advantage of using sBCMA, as it allows physicians to rapidly evaluate ineffective treatment regimens, as well as monitor and quickly determine when a previously effective treatment regimen is ineffective due to drug resistance within the patient. Thus, this should allow treating physicians to change to a new/different treatment regimen much sooner for patients failing their current therapies. For rapidly progressing MM patients, this is critical as switching them to a different treatment regimen more quickly could potentially increase the efficacy of their new treatment rather than allow them to remain on ineffective treatment with worsening disease, as this could potentially allow the MM tumor cells additional time to develop resistance. Additionally, ineffective treatment regimens may result in deterioration of the patient’s health so that they are unable to tolerate their next treatment regimen. The half-life of another serum marker to
track MM patients, serum free light chain (sFLC) is \( \leq 6 \) hours [23]. Thus, changes can theoretically be detected even faster with sFLC than with sBCMA levels. Currently, sFLCs have become part of routine laboratory testing for MM diagnosis [24], monitoring treatment regimen response and as one of the criteria to establish stringent CR [25]. Additionally, it is particularly important among patients with AL-amyloidosis, as these patients usually have a very low tumor burden and little or no M-protein detectable in their blood or urine [26]. However, controversy exists as to the accuracy and reliability of results from sFLC testing. Jagannath et al. stated that “studies are underway to determine the value of (sFLC) testing as a monitoring tool to replace urine [M-protein] testing in patients with myeloma, and initial observations have shown mixed results” [23]. Specifically, Alyanakian et al. stated that “a general relationship between serum concentrations and 24-hr urinary excretion of FLC is prevented by the remarkable variability between patients perhaps due to glomerular filtration rates” [27].” sBCMA levels, on the other hand, are independent of renal function [28]. Additionally, within the Jagannath manuscript [23], Dispenzieri et al. reported that a “mediocre correlation was observed between the urine M-spike and change and the involved free light chain ratio change after 2 months’ treatment in 33 patients with disease not measurable in serum, and the investigators concluded that free light chain testing is not an adequate replacement for urine studies when monitoring response to treatment.” For many MM patients with low tumor burden, sFLC is not measurable; and, thus, cannot be used to track the status of their response to therapy. In contrast, sBCMA can be detected among patients with low tumor burden, thus allowing physicians to monitor these patients’ response to therapy [6,21,28]. Another advantage of sBCMA involves its use for patients with nonsecretory disease, those which do not produce any detectable paraprotein including sFLCs in the blood or urine. There is currently no easy or accurate way to monitor these patients’ response to drug treatment. However, recent studies show that sBCMA is present in the sera of nonsecretory patients and in a nonsecretory MM xenograft model, but most importantly its levels correlated with tumor volume burden/measurements [6,18]. It has been shown that changes in sBCMA levels correlated with results from changes in the percentage of BM plasma cells and PET CT scan findings which are the two current modalities used to monitor nonsecretory patients’ disease status [21,28]. Overall, the short half-life (24-36 hours) of soluble BCMA [18] allows for quick determination of changes in clinical status and is a new biomarker which can predict outcomes [6,18,21,28].

Possibly because of its recent discovery, currently there is no FDA-approved BCMA ELISA kit for diagnosing and/or monitoring humans with B- and plasma cell malignancies. To date, all BCMA ELISA kits that are commercially available are for research use only, and they detect soluble BCMA by means of a capture/primary Ab and
a detection/secondary Ab. Solubilized BCMA is an excellent marker given that it is significantly elevated in the sera of humans with MM [21] with minimal amounts of serum required for its assessment (4 μL) and is stable at room temperature for months; and, thus, it is a convenient and inexpensive assay for monitoring patients with B- and plasma cell malignancies or diseases. These protein levels can be determined quickly with ease [6,21,28]. Soluble BCMA has a short half-life in blood [18] relative to the half-life of IgG, thus making it clinically useful for determining changes in clinical status (response vs disease progression) much more quickly among MM patients receiving new myeloma treatments when compared with IgG paraprotein levels [21,28]. Recent results confirm previous findings [6], and validate sBCMA levels as a reliable marker for predicting outcomes and monitoring MM patients [21,28]. It is theoretically possible that BCMA-directed treatments will eliminate BCMA-expressing MM plasma cells but also lead to the emergence of sub-clones that do not express this protein. Because of this, the monitoring of conventional serum markers should not be abandoned at this point. However, there are no reports of the development of BCMA-negative MM tumor cells occurring among patients treated with a variety of different BCMA-targeted therapies.

