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REVIEW

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CD38 as theranostic target in oncology

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Abstract

CD38 is a multifunctional transmembrane glycoprotein found in multiple tissues and overexpressed in many cancer cells, notably in hematological malignancies such as leukemia and multiple myeloma (MM). Therefore, targeting CD38 remains an attractive strategy for cancer treatment in hematological malignancies as well as in solid tumors. It plays a critical role in the progression of these diseases through its ADP-ribosyl cyclase and cADPR-hydrolase activities. Its importance has led to the development of various anti-CD38 monoclonal antibodies (mAbs), including daratumumab and isatuximab, approved for MM treatment. These mAbs exert their anti-tumor effects through Fc-dependent immune mechanisms and immunomodulation, enhancing T-cell and NK-cell-mediated responses. However, resistance mechanisms arise during the treatment with daratumumab, creating the necessity for new therapies. This review explains current knowledge about the role of CD38 as a target in oncology and aims to delineate the use of single domain antibodies (sdAbs) as innovative theranostic tools in nuclear medicine. For diagnostic purposes, PET radionuclides like ^{68}Ga, ^{64}Cu, and SPECT radionuclides like ^{99m}Tc and ^{111}In, are commonly used. Significant progress has been made in anti-CD38 radioligand therapy (RLT), with anti-CD38 antibodies providing insights into tumor biology and treatment efficacy. In terms of therapy, RLT is a promising approach that offers precise targeting of malignant cells while minimizing exposure to healthy tissue. This involves the use of radionuclides emitting α particles, like ^{225}Ac, ^{212}Pb or ^{211}At, and β^{-}-particles like ^{90}Y, ^{131}I, or ^{177}Lu, to exert cytotoxic effects. Derived from *Camelidae* heavy chain antibodies, sdAbs offer advantages over conventional mAbs such as small size, high stability, specificity, and ability to recognize hidden epitopes. CD38-specific sdAbs, such as sdAb 2F8, characterized by our laboratory, showing excellent tumor targeting and their engineered constructs, such as biparatopic antibodies and chimeric antibodies, represent a new generation of theranostic agents for diagnosis and treatment CD38-expressing malignancies.

CD38 structure and functions

CD38 was first described in 1980 by Reinherz and Schlossman in their studies of thymocytes and peripheral T cells [1]. This protein is a type II transmembrane glycoprotein encoded on chromosome 4 (4p15.32) and consists of three domains: an intracellular domain (N-terminus) of 21 amino acids, an alpha-helix transmembrane domain and an extracellular domain (C-terminus) of 256 amino acids. This extracellular domain has a multifunctional enzymatic activity. Initially described as an ADP-ribosyl cyclase, CD38 cyclizes nicotinamide adenine dinucleotide (NAD) to cADPR. However, it can also hydrolyse cADPR to produce ADP-ribose. This reaction appears to occur at neutral or alkaline pH. Interestingly, at acidic pH, the protein uses NAD phosphate (NADP) as a substrate to produce nicotinamide adenine dinucleotide phosphate (NAADP), which is hydrolyzed to ADP-ribose 2'-phosphate (ADPRP) [2]. These two metabolites are involved in different pathways and play a key role as

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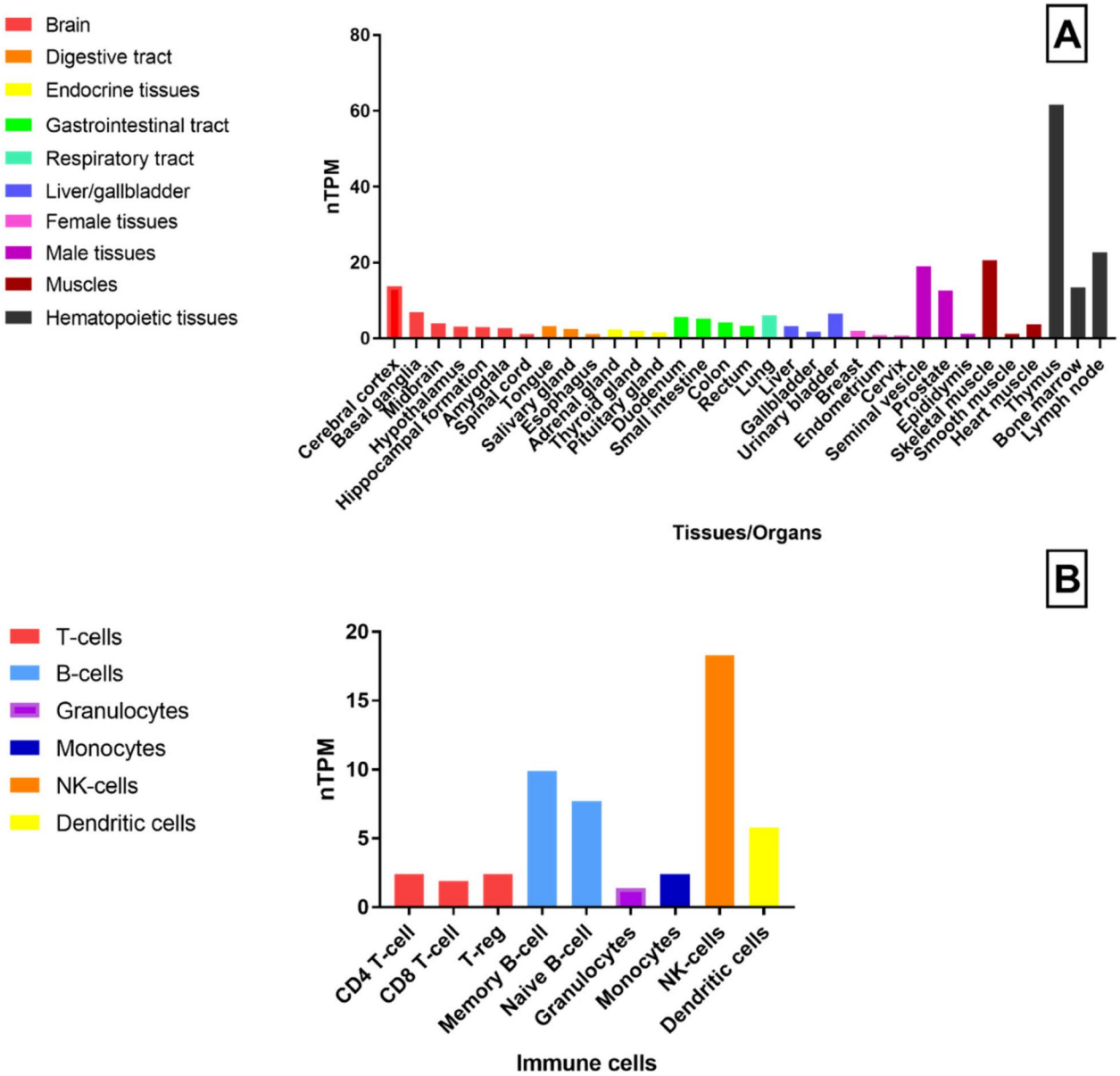

Fig. 1 Schematic representation of the CD38 distribution in human body based on data from "The Human Protein Atlas" (https://www.proteinatlas.org/ENSG00000004468-CD38). The RNA-seq data are reported as nTPM (normalized transcript expression values) for different types of human tissues. **A** CD38 RNA expression detected in different organs, showing a predominance in hematopoietic tissues. **B** CD38 expression at immune cells level, grouped by types of immune cells

second messengers in cellular Ca^{2+} mobilisation. However, it has been described that these substrates are produced in the cytosol to induce Ca^{2+} release mainly from the endoplasmic reticulum or the extracellular space by activating Ca^{2+} channels. That's why some studies have highlighted a cytosolic CD38 orientation, called type III CD38. This protein could be expressed on the surface of the plasma membrane as well as on the endoplasmic reticulum membrane.

