Immunotherapies targeting CD38 in Multiple Myeloma

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ABSTRACT

Recently, the monoclonal antibody daratumumab was approved as a single agent for the treatment of patients with relapsed/refractory Multiple Myeloma (MM). Daratumumab is an antibody targeting surface molecule CD38 on myeloma cells and the agent is already widely being used based on its good tolerability and proven efficacy. We believe, however, that the efficacy of this drug and other anti-CD38 monoclonal antibodies can be further improved by combining it with other types of immunotherapies. Furthermore, surface molecule CD38 can be used as a target for immunotherapies other than just naked monoclonal antibodies. In this report, we review the expression pattern of CD38 among normal tissues and in different types of plasma cell dyscrasias including their progenitor cells, minimal residual disease, and circulating tumor cells. We summarize the physiological role of CD38 as well as its role in the pathophysiology of MM and we present the most recent clinical trials using CD38 as a target. In addition, we highlight possible combination immunotherapies incorporating anti-CD38 monoclonal antibodies and we demonstrate alternative immunotherapeutic approaches targeting the same antigen such as CD38-specific chimeric antigen receptor (CAR) T cells.

Introduction

Multiple Myeloma (MM) is an incurable plasma cell malignancy which develops in the patient’s bone marrow (BM) and causes renal failure, immunosuppression with repeated infections, anemia, hypercalcemia, and bone lesions.1 In the past 15 y, we have experienced a significant improvement in the patients’ overall survival, however, most patients will still develop refractory disease and eventually suffer a fatal relapse. The treatment of patients with high-risk MM in particular has been disappointing and so far we have not been able to identify a convincing way of dealing with this more therapy-resistant and aggressive subtype of myeloma. Immunotherapies targeting surface antigens such as CD38 carry the potential to overcome high relapse rates and the more aggressive clinical course of MM with high-risk cytogenetics because these novel approaches potentially target the tumor cell irrespective of its biologic characteristics.

The first step in designing a novel immunotherapeutic approach is always to identify a promising target antigen. We have defined a few characteristics, which can help to determine the most promising targets expressed by myeloma cells. For example, we believe that an ideal myeloma-associated antigen for antibody-based approaches (1) must be expressed on the surface of myeloma cells, (2) should be expressed by as few normal tissues as possible, (3) should be expressed by a sufficiently large proportion of myeloma patients, (4) should homogeneously be expressed by the tumor cells of a given patient, (5) should have a central function in the biology and/or pathophysiology of myeloma in order to prevent its downregulation under the selection pressure of an effective immunotherapy.

In this review, we will determine whether the myeloma-associated surface antigen CD38 fulfills the criteria listed, we will summarize the most recent clinical trials using CD38 as a target, and we will delineate ways in which alternative CD38-targeting approaches could be used and how current approaches using CD38-specific antibodies can be optimized.

Target molecule CD38

CD38 was first identified in 1981 as a cell surface antigen structurally related to the human major histocompatibility (HLA) antigen. It was described as a 45 kDa protein associated with a small subunit distinct from β2-microglobulin2,3 and is a single-chain transmembrane type II glycoprotein with a short cytoplasmic tail, a single membrane-spanning region, and a long extracellular C-terminal domain.4 The gene that encodes human CD38 has been mapped to chromosome 4p15.5

CD38 expression in normal and tumor tissues

Among normal cells, CD38 is expressed on approximately 20% of all human BM cells6,7 with an expression mainly restricted to plasma cells,8,9 myeloid10,11 and erythroid,11 precursors, and a small subset of T cells.7 In addition, CD38 is highly expressed on thymocytes2,6,7,11. In human lymph nodes, CD38 shows a comparably low expression on germinal center B cells11 and an
increased expression on terminally differentiated B cells. In the peripheral blood, CD38 expression can be found on approximately 90% of normal plasma cells, approximately 60% of natural killer (NK) cells, 60% of monocytes, and a small subset of normal T cells. In contrast, CD38 is absent from normal peripheral blood and BM B cells and neutrophils.

A large body of evidence suggests that CD38 expression on normal T cells is dependent on the activation status of the cells. Accordingly, CD38 has been used as an “intermediate” to “late” T cell activation marker and an increased expression of CD38 has been observed on T cells of patients after allogeneic stem cell transplantation and in patients with autoimmune diseases such as rheumatoid arthritis.