1.2. sBCMA binds BAFF, forming sBCMA-BAFF complexes, resulting in immune deficiency

The absence of normal, or uninvolved/polyclonal Abs, is a hallmark of humans (and mice) with MM [29-33]. It has been demonstrated in vitro, by independent laboratories, that BAFF binds membrane-bound BCMA on normal human or mouse B-cells, resulting in their proliferation, differentiation and production of Abs [34,35]. However, BAFF does not induce human or mouse B-cell proliferation, differentiation and production of Abs in these cell cultures in the presence of exogenously added recombinant human BCMA (BCMA-Fc or BCMA-Ig) [34,35]. The ability of rhBCMA-Fc to suppress BAFF's B-cell stimulatory effect on polyclonal Ab production, by way of formation of rhBCMA-mouse BAFF (mBAFF) complexes, was recently demonstrated in vitro (Figure 1) [18].

Based on the in vitro studies [34,35] and knowing that sBCMA is elevated among MM patients [6], it was hypothesized that sBCMA would bind and neutralize/inhibit BAFF, preventing its stimulatory effects on normal B-cells; and, thus, this would account for the lack of polyclonal Ab production and resulting immune deficiency that commonly occurs among MM patients [18]. To evaluate this, immune competent mice were dosed with rhBCMA, and significantly reduced normal Ab levels and free murine (m) BAFF levels were observed in the blood [18]. Work from previous in vitro studies [34,35] was expanded and independent experiments demonstrated that rhBCMAFc (or rhBCMA-Ig) prevents BAFF-induced normal B-cell proliferation and Ab production in vitro through
The direct formation of rhBCMA-mBAFF complexes [6]. As a result, the lack of free BAFF in these mice prevented its B-cell stimulatory effects on proliferation, differentiation and finally Ab production [6,18]. Additionally, human serum containing high levels of BCMA (or rhBCMA) prevented BAFF from binding to B-cells. Furthermore, an inverse relationship between sBCMA levels and uninvolved, polyclonal Ab levels among MM patients was also observed [18]. These experiments and observations collectively show that BCMA (whether rhBCMA or endogenous human sBCMA) directly binds to BAFF (whether endogenous human BAFF or mBAFF), forming BCMA-BAFF complexes, preventing BAFF-induced B-cell proliferation, differentiation and Ab production (Figure 2) [18]. Thus, the authors hypothesize that elevated levels of sBCMA contribute to the immune deficiency which frequently occurs in MM patients.

The cause through which sBCMA is released into the blood was initially unknown. However, it has recently been shown that the enzyme γ-secretase (GS) causes membrane-bound BCMA ectodomain shedding, resulting in soluble BCMA [36]. Following the dosing of mice with a GS inhibitor (I), an increase in membrane-bound BCMA on plasma cells and more plasma cells in the bone marrow was observed [36]. The potential clinical relevance of these observations for treating patients with anti-BCMA targeted therapies is discussed below.

2. Solubilized BCMA in Chronic Lymphocytic Leukemia

2.1. pBCMA levels in CLL are elevated, correlate with disease activity, time to first treatment and overall survival

It was demonstrated that the pBCMA levels among CLL patients were significantly higher when compared to samples obtained from healthy subjects [37]. To determine if pBCMA levels could be used as a marker for disease activity in CLL, analysis of serial samples (from the same patients) demonstrated that pBCMA correlated with changes in their clinical status. Further support for its use as a marker for disease activity was obtained when pBCMA levels were compared with known indicators of disease activity, where a strong correlation was observed with beta 2 microglobulin (β2M) levels and white blood cell (WBC) counts. Also of clinical importance, it was shown that if untreated patients had pBCMA levels below 39.0 ng/mL, their median time to first treatment (TTFT) was a median of 160 months, whereas those with levels above that threshold showed a median of only 22 months (Figure 3) [37]. When sBCMA was obtained prior to start of any therapy, CLL patients in the highest quartile (>105.8 ng/mL pBCMA) also had a much shorter OS when compared to those with pBCMA in the lower three quartiles (< 105.8 ng/mL).
Current traditional prognostic markers of CLL are immunoglobulin variable region heavy chain (IgHV) mutational status, % homology to the germline immunoglobulin heavy chain gene expressed in the malignant cells, and ZAP-70 expression. Patients with CLL cells with unmutated IgHV genes (> 98% homology to germline) are known to have a poor prognosis [38]. Consistent with this, those with CLL cells containing unmutated IgHV genes also had significantly higher pBCMA levels when compared to those patients with mutated IgHV genes. Another prognostic marker in CLL is ZAP-70, where patients with ZAP-70 expressed in more than 20% of their tumor cells have a worse prognosis [39]. It was shown that patients whose CLL cells had ZAP-70 expression (> 20% of cells) also had higher pBCMA levels when compared to patients with a lower proportion of cells with ZAP-70 expression. In a phase 2 study (NCT01500733) assessing sBCMA levels among CLL patients (n = 46) who received targeted therapy with the Bruton tyrosine kinase inhibitor ibrutinib as a single agent, serum samples were obtained at baseline and at day 2 and 1, 2, 6, 12, 24, 36, and 48 months following the start of this therapy [40]. As early as day 2 after starting ibrutinib, a significant decrease in sBCMA was observed when compared to baseline (P = 0.003), which continued to decrease and showed levels that were similar to age- and sex matched healthy subjects (approximately 30 ng/mL) at approximately 2 months [40]. Among those patients with ongoing responses, these levels were maintained throughout the duration of the 4-year clinical study (Figure 4). Among patients who developed PD during ibrutinib treatment, an increase in sBCMA was observed at a median of 8.4 months before a clinical progression was identified and confirmed using conventional indicators of CLL tumor burden. Further analysis of PD patients demonstrated an increase in sBCMA by a median of 153% from its nadir. Although sBCMA correlated with absolute lymphocyte count (ALC) in CLL patients, it rapidly assessed changes in clinical status before significant changes occurred in ALC. As is well recognized, total body tumor burden in CLL patients involves more than just the peripheral blood, as CLL cells are also found in the spleen, lymph nodes and bone marrow. Thus, the rapid change in sBCMA likely reflects rapid changes in total body tumor burden as has been observed in MM, where these changes in sBCMA occur much more rapidly than changes in M-protein [21,22]. However, at this point, this is speculative and may involve multiple mechanisms, including reduction in total body tumor burden as mentioned above. There is no evidence to date that ibrutinib or any other drug except, specifically, gamma secretase inhibitors, modulate shedding of BCMA from the cell surface, as shown by independent results from laboratories [17,36].
In Waldenstrom’s macroglobulinemia (WM) patients, levels of sBCMA have also been found to be elevated, and correlated with clinical status [41]. It is likely that this protein will also represent a novel marker to predict outcomes and monitor the course of disease for these patients. However, further research will need to be conducted to confirm this. Membrane-bound BCMA has also been shown to be expressed in Hodgkin lymphoma cells, and linked to Hodgkin and Reed-Sternberg cell proliferation and survival [42]. Despite this, levels of sBCMA were not elevated among these patients (data not shown).