CD38 expression

Over the past forty years, extensive research has elucidated the multiple roles of CD38 and revealed its expression in various tissues under healthy conditions (Fig. 1). Predominantly observed in bone marrow, lymph nodes, thymus, spleen and tonsils, CD38 is also present in not hematopoietic tissues such as male tissues in the prostate and seminal vesicles [3, 4] (Fig. 1A). Notably, immune cells have the highest levels of CD38 in the human

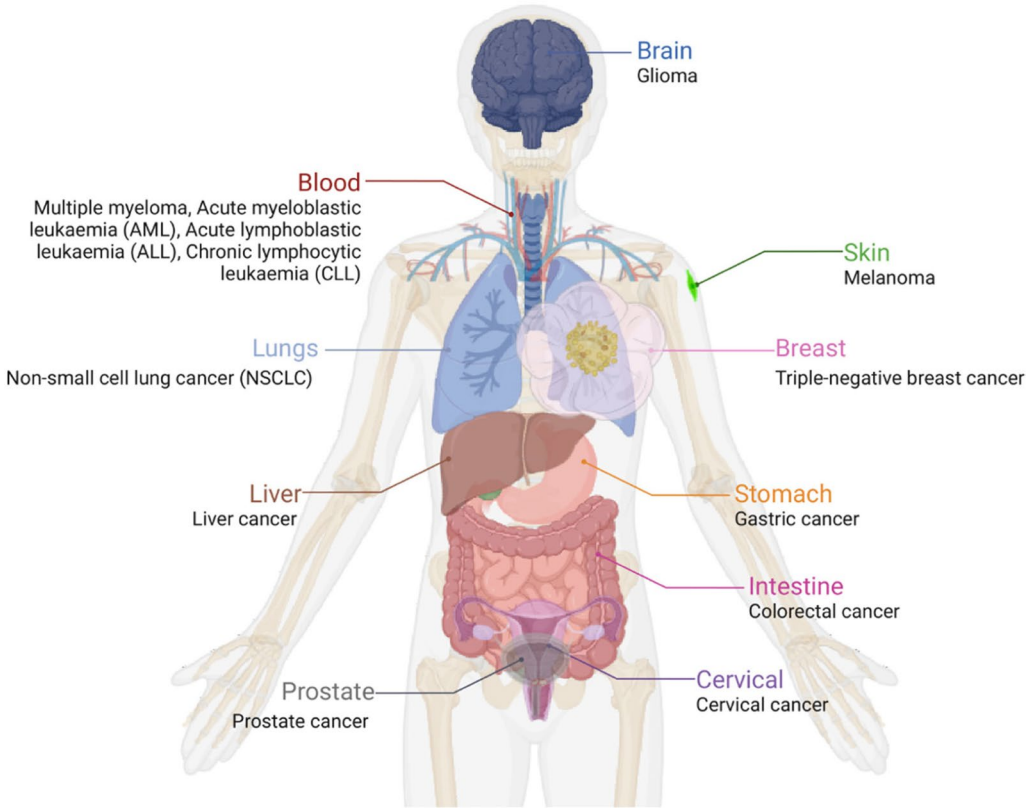

Fig. 2 Distribution of CD38 glycoprotein in the human body and the cancer related to its presence. CD38 is involved in the development of various malignancies, both solid and liquid tumors, related to various organs. Created with BioRender.com

body. Lymphoid cells—such as natural killer (NK) cells, memory and naive B cells—and myeloid cells, including dendritic cells and plasma blasts, express high levels of this molecule (Fig. 1B), confirming is role in immune response. Indeed, CD38 is mainly involved in the maturation of B cells. CD38 has also been studied and analyzed for its involvement in various pathologies over the last 30 years, in particular it is significantly involved in the pathogenesis of hematological neoplasms (Fig. 2). As abnormal CD38 expression in these malignancies correlates with cellular proliferation and disease progression, it can be considered relevant both as a marker of disease progression/prognosis and as a target for therapy.

CD38 expression in lymphoma

CD38 is involved in the pathogenesis of several lymphoid cancers for which it is currently used as a prognostic marker or therapeutic target [5]. Classical Hodgkin's lymphoma (HL), which accounts for approximately 10% of all diagnosed lymphomas, is a B-cell lymphoid disease that is subdivided into classical HL and nodular lymphocyte-predominant HL [6]. In this malignancy, CD38 is mainly expressed on tumor-infiltrating lymphocytes where it acts as an immunomodulator [7]. Burkitt lymphoma is a highly aggressive cancer of mature B cells associated with a translocation of the MYC gene, resulting in an uncontrolled cell growth [8]. The rearrangement of the MYC gene, located on chromosome 8, with the immunoglobulin heavy chain (IGH) or light chain genes results in overexpression of *CD38* [9] and downregulation of the *LMO2* gene [10]. Thus, the combination of LMO2-negative and CD38-positive may be used to diagnose Burkitt lymphoma [10]. Similarly, in diffuse large B-cell lymphoma (DLBCL) and high-grade B-cell lymphoma (HGBL), *MYC* overexpression is associated with *BCL2* and/or *BCL6* rearrangements [11, 12]. These subtypes, known as double/triple-hit lymphomas, are known as more aggressive lymphomas. Dysregulation of BCL2 and BCL6, both of which have anti-apoptotic functions, confers a survival advantage to cancer cells [12]. The detection of CD38, TCL1 and CD44 in DLBCL and HGBL allows the differentiation of lymphoid tumors with presence of *MYC* and absence of *MYC* translocations [13]. In addition, there is a higher intensity of CD38 expression (measured by flow cytometry) in the more aggressive multi-hit variants, which allows further differentiation of the diseases

and the use of CD38 as a biomarker for early diagnosis [14]. In mantle cell lymphoma (MCL), CD38 expression correlates with poorer prognosis and promotes clonal B-cell accumulation, making it an attractive therapeutic and diagnostic target [15]. Another form of lymphoma is Waldenström's macroglobulinemia (WM), which is characterized by the accumulation of malignant IgM-secreting lymphocytes in the bone marrow and in other organs [16]. CD38-positive cells were detected in 20 out of 32 patients, with expression levels essentially identical to those seen in MM [17]. Finally, peripheral T-cell lymphoma (PTCL), which accounts for approximately 10% of aggressive lymphoid tumors [18], includes two common subtypes: Peripheral T-cell lymphoma (PTCL-NOS) and angioimmunoblastic T-cell lymphoma (AITL) [19]. CD38 was found to be overexpressed in approximately 80% of AITL and 60% of PTCL-NOS cases [20]. Also in NK-T lymphoma, CD38 expression on tumor cells is significantly correlated with a poor survival for patients with nasal type of NK-T cell lymphoma [21]. In conclusion, CD38 can be considered as a biomarker for diagnosis and a potential target for treatment in these forms of B- or T-cell lymphoma.