Although CD38 was first identified as a surface antigen expressed on a human T cell leukemia line, its expression is not restricted to T cells, but it was also detected on all T and B lymphoblastoid cell lines analyzed. Accordingly, among malignant cells CD38 has been described to be expressed on cell lines and primary tumor cells from patients with B cell and T cell leukemias and high-grade B cell lymphomas such as diffuse large cell lymphoma. In contrast, it is absent from the tumor cells of patients with B cell chronic lymphocytic leukemia (CLL) and many patients with Waldenström’s Disease.

Importantly, the strongest and most frequent CD38 expression out of all hematologic malignancies was consistently observed on the malignant plasma cells of MM patients by a variety of modalities, including immunotherapeutic treatment, survival, and progression of MM. We consider it very possible that MM needs to be attacked from different biological angles by a variety of modalities, including immunotherapeutic approaches, to destroy the tumor bulk as well as MM-promoting precursors and eventually achieve cures. For example, the presence of circulating tumor cells (CTCs) has been associated with an increased risk of malignant transformation to symptomatic MM in both MGUS and smoldering MM, as well as with an inferior survival among symptomatic newly diagnosed and relapsed/refractory MM. Functionally, CTCs seem to be mostly quiescent, these cells are associated with a higher clonogenic potential, and they seem to be more chemotherapy-resistant than conventional myeloma cells. Importantly, CTCs show a downregulated expression of CD38 when compared to the patients’ BM plasma cells. It is, therefore, at least possible that an otherwise effective anti-CD38 therapy might select for clones with an increased potential for spreading to other sites in the BM or even peripheral tissues.

It has been suggested that the fact that most myeloma patients will eventually suffer a fatal relapse even after an initially effective therapy is due to the persistence of chemotherapy-resistant precursor cells in the BM even after destruction of the bulk of tumor cells. Accordingly, it has repeatedly been shown that the persistence of minimal residual disease (MRD) with CD38 aberrant plasma cells in the BM after autologous transplantation results in a reduced progression-free survival in MM patients. Since, these remaining tumor cells express CD38 they could be targeted by anti-CD38 immunotherapies, which could potentially result in the eradication of the disease from the BM.

It is an ongoing debate whether myeloma stem cells really exist and what their phenotype looks like. However, it was already shown close to 40 y ago using a newly developed colony forming assay that in MM clonogenic cells represented 0.001–0.1% of the total tumor cell number. It has repeatedly been suggested that myeloma progenitor cells might be enriched in CD138-negative but CD38-positive plasma cells. In addition, it has been shown that CD138–CD19–CD38+ plasma cells are capable of engrafting in immunocompromised mouse models. It is, therefore, important to emphasize that the clonogenic myeloma precursors should they indeed exist, are potentially negative for plasma cell marker CD138 while they are most likely positive for CD38 and could probably be targeted by anti-CD38 approaches.

**Biological function of CD38**

It seems that CD38 ligation has stimulatory effects on normal mature lymphocytes. For example, it has been shown that CD38 ligation by specific monoclonal antibodies (mAb) on normal peripheral blood T cells induces the secretion of different cytokines such as IL-6, granulocyte-macrophage colony-stimulating factor (GM-CSF), IFN-γ, and IL-10.

CD38 ligation on human B cells results in different effects depending on the differentiation stage of the cell. For example, Zupo and coworkers showed that the agonistic anti-CD38 monoclonal antibody IB4 is capable of preventing apoptosis in human tonsillar germinal center (GC) B cells. On the other hand, ligation of CD38 inhibits cell growth and induces apoptosis in normal human B cell precursors in the BM environment. Accordingly, CD38 ligation inhibits proliferation and induces apoptosis through the activation of the syk tyrosine kinase and the phosphatidylinositol 3-kinase pathway in human immature B cell lines.