2.2. sBCMA is less characterized but also found in autoimmune disorders B- and plasma-cell malignancies

Within autoimmune disorders, membrane-bound BCMA expression and elevated sBCMA levels have been observed. Soluble BCMA levels strongly correlated with disease activity among those with systemic lupus erythematosus (SLE) patients; those with more active disease demonstrated higher sBCMA levels than healthy controls [36]. Contrary to what one might expect given that SLE is a disease of overactive B-cells, and not terminally differentiated plasma cells, the mean sBCMA levels were higher among SLE patients than the mean soluble BAFF (sBAFF) levels. Experiments both in vitro and in vivo demonstrated that with increasing rhBCMA-Fc concentrations, the detection of free BAFF decreases, because of the sequestering of BAFF by BCMA [18]. Thus, these experiments demonstrate that when BCMA is added, only free BAFF is detected when using the BAFF ELISA as indicated above. This may be due three possibilities: 1) when sBCMA binds to the BAFF protein, it changes the protein configuration of BAFF, thus not allowing the BAFF detection/secondary Ab to bind this ligand, 2) when sBCMA binds to the BAFF protein, it binds to the same location where the BAFF detection/secondary Ab binds, or 3) when sBCMA binds to the BAFF protein, it binds to the same location where the BAFF capture/primary Ab binds. Any of these possibilities would prevent the binding of either the capture/primary or detection/secondary Ab to BAFF; and, thus, it would appear that BAFF is not present. However, BAFF is present but cannot be detected when bound to BCMA, as has been demonstrated previously [18]. Thus, in the publication above [36] sBAFF levels were present at higher concentrations than reported; however, given the presence of sBCMA which bound sBAFF, sBAFF could not be detected using the BAFF ELISA. Recently, patients with multiple sclerosis (MS) and SLE have been shown to have significantly higher sBAFF levels than among controls [36]. These patients were found to have a variant mutation that controls BAFF production, resulting in increased levels of sBAFF resulting in increased amounts of B lymphocytes and immunoglobulins [43]. Elevated sBCMA levels were shown among patients with neuroborreliosis and linked to central nervous system-localized IgG levels [36]. Multiple sclerosis patients were not shown to have elevated sBCMA levels in their
blood, but instead were found to have elevated amounts in their cerebrospinal fluid [36]. For patients with autoimmune disorders, TACI-based and anti-BAFF and anti-APRIL approaches are now being used to effectively treat these patients [44]. Thus, sBCMA and membrane-bound BCMA, along with its ligands BAFF and APRIL and their associated/received receptors TACI and BAFF-R, have been shown to be important in not only patients with late B-cell malignancies but also those with a variety of autoimmune disorders [1-18,36,37,41-44,46-50-52].