CD38 expression in chronic acute leukemia

CD38 plays an important role in the pathogenesis of several types of leukemia. Acute myeloblastic leukemia (AML) is an aggressive malignancy characterized by the clonal proliferation of primitive, immature hematopoietic stem cells in the blood and bone marrow, leading to ineffective erythropoiesis and bone marrow failure [22]. Notably, AML cells exhibit high levels of CD38, which has been implicated with the regulation of cytokine release [23]. Studies by Farber et al. have shown that treatment with the monoclonal anti-CD38 antibody daratumumab is a promising anti-leukaemic strategy that induces leukemic cell phagocytosis and blocks cell migration [24]. CD38 is also important in acute lymphoblastic leukemia (ALL), an aggressive cancer of B or T lymphoblasts characterized by uncontrolled proliferation of immature lymphocytes [25]. In pediatric T-cell acute lymphoblastic leukemia, CD38 expression remains present from diagnosis to relapse, providing a potential avenue for targeted immunotherapy with anti-leukaemic efficacy [26]. Conversely, in Philadelphia chromosome-linked B-cell acute lymphoblastic leukemia CD38 is emerging as an independent adverse prognostic factor useful for diagnostic purposes [5].

CD38 expression in chronic lymphocytic leukemia

CD38 is also involved in chronic lymphocytic leukemia (CLL), a malignancy characterized by the accumulation of malignant mature lymphocytes in the peripheral blood, bone marrow and lymphoid tissues [27]. Overexpression of CD38 in these leukemic cells correlates with a hyperproliferative phenotype, facilitating cancer cell division upon interaction with activated T cells in the tumor microenvironment [28]. CD38 mediates cancer cell migration and homing within this supportive environment, synergizing with the B cell receptor (BCR) pathway to promote malignant cell proliferation and survival [29, 30]. Specifically, CD38 acts as an auxiliary factor that further activates downstream signaling pathways that were initially triggered by the CXCR4 and BCR complex. In CLL, leukemia progression consists of recirculation of cancer cells from the blood to the lymphoid organs, and this process is coordinated by the chemokine CXCL12 and its receptor CXCR4 [31]. However, there are no differences in CXCR4 expression in patients with migrating and non-migrating CLL, suggesting that the diverse responses to CXCL12 are related to CD38 expression [31]. In addition, CD38 promotes the stimulation of CLL cells by activating the AKT/SYK/Mcl-1 signaling pathway. Ligation of CD38 to the BCR complex in lipid rafts provokes tyrosine phosphorylation of several intracellular proteins, including spleen tyrosine kinase (SYK). SYK activates the downstream AKT pathway and induces the upregulation of MCL-1 expression, resulting in cell survival and migration [32]. Taken together, these results confirm the involvement of CD38 in cancer cell proliferation, chemotaxis and survival, and highlight its potential as a target for therapy in CLL [33].

CD38 expression in multiple myeloma

It is important to highlight the involvement of CD38 in MM, one of the most extensively studied CD38-associated diseases. MM is a hematological neoplastic disorder characterized by the accumulation of malignant plasma cells in the bone marrow, where the disease may cause complications that are often referred to as CRAB features (hyper-calcinemia, renal insufficiency, anemia and/or bone disease) or SLIM biomarkers (clonal bone marrow plasma cell percentage $\geq$ 60%; Involved: uninvolved serum free light chain ratio $\geq$ 100 and/or > 1 focal lesions with magnetic resonance imaging) [34]. The aberrant interactions between MM cells and stromal cells promote the reciprocal activation of myeloma cells and creates an environment that supports the progression of the disease [35]. The treatment of MM is based on the use of autologous stem cell transplantation and the combination of proteasome inhibitors and immunomodulatory agents. Although these strategies improved the outcome of MM patients, the majority of them still relapse and have a poor prognosis [36]. Consequently, the search for new therapies has focused on targets, particularly surface

proteins that are preferentially present or overexpressed on cancer cells compared to normal cells.

Among these targets, CD38 has emerged as particularly promising due to its higher expression on MM cells compared to other, normal cells [37]. Several studies have shown robust and high CD38 expression on malignant plasma cells in BM samples of MM patients [38]. While the precise role of CD38 in MM cell biology remains incompletely understood, several investigations have explored its function as a glycoprotein and ectoenzyme in MM pathogenesis. As a glycoprotein, CD38 interacts with CD31, which is co-expressed on MM cells, and contributes to various cellular processes. These include T cell activation and proliferation, B cell differentiation and chemotaxis of neutrophils and monocytes [38]. In addition, as an ectoenzyme, CD38 modulates intracellular NAD + concentration, which is critical for maintaining low glycolytic activity that supports cell proliferation and survival [39]. Depending on pH conditions, CD38 catalyzes the conversion of NAD + to adenosine (ADO), a mediator of calcium signaling that promotes tumor survival and immune evasion. In addition to CD38, other ectoenzymes such as CD39, CD73 and CD203a are involved in the extracellular production of ADO and their levels reflect disease progression [38]. Indeed, macrovesicles (MVs) isolated from bone marrow plasma samples of MM patients have shown higher levels of ectoenzyme expression compared to patients with MGUS and SMM, resulting in enhanced ADO catabolism [40]. In addition, CD38 serves as a metabolic sensor by interacting with osteoclasts (OCs) in adult skeletal remodeling [41]. OCs, which are fundamental to bone remodeling, are affected by CD38 inhibition, which not only inhibits bone resorption [41] but also restores T-cell function, thereby averting the progression of bone disease [42]. As CD38 is expressed on the surface of early OC precursors, CD38 blocking may also prevent early OC formation [43]. Furthermore, in MM, a subpopulation of regulatory T cells (Tregs) shows increased CD38 expression compared to T constitutive cells (Tcons), which enhances their immunosuppressive effect. MM patients have a significantly higher Treg phenotype than healthy individuals [44].

CD38 expression in solid tumors

In addition to hematological malignancies, CD38 plays an important role in several other types of cancer, but data on its functional role are conflicting. Indeed, in some cancers, CD38 overexpression is correlated with tumor progression, while in others the opposite has been described.

In non-small cell lung cancer (NSCLC), CD38 is overexpressed in 40% of primary tumors and in many human lung cancer cell lines [45]. Knocking out the CD38 gene inhibited oncogenesis in vitro and in vivo. In fact, the NAD + glycohydrolase activity of CD38 was essential for tumor progression by downregulating tumor suppressors (such as KEAP1) and upregulating oncogenes (such as NRF2) [46]. In addition, tumors treated with PD-1/PD-L1 blocking antibodies develop resistance through upregulation of CD38 induced by all-trans-retinoic acid and IFNβ in the tumor microenvironment [47]. In NSCLC, a subset of cancer stem cells induces bone metastasis by stimulating adenosine production and osteoclast activity. The high expression of CD38, PC-1 and CD73 in these cells favors pre-tumoral activity [48]. In other cancers, CD38 is mainly expressed by cells in the tumor microenvironment. In triple-negative breast cancer, CD38 is expressed on tumor-infiltrating plasma cells. In fact, a retrospective study has shown that higher expression of CD38 on tumor-infiltrating plasma cells correlates with increased disease-free survival and overall survival [49]. In melanoma, CD38 expression has been found on cancer-associated fibroblasts (CAFs). By injecting melanoma cells into CD38−/− mice, the function of CD38 could be studied and was found to be involved in (1) resistance to cell death, (2) tumor vascularization through a reduction in CD34, (3) recruitment of CAFs and (4) metastatic properties [50]. As described previously, CD38 inhibits the proliferation of memory CD4 + and CD8 + T cells via the alternative ectoenzymatic pathways that produce ADO—responsible for its suppression [51]. In colorectal cancer, Walterskirchen et al. showed that metastasis-associated fibroblasts (MAF) expressed high levels of CD38 and activated macrophages into a pro-tumoral M2-like macrophage [52]. These macrophages activated by MAF are unable to activate T cells. However, knocking down CD38 expression with siRNA did not affect macrophage polarization or T-cell activation. In esophageal cancer, CD38 expression on myeloid-derived suppressor cells (MDSC) discriminates between MDSC with high and low immunosuppressive function on T cells [53]. This CD38 expression was induced by inflammatory cytokines such as IL-6, IFNy, TNFa, CXCL-16, secreted by tumor cells. Therefore, the use of an anti-CD38 antibody could be an interesting strategy to restore the anti-tumor immune response by acting on MDSCs [54]. CD38 is also involved in gliomas where it supports the activation of tumor microglia and macrophages and subsequent tumor progression [55]. In this case, CD38 through its cADPR directly promotes microglial activation by increasing Ca2 + levels. The role of CD38 in the tumor microenvironment on glioma progression was also investigated by comparing WT and CD38-deficient mouse models. The latter group showed attenuated