**Expression on circulating tumor cells, minimal residual disease, and myeloma progenitor cells**

The question whether CD38 is expressed on the bulk of tumor cells from MM patients is of major relevance, however, it is of equal importance to determine whether the antigen is present on the subpopulation of tumor cells promoting the development, survival, and progression of MM. We consider it very possible that MM needs to be attacked from different biological angles by a variety of modalities, including immunotherapeutic approaches, to destroy the tumor bulk as well as MM-promoting precursors and eventually achieve cures.
Later, Deaglio et al. showed that CD31, a member of the Ig gene superfamily characterized by six Ig-like domains and by a unique adhesive ability mediated by homo- and heterophilic mechanisms, is the ligand for CD38. Their results also suggested that the interplay between CD38 and its ligand CD31 is an important step in the regulation of cytoplasmic calcium fluxes identical to the synthesis of different cytokines such as IL-6 and IL-10. Importantly, CD31/CD38 interaction probably also regulates the migration of leukocytes and CD387 cancer cells through the endothelial cell wall. Interestingly, it has been shown that the vast majority of patients with MGUS and MM not only express CD38 on their malignant plasma cells but they are also positive for CD31. In contrast, expression of CD31 was only very rarely detected on the tumor cells of patients with plasmablastic MM and plasma cell leukemia.66

Monoclonal antibodies targeting CD38

The first anti-CD38 monoclonal antibody (Fig. 1) was presented in 1991 when Stevenson and coworkers published a preclinical study on a chimeric mouse Fab-human Fc monoclonal antibody they had prepared from the diagnostic mouse anti-CD38 antibody OKT10. They showed that, in contrast to the parent antibody, the chimera molecule mediated antibody-dependent cellular cytotoxicity (ADCC) very efficiently with human blood mononuclear effector cells in vitro. Importantly, they also demonstrated that, even though CD38 is present on NK cells, there was little deleterious action of the antibody on effector cell function. The antibody also did not affect the growth of progenitor cells of the granulocyte/macrophage or erythroid lineages despite the fact that these cells express CD38.35

In 1995, Ellis et al. reported on the production of a novel high-affinity monoclonal antibody (AT13/S) against CD38. They prepared two engineered forms of the antibody: a humanized IgG1 and a chimeric mouse Fab/human Fc chimeric antibody. They found both constructs to efficiently direct ADCC against CD38-positive cell lines while complement was activated only poorly. Neither construct caused down-modulation of CD38, nor did they affect the NADase activity of CD38.67

In 2011, de Weers and coworkers first described daratumumab, a novel human IgG1 kappa anti-CD38 monoclonal antibody, which was generated by immunizing human Ig transgenic mice with recombinant CD38 protein and CD38-transfected NIH 3T3 cells.68 Daratumumab was of high affinity and, importantly, was capable of inducing strong ADCC and complement-dependent cytotoxicity (CDC) against myeloma cells in vitro and in a mouse model.68 It was also shown that daratumumab-induced ADCC and CDC were not affected by the presence of BM stromal cells, indicating that daratumumab can effectively kill MM tumor cells in the BM microenvironment. Moreover, no daratumumab-mediated lysis of primary human B and T cells, activated T cells, NK cells, and monocytes was observed, suggesting that daratumumab selectively kills MM tumor cells.68

Accordingly, Nijhof et al. showed that the level of CD38 expression is an important determinant of daratumumab-mediated ADCC and CDC. Importantly, they also demonstrated that all-trans retinoic acid treatment led to an upregulation of CD38 expression and a reduced expression of the complement-inhibitory proteins CD55 and CD59 on MM cells, which improved the efficacy of daratumumab.69 However, it remains to be determined if clinically such an intervention, which is potentially associated with significant toxicity in the form of retinoic acid syndrome, might even be necessary given the relatively consistent CD38 expression levels in MM.

More recently, it was shown that, in addition to exerting CDC and ADCC, daratumumab is capable of efficiently inducing macrophage-mediated phagocytosis. Phagocytosis contributed to the antibody’s antitumor activity in a xenograft mouse model and it induced macrophage-mediated phagocytosis of tumor cells isolated from MM patients. These findings indicated that phagocytosis is a clinically relevant mechanism of action that may contribute to the therapeutic activity of daratumumab in myeloma.70

In 2014, Deckert et al. presented their first full-length report on the development of another novel humanized monoclonal antibody named SAR650984.71 The antibody was generated by standard hybridoma technology following immunizations with murine 300-19 cells transfected to express the full-length human CD38 antigen. The authors showed that this antibody was very potent in inducing apoptosis without the addition of an external cross-linking agent and inhibited the ADP-ribosyl cyclase activity of CD38, likely through an allosteric antagonism. Importantly, SAR650984 was also active in B cell lymphoma and MM xenograft mouse models.71 Very recently, it was shown that SAR650984 also decreases MM cell adhesion to BM accessory cells via blockage of CD31–CD38 interaction, rendering the tumor cells more sensitive to antibody-induced cytotoxicity.72