3. Distinguishing Patients with Primary Antibody Deficiency Based on Reduced Serum BCMA Levels

Not only can sBCMA levels be used to track B- or plasma cell malignancies and autoimmune disorders, it has been shown that for those on the opposite end of the immunologic spectrum clinically (those containing few or lacking plasma cells), it can also be very useful. We have shown that a subset of patients with MM in CR show reduced uninvolved, polyclonal Ig levels, and also have very low levels of sBCMA most likely reflecting their decreased numbers of normal late B- and plasma cells. Thus, patients with primary immune deficiencies (PID) should also have reduced sBCMA levels reflecting their lack of late B-lymphocytes and plasma cells. This was confirmed in recent studies which showed sBCMA levels to be markedly reduced when compared to age- and sex-matched control subjects [45]. Patients with two specific common types of PID, common variable immunodeficiency (CVID) and X-linked agammaglobulinemia (XLA), showed very low levels of sBCMA when compared to age- and sex-matched control subjects (Figure 5) [45]. Within the CVID subset of immune deficient patients, those with any chronic complication or enteropathy specifically also had significantly lower sBCMA levels than those without these complications. When compared to CD19+ B-cells or IgA, IgG or IgM levels, no correlation was observed with sBCMA.

4. Anti-BCMA Antibodies as Therapeutics for B- and Plasma Cell Malignancies

4.1. The first anti-BCMA Ab developed as a naked Ab alone (cSG1) and conjugated to a drug

Given the expression of membrane-bound BCMA exclusively on late stage/mature B-lymphocytes and plasma cells (normal and malignant), membrane-bound BCMA can be exploited to target B- and plasma cell malignancies [3,5,6,17,46]. The first investigation of BCMA as a therapeutic target in MM was initiated in 2007 by Seattle Genetics. Their
anti-BCMA Ab was called cSG1 and it was developed as a naked Ab as well as with an Ab drug conjugate (ADC) targeting BCMA [47]. This antagonist mAb directed against BCMA (cSG1), was shown to increase antibody-dependent cell mediated cytotoxicity (ADCC) by approximately 100-fold [47]. Given the robust anti-MM effects of cSG1 in MM, it provided hope that this would be an effective therapy for patients with late B- and plasma cell malignancies. Unfortunately, no additional studies evaluating cSG1 alone or as an ADC have been conducted.

4.2. Anti-BCMA ADC: GSK2857916

Recently GSK2857916 was developed by GlaxoSmithKline. It is an anti-BCMA afucosylated mAb conjugated to monomethyl auristatin F (MMAF) [48]. Similar to cSG1, GSK2857916 shows robust in vitro activity. It causes apoptosis of MM cells, inhibits MM colony formation and induces ADCC (natural killer cell mediated) of primary MM cells and cell lines [48]. The administration of GSK2857916 to mice containing MM xenografts resulted in significant inhibition of tumor growth, and it was reported that some mice had no detectable tumors during the entire study [44]. Currently, clinical trials for relapsed or refractory (RR) MM patients have begun with early promising results but ocular problems have developed from the MMAF toxin [49].

4.3. Bispecific antibodies (BI 836909, BiFab-BCMA) targeting BCMA on MM cells and CD3ε on T-cells

Bispecific anti-BCMA Abs have recently been developed as potential therapies for patients with plasma cell malignancies. These Abs contain two different recognition domains, one recognizes BCMA on MM cells and the other binds CD3ε on T-cells. Once bound, the CD3ε region results in T-cell recruitment and activation, resulting in target cell lysis. For example, Boehringer Ingelheim/Amgen has developed BI 836909 which is a bispecific T-cell engager (a.k.a., BiTE) that binds to BCMA as well as CD3ε on T-cells. Co-culturing of a panel of 12 MM and plasma cell leukemia (PCL) lines with purified T-cells and BI 836909 demonstrated cell-mediated lysis [50]. The concentration of BI 836909 at which 50% of the cell lines (panel of 12 MM and PCL) were killed (EC50) was found to be 190 pg/mL, and extracellular phosphatidylserine (a marker for dead cells) on MM1R cells (a MM cell line) was observed with flow cytometry [50]. Additionally, experiments demonstrated that BI 836909 did not cause target cell lysis in the absence of T-cells [50].

Using a MM xenograft model derived from the MM cell line NCI-H929 in which mice were injected with human T-cells before treatment initiation with BI 836909, BI 836909 was also evaluated in vivo. At the highest dose used, BI 836909 at 0.5 mg/kg, it did not inhibit tumor growth in this MM xenograft model [50]. Using a disseminated model, the
MM cell line L-363 was injected intravenously 5 days prior to intraperitoneal injection of human T-cells, and allowed to grow prior to treatment with BI 836909. The efficacy of BI 836909 was uninterpretable as standard graphs (mean of group, standard error of the mean) and their corresponding P-values were not shown [50].