tumor growth and prolonged survival, suggesting the central role of CD38 in glioma expansion and its feasibility as a target for glioma therapy [55].

Treatment with anti-CD38 monoclonal antibodies

The overexpression of CD38 on cancers cells and their implication in cancer progression has led researchers to develop several monoclonal antibodies (mAbs) targeting CD38 [56–58]. Commercially available CD38 mAbs for MM treatment include daratumumab (fully human, approved by FDA in 2015 and by EMA in 2016) and isatuximab (fully human, approved in 2021). Other new agents are undergoing clinical trials, such as MOR202 (Felzartamab) (fully human) [59], TAK079 (Mezagitamab) (fully human) [60], FTL004 (humanized Ig1) [61], SAR442085 (fully human engineered) [62] and TNB-738 (fully human) [63]. Their anti-tumor activity depends on Fc-dependent immune effector mechanisms and immunomodulatory effects that eliminate CD38 regulatory T cells and restore T-cell- and NK-cell-mediated antitumor immune responses [56].

Daratumumab

Despite significant therapeutic advances against MM over the past decades, a large proportion of patients relapse, resulting in poor overall survival [64]. The need to find new agents for refractory patients has led to the development of mAb-based therapies. Daratumumab (Janssen Pharmaceuticals) is the first high-affinity CD38-targeting Ab to be approved by the FDA and EMA as a monotherapy for relapsed/refractory MM (RRMM) [65]. Daratumumab has direct and indirect anti-cancer activity by binding to CD38, inducing direct apoptosis of tumor cells and triggering complement-dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP) [64]. Complement-dependent cytotoxicity involves the activation and deposition of complement components on the target cell surface, ultimately leading to the formation of the membrane attack complex (MAC). This permeabilizes the cell membrane, overcoming the cells' defenses and leading to cell death [66]. In addition, the anti-tumor activity is due to ADCC, whereby Ab-coated tumor cells are lysed by NK cells [67]. In fact, when the Fc portion of daratumumab binds to FcγRs on NK cells, cytotoxic proteins (granzymes and perforins) are released, leading to cell death [68]. In addition, ADCP induced by daratumumab binding to monocytes and macrophages enhances cell killing mechanisms, as mAb-coated cancer cells are rapidly phagocytized by macrophages [69]. Finally, daratumumab induces direct myeloma cell apoptosis by modulating CD38 and via Fc receptor-mediated cross-linking. It binds to activating FcγRs on immune effector cells such as macrophages, NK cells and polymorphonuclear cells, inducing programmed cell death [70]. The induction of apoptosis was mainly studies on CD38 + MM cells and was verified by studying the morphological changes, phosphatidylserine translocation, loss of mitochondrial membrane potential and loss of membrane integrity after treatment [70]. In addition to its direct antitumor effects, daratumumab exerts an indirect immunomodulatory role by eliminating CD38 + immunosuppressive cell types (Treg, Breg and myeloid-derived suppressor cells) in the tumor microenvironment (TME), thereby promoting T-cell proliferation and effector functions [44]. Together, these multiple mechanisms contribute to the efficacy of daratumumab, which was proven in the subsequent clinical trials as an effective and durable antitumor drug in MM patients. Daratumumab earned approval for monotherapy due to its efficacy, safety profile and durable responses demonstrated in the phase 1/2 GEN501 and the phase 2 SIRIUS clinical trials on heavily pretreated RRMM patients [71, 72]. Moreover, daratumumab's activity was validated in newly diagnosed MM patients [73] and those with intermediate-risk/ high-risk smoldering MM [74]. Following the encouraging results as single agent, daratumumab was evaluated in combination with immune checkpoint inhibitors, immunomodulatory drugs and proteasome inhibitors (Fig. 3). Combining daratumumab with these therapies improves treatment efficacy, reduces treatment resistance, and extends patient survival [75]. Combinations with immune checkpoint inhibitors, such as PD-1/PDL-1 axis and CD47 inhibitors, are still under investigation. Inhibiting these inhibitory pathways emerges as a promising strategy to enhance the immune response against cancer cells (Fig. 3A). Preliminary data from trials involving daratumumab with Pembrolizumab (a PD-1 inhibitor), Atezolizumab (another PD-1 inhibitor), and TTI-622 (a CD47 inhibitor) suggest potential synergistic effects, offering new avenues for MM treatment [77]. Additionally, significant improvements in ORR and clinical outcomes have been seen with combinations with immunomodulatory drugs (IMiDs), such as lenalidomide and pomalidomide, and the proteasome inhibitors (PI) bortezomib and carfilzomib (Fig. 3C). The quadruplet regimen Dara-VRd (Daratumumab + Bortezomib + Lenalidomide + Dexamethasone) has recently shown superior response rates, higher minimal residual disease (MRD) negative rates, and improved progression-free survival in newly diagnosed multiple myeloma patients. In this regimen, lenalidomide can be replaced with cyclophosphamide [76]. Lastly, the feasibility of combining anti-CD38 mAb with NK cellular therapy is currently being studied in clinic (NCT04614636). It has been reported that $CD38^{low}$NK-cells exert a higher tumoricidal response