Mor202 (MOR03087) is another fully human anti-CD38 antibody. In addition to ADCC as a potent effector mechanism
of MOR020, it was shown that the antibody is capable of inducing killing of MM patient cells via ADCP.\textsuperscript{73}

Van Bueren \textit{et al.} performed an \textit{in vitro} comparison of daratumumab with alternative antibodies targeting CD38 including MOR03087, SAR650984, and Ab79.\textsuperscript{74} They found that daratumumab and the surrogate antibodies showed comparable affinity and induced similar amounts of ADC. SAR650984 was the only antibody that was able to directly induce apoptosis without Fc crosslinking, a finding which was later confirmed by a different group that showed that SAR650984 can trigger caspase 3/7-dependent apoptosis in MM cells including those with a p53 mutation.\textsuperscript{75} In addition, SAR650984 also showed the most significant inhibition of the enzymatic activity of CD38. The most striking difference, however, was observed for the ability to induce CDC, the mechanism of action, which seems to be very important for the \textit{in vivo} anti-myeloma activity of a given antibody. Daratumumab efficiently induced high levels of CDC at low concentrations, whereas the other CD38 mAb were unable or less capable to induce CDC.\textsuperscript{74}

Interestingly, the different monoclonal antibodies seem to be significantly more effective in preclinical models when combined with IMiDs such as lenalidomide. Accordingly, the Utrecht group showed that daratumumab-dependent ADCC using primary multiple myeloma cells was significantly augmented after lenalidomide pre-treatment of the effector cells but not the myeloma cells themselves. They also observed that daratumumab-dependent ADCC was significantly amplified using peripheral blood mononuclear cells from lenalidomide-treated MM patients as effector cells.\textsuperscript{76}

In their recent analysis, Nijhof \textit{et al.} provided first preclinical evidence for a potential benefit of the daratumumab/lenalidomide combination in lenalidomide- and bortezomib-refractory patients. They showed that daratumumab induced significant lysis of lenalidomide/bortezomib-resistant multiple myeloma cell lines and primary multiple myeloma cells from refractory patients. In these assays, lenalidomide but not bortezomib, synergistically enhanced daratumumab-mediated tumor lysis through activation of NK cells. In an \textit{in vivo} xenograft model, only the combination of daratumumab with lenalidomide effectively reduced the tumorigenic growth of primary multiple myeloma cells from a lenalidomide- and bortezomib-refractory patient.\textsuperscript{77}

An additive or synergistic effect of the combination of an IMiD with an antiCD38 mAb has not only been demonstrated for daratumumab and lenalidomide. It was shown recently that the next-generation IMiD pomalidomide is capable of enhancing SAR650984-induced direct and indirect killing even in MM cells resistant to pomalidomide and lenalidomide alone.\textsuperscript{78} The combination with lenalidomide also induced additive to synergistic enhancement of the activity of antibody MOR202, which was mediated by enhanced direct cytotoxicity and increased CD38 expression levels on MM cells.\textsuperscript{79} Similar effects were observed when MOR202 was combined with the next-generation IMiD pomalidomide.\textsuperscript{79,80}

Finally, it has been shown that antiCD38 antibodies cannot only effectively be combined with IMiDs. Since antibody-dependent cell-mediated cytotoxicity is an important effector mechanism of daratumumab, Nijhof \textit{et al.} explored the possibility of improving ADCC by blocking NK cell inhibitory receptors with the human monoclonal anti-KIR antibody IPH2102 in addition to activating NK cells with lenalidomide. They showed that IPH2102 alone significantly augmented daratumumab-induced myeloma cell lysis. A further synergistically improved myeloma cell lysis with the daratumumab-IPH2102 combination was observed by adding lenalidomide,\textsuperscript{81} which suggests that anti-CD38 strategies can be optimized by combining the respective antibodies with agents that independently modulate NK cell function.