BiFab-BCMA, is also a bispecific Ab (binds BCMA on MM and CD3ε on T cells) developed by Ramadoss and colleagues [51]. Culturing of healthy donor T-cells with a BCMA-expressing MM cell line (OPM-2) plus BiFab-BCMA, demonstrated T-cell activation via an increase in levels of cytokines (IFNγ, TNFα, IL2, IL-4, IL-6, and IL-10) and activation of the T-cell surface markers CD69, CD25 and CD107a [51]. It was demonstrated that tumor regression was similar in BiFab-BCMA-treated mice when compared to CAR T-cell therapy against BCMA [50]. EM801 is another bispecific Ab (against BCMA and CD3ε on T-cells) [5]. T-cells co-cultured with the BCMA-expressing MM cell line in the presence of EM801 resulted in an increase of the activated T-cell cytokines IFNγ, TNFα and IL-2 and surface markers for CD69 and CD25 [5]. These results are similar to those using the BiFab-BCMA outlined above [51]. At concentrations from 10 pM to 30 nM, EM801 induced significant death of CD138-selected primary MM cells in 75% to 80% of previously untreated and RRMM patients [5]. At the highest doses used in vitro (2.6 nM/kg = 0.5 mg/kg), significant tumor growth inhibition was observed in six of nine EM801-treated mice, when compared to the control group [5].

Circulating BCMA potentially may reduce the efficacy of BCMA-directed therapies, and recent studies support this possibility from past and ongoing clinical studies [35]. Recently, it was reported that sBCMA inhibited the efficacy of BI 836909 [50], by causing “a shift in the EC50,” on the percentage of cell lysis in two BCMA-expressing MM cell lines. Clinical signs of toxicity observed in cynomolgus monkeys were observed at doses ≥ 15 µg/kg/day, and symptoms consisted of vomiting and increased body temperatures which were accompanied by transient increases in serum cytokines (IFNγ, IL-2, IL-2, and/or MCP-1) [50]. The EC50 of BI 836909, using the BCMA-positive MM cell line NCI-H929, was at approximately 5 ng/mL. Interestingly, in the presence of clinically relevant concentrations of sBCMA (176 ng/mL), the anti-MM efficacy of BI 836909 was reduced and only 20% of the MM cells were killed (in contrast to 50% previously killed at this concentration without soluble BCMA) [50]. Given that the median sBCMA level in previously untreated patients with active MM is over 500 ng/mL and in the RR state 176.0 ng/mL [17,22], higher doses of BCMA-directed therapies may be necessary as circulating BCMA in the blood of MM patients are likely to bind and neutralize these drugs, before they are able to reach their intended late B-cell tumor target in the bone marrow. In fact, binding of anti-BCMA Ab at clinically achieved concentrations to primary MM tumor cells occurs consistently among patients with
sBCMA concentrations ≥ 156 ng/mL [17] so that the majority of both previously untreated MM patients and those with RR disease are likely to show reduced ability to respond to BCMA-directed therapies because of the circulating BCMA in the blood. The anti-MM effects of EM801 on cultured BM aspirates from MM patients was also evaluated. Unlike their first study [5], it was not indicated whether the cultured BM cells were CD138-selected [52]. Although the concentrations of sBCMA were not provided in the abstract, it was reported that sBCMA levels obtained from the cultured BM cells had no impact on MM tumor cell lysis from these co-cultures of bone marrow and T-cells incubated with their drug (EM801), when comparing the tumor cell lysis from responding patients (n = 11) to nonresponding patients (n = 5) [52] but only 16 patients were evaluated. However, it has previously been reported that the levels of BCMA in supernatant fluid obtained from cultured MM BM cells is very low, and several logs lower than the levels present in serum from these same patients [6]. A recent study performed by Hipp et al. (2017) supports the fact that sBCMA concentrations may inhibit anti-BCMA-directed therapies [50]. Experiments showed that higher sBCMA concentrations inhibited the efficacy of BI 836909 [50], “a shift in the EC<sub>50</sub>,” on MM cell lysis in both BCMA-expressing MM cell lines tested (NCI-H929 and L363) [50]. Higher concentrations of their anti-BCMA-directed therapy (BI 836909) were needed to achieve 50% percentage cell lysis in the presence of sBCMA, when compared to lower concentrations of drug needed in the absence of sBCMA in the cultures. The routinely detected BCMA levels in the serum from many MM patients in the clinic tend to be at or above the sBCMA concentrations used in these experiments (100-200 ng/mL) [21,28]. Thus, it is likely that the higher sBCMA levels present in many MM patients are clinically and therapeutically relevant, and may inhibit the efficacy of the various BCMA-directed therapies that are currently in development [5,48,50,52].