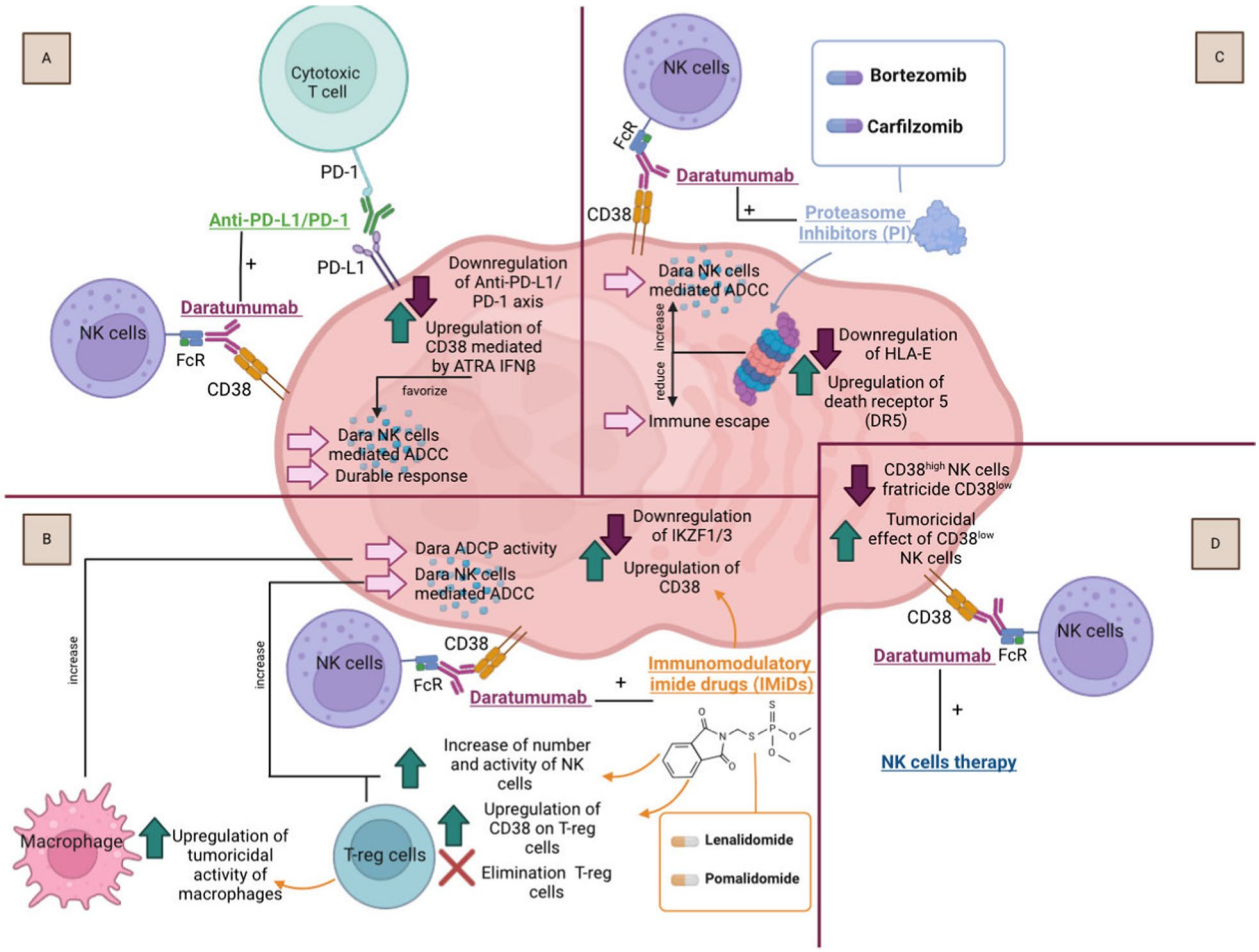

Fig. 3 Schematic representation of molecular mechanism of daratumumab combined therapy. **A** Dara with checkpoint inhibitors. The downregulation of the PD-L1/PD-1 pathway in MM cells, leads to the upregulation of CD38 on NK cells and their ADCC, favorizing a durable response. **B** Dara with IMiDs. IMiDs work by reducing IKZF1/3 levels, which increases CD38 expression, improving the effectiveness of Daratumumab treatment. NK cell-mediated ADCC is increased by enhancing both the number and activity of NK cells and reducing regulatory T cells (Tregs). Additionally, it stimulates macrophage-mediated ADCP. **C** Dara with PIs. The combination increases DR5 levels and decreases HLA-E levels, which strengthens NK cell-mediated ADCC and reduces immune evasion. **D** Dara with NK cells therapy. The tumor-killing ability of CD38low/- NK cells is improved by reducing the fratricide CD38high/- NK cells. Created by Biorender.com

compared to $CD38^{high}$NK-cells. Daratumumab depletes $CD38^{high}$NK-cells favorizing the cytotoxic activity of $CD38^{low}$NK-cells. Therefore, combining $CD38^{low}$ NK cells or engineered $CD38^{KO}$NK cells (without CD38 expression) with daratumumab can enhance treatment efficacy (Fig. 3D).

Mechanisms of daratumumab resistance

However, after several months of treatment with daratumumab, patients face a progression of their disease because of acquired resistance [78]. This resistance is mediated by different causes and mechanisms, (that are resumed in Table 1 and excellently summarized in different review articles [78].One of the most obvious and intriguing mechanisms of resistance involves alteration in CD38 expression, a crucial factor in both primary and acquired resistance to anti-CD38 therapies. The efficacy of ADCC and CDC, and consequently response rates observed in GEN501 and SIRIUS trials, are directly proportional to CD38 expression levels on MM cells.

Isatuximab

Isatuximab, developed by Sanofi, is the second CD38-targeting antibody approved by both the EMA and FDA in 2020/2021 for the treatment of MM. Presently, isatuximab is approved for the treatment of RRMM in combined therapy with pomalidomide and dexamethasone [79] and, in patients who have undergone two prior

Table 1 Mechanisms of resistance to daratumumab treatment

Daratumumab mechanisms of resistance		
Resistance factors	**Mechanisms**	**Consequences**
1) Reduction of CD38 expression	- The depletion of high-CD38 expressing cells allows expansion of low-CD38 expressing cells CD38 direct internalization - Trogocytosis	CD38 expression influences daratumumab activity Immunosuppression Escape from immune system
2) NK-cells activity	- NK-cells activity favorize ADCC - High ADCC leads to rapid NK-cell fratricide	Daratumumab efficacy reduced
3) CD47-SIRP α action	- CD47 is overexpressed on MM cells, inhibiting of phagocytosis	Inhibition of ADCP Immune escape of tumor cells
4) Complement regulatory proteins	- Regulatory proteins are overexpressed on cancer cells, inhibiting the complement system	Less sensitivity for CDC induced by daratumumab
5) Tumor microenvironment	- Stromal cells make contact to MM cells protecting them	T-cell cytotoxicity reduced

therapies, in combination with carfilzomib and dexamethasone [80]. Distinguished by its unique ability to inhibit the ectoenzymatic activity of CD38, Isatuximab diverges from Daratumumab by binding to a distinct epitope on CD38, which encompasses the CD38 ectoenzyme catalytic site. Through allosteric antagonism, Isatuximab effectively inhibits both CD38 hydrolase and cyclase activity [81]. Its anti-tumor activity manifests through various mechanisms, including the induction of direct homotypic aggregation (HA)-related apoptosis, mediated by via actin cytoskeleton polymerization, caspase-dependent pathways, lysosome-associated pathways, and the activation of immune mechanisms [82].

Like daratumumab, isatuximab engages in Fc-dependent effector mechanisms such as ADCC, ADCP, and CDC [58]. Moreover, isatuximab exhibits immunomodulatory effects by blocking the suppressive function of Tregs, consequently fostering NK- and T-cell-mediated antitumor immune responses [83]. Pomalidomide augments isatuximab's capacity to reduce the proliferation of Tregs in a dose-dependent manner and to enhance T- and NK-cell-mediated lysis of MM [84]. Lastly, isatuximab depletes B-lymphocyte precursors, basophils, and NK lymphocytes in bone marrow cells [58]. Furthermore, it is able to induce apoptosis independently of cross-linking [58]. Just like daratumumab, isatuximab can also be used in combination therapy with IMiDs and proteasome inhibitors. Isatuximab has shown comparable efficacy when combined with carfilzomib and dexamethasone (Isa-Kd) [76].