\section*{Anti-CD38 immunotoxins and bispecific antibodies for the treatment of MM}

In addition to their application as naked monoclonal antibodies, anti-CD38 approaches have also proven useful in preclinical models when used in the form of immunotoxins or bispecific antibodies (Fig. 1). In 1994, Goldmacher \textit{et al.} reported the development of an immunotoxin composed of the anti-CD38 antibody HB7 conjugated to a chemically modified ricin molecule. They showed that the conjugated antibody was capable of effectively killing CD38\textsuperscript{+} human myeloma and lymphoma cell lines as well as primary patient-derived myeloma cells \textit{in vitro}. Importantly, the antibody did not affect the growth of progenitor cells of the granulocyte/macrophage or erythroid lineages, which to a certain extent also express CD38.\textsuperscript{82}

Later, Mehta and coworkers showed that \textit{in vitro} treatment with retinoic acid induced high levels of CD38 antigen expression on leukemia cells. As a consequence, the combination of retinoic acid and anti-CD38 gelonin immunotoxin, made of the murine antihuman CD38 mAb IB4, induced a synergistic killing of the tumor cells including multidrug-resistant variants.\textsuperscript{83} Accordingly, Bolognesi and coworkers showed that an immunotoxin consisting of the same anti-CD38 mAb IB4 and the type 1 ribosome-inactivating protein saporin-S6 was capable of exerting strong and specific cytotoxic effects on selected CD38-positive lymphoma cell lines.\textsuperscript{84}

Recently, the Seattle group presented a preclinical study assessing both conventional radioimmunotherapy (RIT), using a directly radiolabeled antibody, and streptavidin-biotin pretargeted RIT (PRIT). Both approaches were directed against the CD38 antigen and were supposed to deliver radiation doses sufficient for MM cell eradication. Complete remissions were observed in all of the mice treated with anti-CD38 pretargeted 90Y-DOTA-biotin and long-term myeloma-free survival was achieved.\textsuperscript{85} More recent biodistribution studies demonstrated myeloma-specific targeting by the antibody with minimal levels of yttrium-90 radioactivity detected in normal organs supporting clinical evaluation of bispecific anti-CD38 PRIT in MM.\textsuperscript{86}

Teiluf \textit{et al.} recently investigated the preclinical efficacy of a different radioimmunoconjugate composed of the \(\alpha\)-emitter\textsuperscript{212}Bi coupled to the anti-CD38 monoclonal antibody MOR03087 in MM. They found that \textsuperscript{212}Bi-anti-CD38 antibody treatment induced DNA damage, which resulted in mitotic arrest and subsequent mitotic catastrophe. The antitumor effect of the immunoconjugate correlated with CD38 expression levels. In myeloma xenografts, treatment with the \textsuperscript{212}Bi-anti-CD38
antibody conjugate suppressed tumor growth via induction of apoptosis and prolonged survival without significant toxicity.\textsuperscript{97}

Compared to immunotoxins fewer results are available for anti-CD38 bispecific antibodies, however, the findings obtained so far look promising. For example, Flavell and coworkers investigated the effectiveness of two different bispecific antibodies, which were based on the OKT10 anti-CD38 antibody, for delivering the ribosome-inactivating protein (RIP) saporin to human T-ALL cell lines. They found that the administration of the antibodies led to a dramatically increased saporin toxicity \textit{in vitro}.\textsuperscript{88} Similarly, French \textit{et al.} generated a bispecific antibody consisting of the anti-CD38 monoclonal antibody AT13/5 and an antibody against the RIP gelonin. They found that the bispecific antibodies were highly efficient at delivering gelonin to B cell lymphoma cells \textit{in vitro} and that cytotoxicity correlated closely with the affinity of the respective antibody. Importantly, they also observed that the anti-CD38 treatment was significantly more effective when combined with a monoclonal antibody specific for gelonin and surface molecule CD22.\textsuperscript{89}

It was demonstrated a little later that “true” bispecific antibodies targeting CD38 (mAb AT13/5) as well as CD59, the major membrane-bound complement inhibitor, were capable of lysing B cell lymphoma cell lines \textit{in vitro}.\textsuperscript{90} Chu \textit{et al.} developed humanized and affinity-optimized Anti-CD38 × Anti-CD3 bispecific antibodies which stimulated potent T cell-mediated killing of human myeloma cell lines in a humanized mouse model and CD38\textsuperscript{+} cells in monkeys.\textsuperscript{91} Finally, Peng \textit{et al.} presented a very interesting study where they showed that an oncolytic measles virus genetically modified to display a single-chain antibody (scFV) against CD38 was capable of mediating cytotoxicity against myeloma cell lines \textit{in vitro} and against CD38-positive tumor cells in a mouse model.\textsuperscript{92}

\textbf{CD38-specific CAR T cells and TCR-transduced T cells as anti-myeloma approaches}