Laurent et al. (2015) recently showed that GS is the enzyme responsible for BCMA shedding from the cell membrane of plasma cells and that GSIs cause retention of BCMA on the membrane of B-cells [36]. It has been demonstrated that GSIs at picomolar concentrations do, in fact, prevent shedding of BCMA from MM and CLL patients in vitro [17], thereby providing evidence that they may enhance the efficacy of anti-BCMA mAbs and anti-BCMA ADC therapeutics for treating patients with BCMA expressing tumors. Given that at least one prior GSI (LY411575) has been shown to increase plasma cells in the BM [36], and that membrane-bound BCMA activation by its ligands BAFF and/or APRIL are pro-B and plasma-cell survival [1-16], caution must be taken during future clinical trials evaluating anti-BCMA mAbs or anti-BCMA ADC in combination with GSIs for patients with malignancies of late B-cell origin.
5. Anti-BCMA CAR-T-cells

5.1. The first report of anti-BCMA-CAR-T-cells

T-cells have recently been genetically engineered to recognize BCMA-expressing cells. T-cells have been transfected with the BCMA CAR and are called anti-BCMA-CAR-T-cells. The first report of anti-BCMA CAR T-cells (2013) showed that when they were cultured in the presence of BCMA expressing cells, increased CD107a on activated T-cells was observed and they produced a large amount of IFN\(_\gamma\) [53]. Anti-BCMA-CAR-T-cells killed MM cell lines \textit{in vitro}, as demonstrated using cytotoxicity assays. \textit{In vitro}, tumor regression was observed in the RPMI8226 MM xenograft model following injection with anti-BCMA-CAR-T-cells, whereas mice which received T-cells expressing the negative control did not show anti-tumor effects [53].

5.2. Bb2121

Bluebird and Celgene are developing Bb2121, an anti-BCMA CAR T-cell therapy. Updated results were recently presented from the current ongoing clinical study, evaluating Bb2121 for RRMM patients [54]. Eighteen MM patients (with BM involvement at baseline) in cohorts 2, 3 and 4 had no detectable MM cells in their BM on day 14 or beyond [54]. However, 3/4\(^{th}\) of patients experienced cytokine release syndrome (CRS), which were mostly grade 1 and 2 [54].

5.3. LCAR-B38M

Early, promising but preliminary results were recently presented using anti-BCMA CAR-T-cells (designated LCAR-B38M) to treat 19 RRMM patients. For more than 6 months, 7 of the 19 patients were monitored and 6 achieved CR and minimal residual disease-negative status [55]. Among the remaining 12 evaluable patients (followed for less than 6 months following infusion of anti-BCMA CART-cells), all achieved a near CR. Additionally, M-protein levels in these patients were observed to decrease further over time. Of the 19 surviving patients, follow-up demonstrated that they all had no myeloma related tumor markers left. Low-grade CRS was occurred in 74\% of patients but all recovered after treatment [55].

6. Conclusion

BCMA is a TNFR present on late B lymphocytes and is shed off the membrane. Because of its high levels in the serum of patients with late B-cell malignancies, it appears to be a promising blood marker to monitor patients with late B-cell malignancies, including MM, WM and CLL. It also predicts their overall outcomes including PFS and OS among MM
and CLL patients and TTFT among patients with the latter B-cell tumor. The rapid turnover of BCMA in the blood makes it an ideal marker to monitor the disease course of patients with these diseases. In support of this, BCMA determines changes in clinical status much more quickly than conventional tumor markers for among MM and CLL patients. Furthermore, the immune deficiency that is a hallmark of MM points to sBCMA as one of the causes of this clinically important problem. Elevated shed protein in the blood sequesters one of its B-cell ligands, preventing normal B-cell development. Patients with XLA and CVID who show reduced numbers of B- and plasma cells have markedly decreased sBCMA levels [45].

Many immune-based therapeutic approaches targeting BCMA are now in clinical development, including Abs, ADCs, BiTEs, and CAR-T-cells. Early clinical results with ADCs and CAR-T-cells show impressive efficacy including many MM patients in which residual tumor is not able to be detected, but their remains concerns regarding their safety especially CRS among patients receiving T-cell-based treatments and ocular problems for those receiving specific ADC-based approaches with the currently used toxic conjugate. BiTEs targeting both BCMA and T-cell activation proteins are now being evaluated in clinical trials.

Importantly, circulating BCMA may interfere with the efficacy of these immune-based approaches targeting this TNF receptor. GS has recently been shown to be the enzyme responsible for its shedding of this protein off the surface of the B-cells [36]. Blocking its activity with GSI's at low concentrations markedly reduces shedding of BCMA from MM and CLL tumor cells; and, thus, these drugs inhibiting this enzyme’s activity may improve the efficacy of therapies targeting this TNFR. They may also reverse the sBCMA induced immune deficiency by preventing the sequestering of B-cell ligands from solubilized BCMA, allowing free BAFF to bind its receptors on late B-cells, resulting in normal B-cell proliferation and Ab production. Given the promising results from preclinical and recent clinical studies, there is now great interest in BCMA for both its biomarker capabilities and therapeutic possibilities for patients with late B- and plasma cell malignancies.