Other anti-CD38 monoclonal antibodies

Both daratumumab and isatuximab are currently FDA and EMA approved for the treatment of MM. Other CD38 antibodies are under investigation for clinical development for the treatment of MM, such as MOR202, SAR442085, TAK079, FTL004 and TNB-738. They differ by the induced mechanisms of cell death, their affinity for FcγRIIIa and subsequent NK cell activation and the epitopes they bind. While MOR202 is currently being evaluated in phase III clinical trials, the development of others (such as SAR442085) has been stopped.

While initially developed to target MM, the scope of anti-CD38 monoclonal antibodies extends beyond hematological malignancies, both within and outside the hematological domain [67, 81]. In addition, ongoing research efforts are exploring the potential of MOR202 for treating membranous nephropathy (NCT04145440), while TAK79 is being investigated for conditions like severe systemic lupus erythematosus and myasthenia gravis (NCT04159805). Lastly, SAR442085 is being considered as a therapeutic option for Cold agglutinin disease (NCT04802057). These expansions into new therapeutic domains underscore the versatility and evolving landscape of targeted therapies.

Anti-CD38 single domain antibodies as new therapeutic agents

Today, while daratumumab and isatuximab have become integral components of first-line MM treatment regimens, some limitations have emerged in the use of mAbs as agents. One of the major drawbacks is their large molecular mass of about 150 kDa, responsible for slow kinetics and limited tumor penetration with only approximately 20% of the administered dose reaching the tumor site [85]. Moreover, mAbs are mainly expressed in mammalian cells because of their complex structure, increasing the cost of large-scale productions [86]. Overcoming these challenges requires the exploration of smaller CD38-binding molecules, with a particular focus on single domain antibodies (sdAbs) derived from the heavy chain antibodies of Camelidae species [87]. These sdAbs offer several advantages, including small size, high stability,

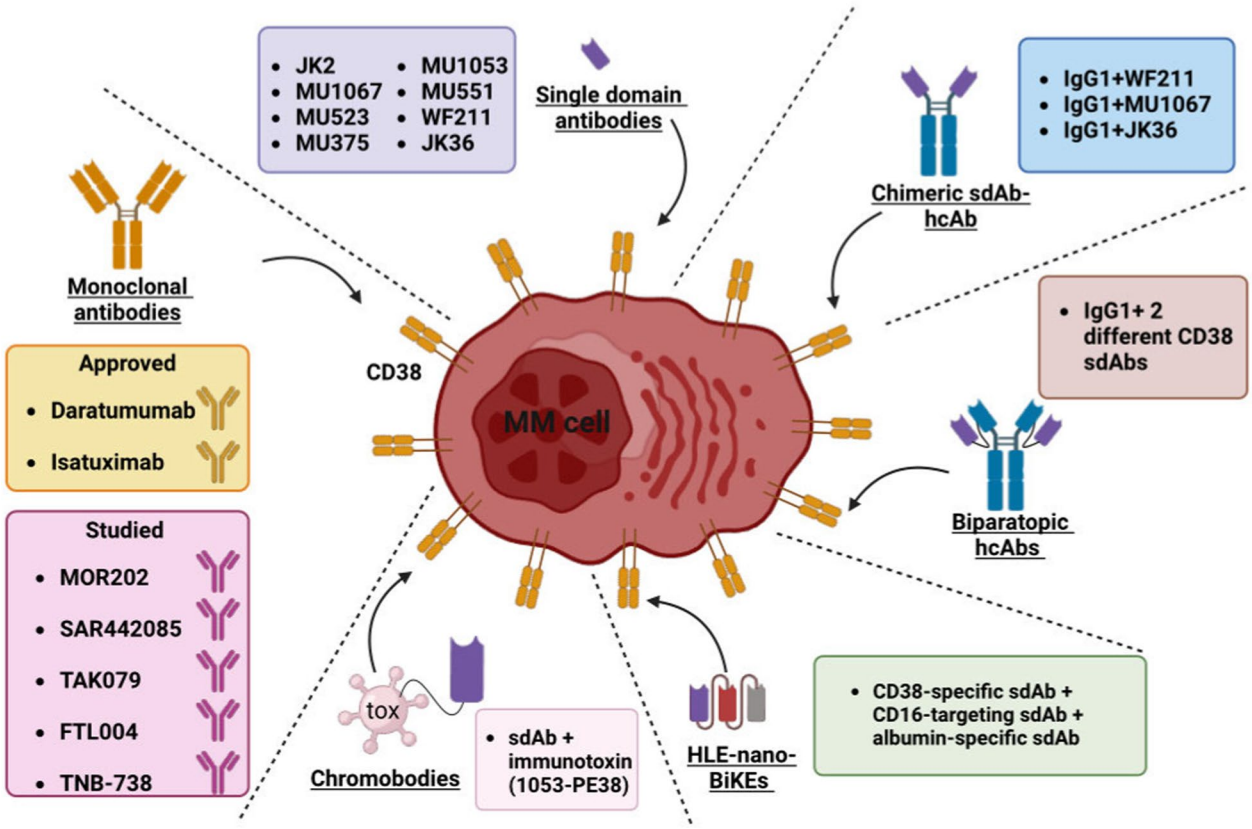

Fig. 4 Different agent created targeting CD38 for treating multiple myeloma. Created with BioRender.com

remarkable specificity and affinity for the target antigen, and the ability to recognize hidden epitopes. Furthermore, sdAbs are expressed in prokaryotic systems, lowering the production's costs [86]. Consequently, they have emerged as promising candidates for a new generation of tumor imaging and therapeutic agents of various CD38-expressing malignancies [85, 88]. In 2017, the first CD38-targeting sdAbs were developed by the group of Koch-Nolte, who identified 22 different CD38-specific sdAb families capable of recognizing the three different epitopes (E1, E2, E3) of CD38. Among these families, three nanobodies (JK2, MU1067 and MU523) showed antagonistic inhibition of CD38 enzymatic activity in a dose-dependent manner, while two families showed potentiating effects. Notably, many sdAbs binding to epitope 2 and 3 interfered with the binding of daratumumab, with MU375, MU1053 and MU551 showing competitive binding. Four sdAbs were found to bind CD38 independently of Daratumumab [89]. These CD38-specific sdAbs have immense therapeutic and diagnostic potential, making them exciting candidates for further research and development (Fig. 4).

Integration of sdAbs in chimeric antibodies

Recent advances in antibody engineering have enabled the development of novel recombinant antibody constructs, including chimeric antibodies. CD38-specific sdAbs and humanized heavy chain antibodies have been extensively studied in preclinical experiments. These recombinant heavy chain antibodies (hcAbs) retain all the functionalities of traditional monoclonal antibodies but have a reduced size and improved solubility [90]. As potential therapeutics for MM, sdAbs such as WF211, MU1067, JK36, genetically fused to the hinge and Fc domains of human IgG1, have demonstrated the ability to elicit antibody-dependent cytotoxicity against CD38 + cancer cells [89]. They were able to reduce tumor growth in murine models of myeloma and to induce ADCC in vitro [89]. In addition, Baum et al. have attempted to generate recombinant chimeric mouse IgG2a heavy chain antibodies, grouped into five distinct families, that bind non-overlapping epitopes of CD38 [91].