CARs are artificial fusion proteins linking the specificity of an antibody-binding domain to the signaling subunits of activating T cell proteins (Fig. 1). Autologous T cells transduced with these constructs exhibit high and HLA-independent target specificity and cytotoxicity as demonstrated in patients with CD19\textsuperscript{+} B cell lymphoma.\textsuperscript{93} In a recent study, 30 patients with acute lymphoblastic leukemia (ALL) received CD19-specific CAR T cells and 90% achieved durable complete remissions.\textsuperscript{94} A major obstacle in the development of CAR T cell therapies for indications other than CD19\textsuperscript{+} B cell lymphoma is their substantial on-target off-tissue toxicity. Unfortunately, virtually no cell surface antigens are exclusively expressed by malignant cells resulting in the undesired targeting of healthy tissues. Therefore, the search for antigens specifically expressed on tumor cells and only non-vital healthy tissues has become a central objective and we believe that CD38 represents one of the most promising targets for CAR T cell treatments in MM.

Mihara and coworkers have provided a first proof-of-principle showing that T cells equipped with an anti-CD38 CAR can be effective \textit{in vitro} and \textit{in vivo} against hematologic malignancies such as B cell non-Hodgkin lymphoma (B-NHL)\textsuperscript{95} even if the tumor cells were highly chemotherapy-resistant.\textsuperscript{96} The same group later showed that T cells harboring the anti-CD38-CAR were equally efficient in eliminating myeloma cell lines as well as tumor cells from myeloma patients.\textsuperscript{97}

More recently, Drent and colleagues generated retroviral CAR constructs based on human CD38 antibodies as antigen recognition domain and CD32eta and 41BB (CD137) as signaling domains.\textsuperscript{98} Their CD38–CAR T cells effectively proliferated, produced inflammatory cytokines and effectively lysed malignant cell lines and primary malignant cells from multidrug resistant MM patients in a cell-dose, and CD38 expression–dependent manner. Importantly, the CAR T cells did not affect the outgrowth of CD34\textsuperscript{+} cells into various myeloid lineages and were, furthermore, effectively controllable with a caspase-9-based suicide gene. Finally, in a novel \textit{in vivo} xenotransplant, in which myeloma cells were grown in a human BM-like microenvironment, administration of CD38–CAR T cells also resulted in significant antitumor efficacy.

Overall, these early results suggest that CD38-specific CAR T cells can be developed into a safe and effective treatment for MM. The advantage of using CD38-specific T cells versus a monoclonal antibody with the same specificity is that such an approach could take full advantage of the specificity of a CD38-targeting antibody combined with the durability and efficacy of a myeloma-targeting memory T cell response.

Unfortunately, clinical results with CAR T cells have thus far been disappointing in tumors other than CD19\textsuperscript{+} B cell lymphomas, probably reflecting the fact that most cancers do not express highly accessible tumor-specific antigens on their cell surface. Therefore, an alternative approach of engineering tumor-specific autologous T cells is to replace their natural T cell receptor (TCR) with an ectopically expressed TCR specific for the aberrantly expressed antigen (Fig. 1). These TCR-transduced T cells recognize intracellular tumor-specific proteins in a given HLA context. Clinical studies have tested T cells transduced with a TCR specific for an HLA-A2-restricted epitope of the cancer-testis antigen family member NY-ESO-1 in HLA-A2\textsuperscript{+} patients with advanced melanoma,\textsuperscript{99,100} synovial cell sarcoma,\textsuperscript{99,100} and MM.\textsuperscript{101} The treatment was generally well tolerated and in sarcoma and melanoma patients an impressive overall response rate of 55–60\% was reported.\textsuperscript{100} So far, CD38-specific TCR-transduced T cells (Fig. 1) have not been introduced into the clinic, however, this could be developed into a valuable alternative, for example in patients who have lost surface expression of the antigen under the selective pressure of an anti-CD38 antibody therapy but still express the intracellular protein.

\textbf{Clinical results with anti-CD38 strategies}

A number of clinical studies paved the way to the approval of daratumumab, in November 2015, as the first monoclonal antibody directly targeting myeloma cells. The US Food and Drug Administration (FDA) granted accelerated approval to the CD38-targeted monoclonal antibody as a monotherapy for patients with multiple myeloma following at least three prior therapies, based on data from two open-label clinical trials, the MMY2002 study and the GEN501 trial.