7. Expert commentary

BCMA now represents both a prognostic and monitoring biomarker, and target for therapeutic approaches for late B-cell malignancies. The soluble form of the protein is present at high concentrations in the blood of these patients, so that its levels can easily
be assessed with only a few microliters of blood. It is also stable at room temperature for months making it convenient to obtain specimens for testing. Specifically, sBCMA represents a new marker to predict important clinical outcomes for patients with MM and CLL, including PFS and OS as well as TTFT for those with CLL. A major additional advantage of assessing sBCMA levels is its ability to more quickly identify changes in the clinical status of patients with MM and CLL than currently available parameters. This allows patients with worsening disease to change therapies more rapidly than currently available modalities, also avoiding the expense and side effects that may develop with unnecessary longer use of drugs that are not effective. The solubilized protein also leads to negative clinical consequences for these patients. It sequesters normal B-cell ligands; and, thus, plays a key role in causing one of the hallmarks of MM, its humoral immune deficiency. Because of the increasing demonstrated clinical efficacy of targeting BCMA with a variety of immune-based approaches, assessment of its soluble form has taken on an additional role for assessing patients with late B-cell malignancies. Specifically, the levels of this circulating protein may adversely impact efficacy of these BCMA-targeted approaches as recently demonstrated from laboratory-based studies. It has also been recently demonstrated that GSIs are capable of preventing shedding of BCMA from MM and CLL tumor cells which should improve the efficacy of these therapeutic approaches. Reducing circulating levels of BCMA may also improve the humoral immune deficient state since it will allow more BAFF to be freely available, rather than BCMA-bound so that it can be capable of driving normal late B-cell development. Because the expression of BCMA is limited to late B-lymphocytes, those with immune deficiencies that show a lack of these cells including patients with XLA and CVID, show markedly reduced levels of this protein in the serum, making it likely that this may also be a diagnostic as well as monitoring tool for patients with these disorders.

Since the expression of BCMA is limited to late B-cells including plasma cells, patients with tumors of these cell types, especially MM, are being treated with therapeutic approaches targeting this protein. Specifically, BCMA is being targeted with multiple immune-based approaches including naked Abs, ADCs, BiTEs, and CAR-T-cells. Early results suggest high levels of efficacy with many patients achieving stringent CRs, including some who achieve minimal residual disease negativity especially among those treated with T-cell therapies. However, concerns exist regarding the safety of this cell-based treatment especially in terms of CRS. Reducing these problems either with less toxic therapeutic approaches or with agents that can mitigate these effects will be an important part of development of these therapies. The Ab-based therapies, especially the ADCs, are also showing clinical activity but there are also ongoing problems with off-target side effects especially ocular problems because of the impact of the conjugated toxin among those receiving this antibody-based approach. It will be important to attach
toxins to the BCMA-targeting vehicle with the least amount of these deleterious effects as new ADCs are developed.

8. Five-year view

Because its expression is limited to late B-cells, BCMA is likely to become the number one target for treating patients with malignancies of these cell types, including MM, WM and CLL. Assessment of its circulating levels will become of increasing importance given its ability to not only predict outcomes for these patients but also to more quickly find changes in clinical status. This will become of increasing importance as drugs become more expensive, so their unnecessary additional usage will be able to be minimized. Because circulating BCMA may sequester drugs targeting this protein, determining its level may have important consequences for patients receiving therapies involving BCMA. As we learn more about how to most effectively use these BCMA-targeted therapies for CLL and MM patients, it is likely to become of increasing importance to monitor circulating levels of BCMA to determine optimal dosing of these drugs, as well as reduce the levels of its blocking effects to improve the efficacy of these treatments. Drugs such as GSIs reduce the solubilized protein and should improve the efficacy of these BCMA-targeted therapies; and, thus, will likely become of increasing importance as part of the therapeutic armamentarium for patients treated with these types of agents. Since sBCMA assesses late B-cell numbers, including among those with immune deficient states, it may become a screening test for determining whether individuals harbor a PID state. In addition, it may be used to monitor their course of disease as well as determine their requirement for specific treatments. It is also possible that assessment of sBCMA may have utility for assessment of immune function in general, so that low levels suggest an impaired immune system. Thus, the next few years are likely to lead to a marked increase in BCMA’s use as both a therapeutic target and monitoring tool for patients with a wide variety of disorders of the immune system.

Key Issues

- BCMA represents a promising new biomarker given its short half-life, which allows for quicker determination of changes in clinical status than conventional/currently used parameters.
- sBCMA predicts PFS and OS for patients with CLL and MM, and can be used to monitor changes in clinical status among these patients more rapidly than currently available tests.
• sBCMA is able to be used to follow patients with nonsecretory MM.