Some of these CD38-specific hcAbs have been shown to inhibit the GDPR cyclase activity of CD38, while others induce mechanisms of cytotoxicity in mouse tumor models [91]. The role of MU1067 and daratumumab

in the conversion of NAD + to ADPR and cADPR by CD38 expressed on MM cells has also been investigated. While Daratumumab shows a weak inhibition of cADPR production, MU1067 strongly inhibits both cADPR production and hydrolysis of cADPR to ADPR. Notably, both daratumumab and MU1067 show detectable inhibitory effects on ADPR cyclase activity but not on NAD + hydrolase activity [92].

Biparatopic antibodies, HLE-nano-BiKEs and chromobodies

Schütze et al. pioneered the development of biparatopic Abs constructed by fusing two different CD38 sdAbs to the hinge, CH2 and CH3 domains of human IgG1 via a flexible peptide linker. These biparatopic hcAbs were highly soluble and induced stronger CDC reactions compared to Daratumumab [93]. By binding to two different epitopes of CD38, these constructs facilitate the formation of C1q-activating oligomers, thereby increasing the interaction with C1q and enhancing the CDC effect [93]. The increased CDC confers potential advantages for therapeutic use, either alone or in combination with Daratumumab, to potentiate its effect on MM cells [93]. In the area of antibody engineering, the therapeutic potential of novel conjugated half-life extended nano-BiKEs (Bispecific Killer-cell Engagers) was evaluated. These nano-BiKEs are composed of CD38-specific sdAbs fused to CD16-targeting sdAbs. These bispecific constructs, characterized by their small size, high solubility and extended half-life facilitated by the integration of an albumin-specific sdAb, have demonstrated potent cytotoxicity against tumor cells, exceeding the ADCC of Daratumumab [94]. Finally, anti-CD38 sdAbs have been engineered with an immunotoxin (1053-PE38) to generate chromobodies capable of selectively killing CD38+ cells [95].

The theranostic approach in nuclear medicine

Among the various approaches, theranostics represents an important alternative technique for developing more specific and individualized therapies. The term "theranostics" defines the ongoing efforts in the clinic to combine diagnostic and therapeutic capabilities in a single pharmaceutical agent [96]. An ideal radiopharmaceutical theranostic agent has both diagnostic and therapeutic potential; however, in practice, the diagnostic and therapeutic components differ by incorporating a different radionuclide (Summarized in Tables 2 and 3), although the binding agent or vector remains the same. The antigen-binding agent is often a small molecule (peptide) or an antibody or antibody fragment that binds to a disease-related antigen. CD38 is a valuable antigen in MM patients, even after relapse or resistance to treatment, because its expression remained elevated in patients with relapsed refractory MM. In a study of 68 paired samples, the average expression level was significantly higher in R/R patients compared to NDMM patients, highlighting the potential of CD38 as a target for imaging and therapy [97].

Table 2 A summary of the main radionuclides used for diagnosis in single photon emission computed tomography (SPECT) and positron emission tomography (PET)

Radionuclide	Emission type	Half-life	Emax (MeV)	Diagnosis
^{99m}Tc	γ	6.0 h	0.14	SPECT
^{67}Ga	γ	78.3 h	0.9, 0.19, 0.30	
^{131}I	γ	8.0 days	0.28, 0.36, 0.64	
^{111}In	γ	67.2 h	0.17, 0.25	
^{89}Zr	Positron	78.4	2.4	PET
^{18}F	Positron	1.83 h	0.64	
^{15}O	Positron	2.07 min	1.72	
^{13}N	Positron	9.96 min	1.19	
^{64}Cu	Positron	12.7 h	0.65	
^{68}Ga	Positron	1.13 h	1.90	
^{124}I	Positron	4.1 days	1.53	

Table 3 A summary of the main radionuclides used for therapeutic purposes

Radionuclide	Emission type	Half-life	Emax (MeV)	Therapy
^{225}Ac	α	10.0 days	5.83, 5.79	Alpha therapy
^{211}At	α	7.2 h	5.87	
^{227}Th	α	18.7 days	6.04, 5.97	
-^{223}Ra		11.4 days		
^{224}Ra	α, β^-, γ	3.6 days	5.44, 5.69	
- ^{212}Pb		10.6 h		
- ^{212}Bi		1 h		
^{90}Y	β^-	2.0 days	2.28	Beta therapy
^{67}Cu	β^-	2.6 days	0.19	
^{177}Lu	β^-, γ	6.7 days	0.49	
^{131}I	β^-, γ	8.0 days	0.28, 0.36, 0.64	
^{153}Sm	β^-, γ	1.9 days	0.82	
^{161} Tb	β^-, γ	6.9 days	0.5, 0.6	

Anti-CD38 agents for diagnosis of MM

In the diagnostic context, radiolabeled anti-CD38 antibodies have emerged as promising tools that provide valuable insights into the biology of tumors. They also allow to evaluate the efficacy of monitoring the cancer cells during the treatment. Understanding the cellular phenotype and heterogeneity of cancers prior to the initiation of cancer treatment is critical, as is monitoring response to therapy during its course [98]. Nuclear imaging

modalities include single-photon emission computed tomography (SPECT) and positron emission tomography (PET), allowing the visualization and quantification of molecular processes using radiolabeled tracers. For diagnostic purposes, PET and SPECT tracers require radionuclide labels, emitting positrons and gamma rays respectively, with half-lives matching the biological half-life of the targeting vector. Thus, mAbs which may take days to reach their target, are radiolabeled with long-lived radionuclides, which is not ideal as the patient should also be scanned several days post-injection. In the case of therapy, this also increases the effective dose to patients [99]. PET is preferred over SPECT due to its higher sensitivity, higher spatial resolution, and higher quantification accuracy [100]. Immuno-PET, or iPET, is the given name sometimes used when it is performed using radiolabeled-mAbs. to reduce the effective dose to patients.

mAbs-based approaches

In MM, radiolabeled daratumumab, known for its high affinity and specificity for CD38, has been used as a diagnostic tracer for imaging purposes in both preclinical [101, 102] and clinical studies [65, 103]. Traditionally, 2-Deoxy-2-[^{18}F]-fluoro-d-glucose ([^{18}F]FDG) PET/CT has been the standard imaging technique for patients with MM. However, it is less sensitive in detecting lesions with low metabolic rates [104]. Therefore, the exploration of radiolabeled daratumumab represents a promising alternative in diagnostic imaging. Through conjugation with the DOTA chelator and subsequent radiolabeling with the positron-emitting radionuclide copper-64 (^{64}Cu), daratumumab has been successfully developed as a molecular probe targeting CD38-expressing MM cells [102]. Preclinical studies, particularly in mouse models bearing CD38 + tumors, demonstrated preferential accumulation of the [^{64}Cu]Cu-DOTA-daratumumab within bone-associated tumor sites. In addition, PET/CT imaging using this radioligand demonstrated improved sensitivity and specificity in detecting MM cell dissemination compared to conventional [^{18}F]FDG PET/CT, while also showing superior sensitivity to bioluminescent signals [102]. These preclinical data were validated in a phase 1 study (NCT03311828) in which [^{64}Cu]Cu-DOTA-daratumumab PET/CT imaging provided whole-body imaging of MM with a safety profile [103].