The phase II MMY2002 study consisted of two parts. In part one, 34 patients were randomized to daratumumab at 8 mg/kg
In part 2 of the study, 90 additional patients were treated with daratumumab at the 16 mg/kg dose level. Those 106 patients who had received the 16 mg/kg dose level had a median number of five prior treatment lines, and 95% were refractory to their last PI and IMiD. Adverse events were mostly mild and the overall response rate was 29.2% with a median duration of response of 7.4 mo. The median time to progression was 3.7 mo and the estimated 1-y overall survival rate was 65%.102

The phase I/II GEN501 study also contained two parts. In the first part, 32 patients were treated with daratumumab at increasing doses and in the second phase, 72 patients received daratumumab at either 8 mg/kg or 16 mg/kg. In each group, patients had a median of four prior therapies, 64% had disease refractory to bortezomib and lenalidomide, and 76% had received autologous stem-cell transplants. The majority of patients experienced some sort of infusion-related reactions, which most commonly occurred during the first infusion. However, reactions were generally mild and the treatment was considered safe. In part 2 of the study, the overall response rate was 36% in those 42 patients that received the highest dose level of 16 mg/kg. The median progression-free survival in these patients was 5.6 mo and among those patients who had a response, the estimated median duration of response was 6.9 mo.103

As indicated by preclinical investigations, the combination of the anti-CD38 antibody with an IMiD resulted in an improved efficacy. For example, in a phase I/II dose escalation study daratumumab was combined with lenalidomide. Of the 32 myeloma patients in the expansion cohort that received daratumumab at a dose of 16 mg/kg 34% had prior exposure to lenalidomide. The most common adverse events included neutropenia in 81% of the patients, as well as muscle spasms, cough, diarrhea, fatigue, and hypertension in less than half of the patients. The overall response rate was 88%, with 34% partial responses and 53% ≥ very good partial responses.104

Chari et al. reported on another phase Ib study where daratumumab was given in combination with next-generation IMiD pomalidomide and dexamethasone to patients with relapsed and refractory multiple myeloma. Patients had ≥2 prior lines of therapy including ≥2 consecutive cycles of lenalidomide and bortezomib and received daratumumab at 16 mg/kg. A total of 77 patients were enrolled and the median number of prior therapies was 3.5. Eighty-eight percent of the patients were refractory to lenalidomide and 65% to both a PTDl1 and an IMiD. There was little additional toxicity when daratumumab was added to pomalidomide/dexamethasone other than daratumumab-specific infusion-related reactions such as chills, cough, and dyspnea. In 53 evaluable patients, the ORR was 58.5% and many responses deepened over time.105

Very recently, Lonial et al. reported results of a multicenter phase II trial where 106 myeloma patients, following a dose-escalation part, received daratumumab at 16 mg/kg. Patients had received a median of five previous lines of therapy, as many as 85% had previously received autologous stem cell transplantation, about 90% were refractory to either lenalidomide or bortezomib, and 83% were double-refractory. In this heavily pretreated and multi-refractory patient population the ORR was still 29.9% with a median duration of the responses of 7.4 mo. As in the other trials with anti-CD38 antibodies, the treatment was well tolerated with 42% of patients experiencing infusion-related reaction, which were generally mild and predominantly occurred during the first infusion.106

There have been a number of clinical trials with alternative anti-CD38 antibodies. In a dose escalation phase I study, the SAR650984 antibody was administered to patients with different CD38+ hematologic malignancies who had progressed on or after standard therapy. A total of 32 patients were treated across all dose levels including 27 patients with MM. The most frequent occurring adverse events were fatigue (46.9%), nausea (31.3%), pyrexia (28.1%), cough (25%), vomiting (21.9%), and hypercalcemia (18.8%). All MM patients had received prior lenalidomide and bortezomib and the median time from diagnosis to first SAR650984 dosing was 6.8 y. Of all myeloma patients 17 were treated at dose levels between 1 mg/kg and 10 mg/kg. At a dose level of ≥1 mg/kg the overall response rate was 25% and at a dose level of ≥10 mg/kg objective responses were seen in 31% of all myeloma patients.107

The same monoclonal antibody was later combined with lenalidomide in a different phase Ib dose escalation trial. Out of the 31 MM patients included 95% had prior exposure to an IMiD and more than 85% had relapsed after or were refractory to at least one prior IMiD-based therapy. With a median follow-up of 6 mo, the overall response rate was 64.5% including two complete responses and eight very good partial responses. Of note, among the 24 patients who were relapsed and refractory to their last regimen containing lenalidomide, the overall response rate was still 62.5%. Median duration of all responses was 23 weeks.108