• Given its limited expression to late B-cells and plasma cells, BCMA is the target of novel immune-based therapeutic approaches for patients with late B-cell malignancies (CLL, WM and MM), including naked Abs, ADCs, BiTEs, and CAR-T-cells.

• Using GSI’s, reducing the shedding of BCMA is now possible and may improve the efficacy of anti-BCMA-directed treatment approaches.

• It is responsible for the immune deficiency observed in MM patients.

• sBCMA levels are lower among patients with PID including X-linked agammaglobulinemia and CVID, thus it has the potential to be used to diagnose and monitor these patients.

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Declaration of Interest

E Sanchez, JR Berenson, M Li, CS Wang and H Chen report that they hold ownership interest (including patents) in OncoTracker, Inc., a company that may be affected by the research reported in the paper. This interest has been disclosed fully to Taylor and Francis, and the authors have an approved plan in place for managing any potential conflicts arriving from that involvement. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed. Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

References

Papers of special note have been highlighted as either of interest (*) or of considerable interest (**) to readers.

Laabi’s group was the first to discover the BCMA receptor. We now are beginning to understand the critical role it plays in normal and malignant B- and plasma cells.


This group was the first to discover that BCMA is solubilized (sBCMA) and found in much higher levels in the blood of MM patients when compared to normal controls. In time, sBCMA will be used to track the response (or more importantly, the lack of response) to anti MM treatments, thus allowing physicians to change the treatment regimen before patients are progressed in a potentially ineffective treatment regimen administered to patients.


This reference was marked of considerable importance because the results are in opposition to what would be expected from the data/experiments in hematological malignancies. In this case, APRIL inhibits solid tumor growth. In hematological malignancies, APRIL stimulates growth.


35. Hong-Bing S, Johnson H. B cell maturation protein is a receptor for the tumor necrosis factor family member TALL-1. PNAS. 2000;97:9156-9161. [PubMed: 10908663]
Discovered that BCMA is solubilized (sBCMA) by the enzyme gamma secretase AND that the use of gamma secretase inhibitors prevents cleavage of BCMA.
This laboratory in collaboration with the highlighted one below (Sun C, et al. 2007) discovered that in CLL, BCMA is also solubilized (sBCMA) and can be used to track the response (or more importantly, the lack of response) to treatments. Physicians will now be able to change ineffective treatment regimens sooner, which will hopefully result in the administration of effective treatment regimens to CLL patients.
40* Sun C, Soof C, Taneja A, Herman SE, Cook E, Udd K, Chen H, Sanchez E, Tian X, Ahn IE, Berenson JR, Wiestner A. Serum B-cell maturation antigen as a biomarker for chronic lymphocytic leukemia treated with ibrutinib. Poster session presented at: Lymphoma biology - non-genetic studies. 59th ASH Annual Meeting and Exposition; 2017 Dec 9-12; Atlanta, GA.
This laboratory in collaboration with the highlighted one above (Udd K, et al. 2005) discovered that in CLL, BCMA is also solubilized (sBCMA) and can be used to track the response (or more importantly, the lack of response) to treatments. Physicians will now be able to change ineffective treatment regimens sooner, which will hopefully result in the administration of effective treatment regimens to CLL patients.
45. Maglione PJ, Li M, Sanchez E, Chen H, Radigan L, Berenson JR, Cunningham-Rundles C. Reduced serum levels distinguish patients with primary antibody deficiency. Poster session presented at: Genetic dysregulation in immunodeficiencies and immune-mediated diseases. 100th Annual Meeting of the American Association of Immunologists; 2016 May 13-17; Seattle, WA.
Figure 1.
Soluble BCMA from MM cells binds BAFF, which then prevents BAFF from binding to normal B-cells and inhibits them from proliferation, differentiation and production of normal/polyclonal/uninvolved immunoglobulins.
Figure 2.
BCMA-BAFF complexes. rhBCMA injected into immune deficient CB17 SCID mice binds mBAFF, forming BCMA-BAFF complexes. Negative controls include samples of mBAFF, hIgG and rhBCMA.
Figure 3.
Untreated CLL patients with pBCMA levels > 39 ng/mL had a significantly shorter TTFT (21.7 months) when compared to patients with pBCMA levels < 39 ng/mL (159.8 months).

Figure 4
Figure 4.
As early as day 2 post-ibrutinib therapy, sBCMA levels in CLL patients decreased and progressively declined during the first year of therapy. After a year, a few patients began to progress, as did their sBCMA levels.

Figure 5
Figure 5.
Patients with XLA, CVID, IgG deficiency, or IgA/IgG subclass deficiency had significantly reduced sBCMA levels when compared to controls. In contrast, sBCMA levels were not significantly reduced in patients with selective IgA deficiency when compared to controls.