Recently, preliminary results were reported on a phase I dose-escalation study with the anti-CD38 antibody MOR202. For the first 38 patients with relapsed myeloma treated, the treatment proved to be safe and well tolerated but no efficacy data were reported at this early stage of the study.109

There is currently no detailed information on the question whether patients can effectively be retreated with an anti-CD38 antibody after an initial response. However, a recent report of two cases suggested that patients who had an initial response to daratumumab can effectively be retreated following a treatment interruption.110 The authors showed that continuous treatment with daratumumab decreases ADCC-mediated antitumor activity using two independent mechanisms, which can both be recovered by treatment interruption. In conclusion, there may be a need for treatment interruption for optimal maintenance of ADCC.

**Immunotherapies targeting CD38 – conclusions, possible limitations, and future perspectives**

Myeloma therapy using anti-CD38 antibodies has already proven effective, however, there are certain limitations we need to address in order to further increase the efficacy of this approach. For example, as outlined above there seems to be a progressive decrease in expression levels of CD38 from normal plasma cells to MGUS plasma cells, MGUS to MM, and finally MM to plasma cell leukemia. Accordingly, it has been reported that myeloma cells may downregulate the expression of CD38 or even completely lose its expression in very aggressive forms.
of the disease with extramedullary MM.\textsuperscript{39} Furthermore, another recent report suggested that myeloma cells can in rare cases lose expression of CD38 at the time of occurrence of relapsed/refractory MM, even without prior treatment with anti-CD38 approaches.\textsuperscript{111} We, therefore, believe that CD38 expression should always be re-evaluated prior to considering CD38 monoclonal antibody therapy for patients with relapsed and refractory MM, even if their tumors initially expressed CD38.

Along the same lines, the presence of soluble CD38 protein, in particular in patients with a significant tumor burden, could undermine the therapeutic efficacy of anti-CD38 approaches. Funaro \textit{et al.} demonstrated the existence of a soluble form of human CD38 derived from the membrane-anchored CD38\textsuperscript{58} and it remains to be seen whether soluble CD38 present in the peripheral blood and BM environment of patients with MM potentially interferes with clinical responses to anti-CD38 approaches.

Accordingly, in their response to a report on the design of an anti-CD38 immunotoxin Vooijs and colleagues considered expression of CD38 by basophils an important drawback. They remarked that a destruction of these cells by an anti-CD38 immunotoxin may result in anaphylactic shock caused by the release of vasoreactive agents. They even concluded that anti-CD38 monoclonal antibodies “are principally not the right candidates for immunotherapy […] in patients with MM”\textsuperscript{112}

Recent clinical data using anti-CD38 monoclonal antibodies clearly show that this approach is safe and, with the exception of infusion-related reactions with the first application, does not cause any major toxicities. However, unspecific expression of the target antigen could become a significant problem especially with the introduction of more potent approaches such as CD38-specific CAR T cells.

Apart from the assessment of antigen expression on myeloma cells, we currently do not have any biomarkers with a predictive value for anti-CD38 approaches. In a very recent study, serum samples of myeloma patients were analyzed before and during treatment with daratumumab to identify proteins and biological pathways associated with a clinical response to the antibody. Profiling of 1,129 serum proteins was performed and the investigators found 51 proteins to be significantly different between clinical responders and non-responders. Interestingly, a subset of the differentially expressed proteins has previously been associated with an immune response and T cell biology. In addition, proteins related to T-cell activity, immune checkpoints, and immune response (such as CCL5, ICOS, Granzyme B, and PD-L1) also showed changes associated with daratumumab treatment, supporting the idea that daratumumab induces a T cell response in MM patients, which may contribute to clinical responses.\textsuperscript{113}

Overall, we believe that the introduction of novel biomarkers assessing CD38 expression on myeloma cells, normal leukocytes, and immune factors within tumor cells and the BM microenvironment will help to further improve the efficacy of anti-CD38 approaches in MM. Furthermore, combining different types of immunotherapies with CD38-specific approaches as well as the introduction of more potent CD38-targeting modalities, such as bispecific antibodies and CD38 CAR T cells, will lead to deeper and more durable responses in patients with MM.

Disclosure of potential conflicts of interest
No potential conflicts of interest were disclosed.

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