In parallel, radiolabeling of daratumumab with [^{89}Zr]Zr-oxalate has been developed as an alternative imaging approach [101]. This innovative strategy aims to verify CD38 expression and to stratify patients for daratumumab therapy, thereby reducing off-target toxicities and avoiding unnecessary treatment of individuals unlikely to respond. [^{89}Zr]Zr-DFO-daratumumab demonstrated high affinity and specificity for MM1.S human myeloma cells both in vitro and in vivo, with immunoreactivity of more than 95%. In mouse models bearing subcutaneous MM tumors, the radiopharmaceutical showed specific uptake in tumors of various sizes with excellent tumor-to-background contrast, particularly evident at 6–7 days post-administration. Furthermore, PET imaging in a disseminated mouse model demonstrated tumor cell localization in femur, tibia and spine, highlighting the potential of this antibody-based PET radiopharmaceutical for non-invasive CD38-positive myeloma imaging [101]. Following these results, a prospective first-in-human imaging phase I study (NCT03665155) was conducted to assess [^{89}Zr]Zr-DFO-daratumumab uptake in MM patients using PET/CT. Patients received 74 MBq (2 mCi) of the radioligand and underwent PET/CT over the next 8 days with no adverse events reported. [^{89}Zr]Zr-DFO-daratumumab demonstrated the ability to detect MM with high sensitivity, localise tumor cell infiltration and quantify disease burden. In addition, it allowed the detection of residual disease after therapy, which is important for prognosis and is often used as an endpoint in clinical trials of MM. Prior to treatment, [^{89}Zr]Zr-DFO-daratumumab could serve as a predictor for the tumor's response to daratumumab therapy, and as biomarker for monitoring the response to daratumumab therapy [65]. Radiolabeled daratumumab has been studied as an imaging tool in lymphoma and lung cancer [97]. A phase II trial of [^{89}Zr]Zr-DFO-daratumumab (NCT04814615) is underway to evaluate the potential clinical applications of this novel imaging agent.

SdAbs-based probes

Although immunoPET, using mAbs as imaging probe, is a powerful diagnostic tool, it presents challenges. First, mAbs are produced in eukaryotic cell lines because of their complex expression and post-translational modifications, increasing the cost of production [105]. In addition, long-lived radionuclides are required to match the biological half-life of mAb-based tracers due to their interaction with the neonatal Fc receptor (FcRn) in endothelial cells, which protects serum IgG from degradation [106]. Furthermore, the size of mAbs (150 kDa) exceeds the renal filtration limit, resulting in prolonged circulation and increased accumulation of these long-lived radionuclides in healthy tissues. Unfortunately, currently available mAb imaging probes for the detection of MM are not suitable for use in patients treated with daratumumab, due to overlapping binding epitopes with daratumumab binding site [101] [102]. This unmet need for alternative constructs has led to the exploration of sdAbs. Whether labelled with radionuclides, fluorophores or fluorescent proteins, sdAbs have proven useful

as tracer tools due to their small size and subsequent clearance from background organs [107]. Specifically, sdAbs have been successfully used in radionuclide-based imaging methods for various cancers and are promising tools for the diagnosis of MM [107]. Our laboratory produced and characterized sdAb#2F8. It was radiolabeled with ^{99m}Tc to evaluate its biodistribution and tumor targeting potential [108]. sdAb#2F8 is minimally internalized after CD38 binding and can be administered without affecting CD38 expression. In addition, it was found that this sdAb specifically binds to the tumor and does not spread to healthy tissue, resulting in a high tumor/normal tissue ratio. This specific and rapid tumor accumulation, the lack of competition with daratumumab and rapid clearance make sdAb#2F8 an ideal candidate for an imaging approach to also assess response during daratumumab treatment [108]. Two subsequent sdAb-based radiotracers, [^{68}Ga]Ga-NOTA-Nb1053 and ^{18}F-labeled Nb1053, have been introduced for CD38-targeted immunoPET imaging for the early detection of MM [99, 109]. In particular, [^{68} Ga]Ga-NOTA-Nb1053 showed to be able to identify tumor infiltration in the bone marrow before the presence of overt bone damage. This not only aids in efficient patient stratification and selection, but also provides a means to assess treatment response and monitor disease relapse in a CD38-dependent manner. The use of sdAbs such as [^{68} Ga]Ga-NOTA-Nb1053 allows same-day imaging one hour after injection with high target-to-background ratios, avoiding concerns associated with non-specific bone accumulation seen with ^{89}Zr-labeled mAb immunoPET techniques. However, [^{68} Ga]Ga-NOTA-Nb1053 has several drawbacks. These include the constraints associated with the short half-life of [^{68} Ga] (impossible to ship to other hospitals or inject many patients in one day) and the accumulation in the kidneys, potentially causing nephrotoxicity when the radioligand would be changed for therapeutic purposes. Consequently, Nb1053 has been radiolabeled with ^{18}F, resulting in a radioconjugate excreted via the hepatobiliary system, that prevents renal accumulation without compromising tumor uptake [99–109]. Another radioconjugate, ^{99m}Tc-labeled CD3813, has been tested as a SPECT radiotracer to image CD38-positive tumors and assess CD38 expression in patients before, during and after daratumumab treatment [110]. It has a smaller molecular weight (15 kDa) compared to the full-length antibody daratumumab, exhibits rapid uptake in tumors with high tumor/background ratios and undergoes rapid metabolism in vivo. Blocking experiments confirmed the CD38 specificity of tumor uptake by CD3813. It is an excellent radiotracer for imaging both MM and CD38-positive lymphoma, outperforming [^{18}F]FDG PET/CT in MM imaging. The unaffected CD38 binding of CD3813 in the presence of excess cold daratumumab suggests that CD3813 and daratumumab have distinct binding sites on CD38 [110].

Peptides for imaging

As an alternative to Ab and Ab fragments, peptides have traditionally been used as imaging probes. Peptides are less immunogenic and have pharmacokinetic advantages similar to those of sdAbs, with excellent uptake in tumor tissue and rapid clearance [97]. In addition, peptides have the advantage that they can be chemically synthesized. They are stable at higher temperatures and under acidic conditions, which may be required for conjugation to radionuclides. CD38 binding peptides were obtained from phage display peptide libraries and synthesized using standard chemistry [97, 111]. Two families of peptides (SL022, SL028, CA-1 and CA-2) were identified and showed high affinity and specificity for CD38. Their conjugation to ^{68} Ga or ^{64}Cu allowed in vivo visualization of CD38 + myeloma or lymphoma tumors in murine xenograft models [97, 111]. Compared to linear peptides, cyclic peptides are less prone to conformational changes and present a larger surface area that can interact with its target [112]. They are more stable and resistant to exopeptidases and endopeptidases due to the lack of terminal amine and carboxylic acid groups and a limited access to the cleavage sites [113]. The CD38-binding cyclic peptide AJ205 was synthesized and conjugated to the NOTA chelator [114]. Standard ^{68} Ga-labeling allowed to evaluate the pharmacokinetics, biodistribution, and in vivo specificity in different xenograft models using cell lines with different expression levels of CD38 and in two patient-derived xenograft models. [^{68} Ga]Ga-AJ206 PET showed specific accumulation of radioactivity in these models that correlated well with the CD38-expression levels in these tumors [114].

Anti-CD38 conjugated with fluorescent dye

Fluorescence imaging has been developed over the last few decades as another type of imaging technique. Most of these tools are used in preclinical studies [115]. They offer many advantages, such as detection of multiple targets using the different fluorophore spectra available and avoidance of isotopes. Fluorescence imaging, especially in the near infrared (NIR) range, has deeper tissue penetration. Combined with a low tissue autofluorescence, this spectrum favors a high resolution for imaging [116]. They are mainly used in cancer surgery to delineate the malignant lesion [116].

In contrast to radiolabeled anti-CD38 agents, conjugation with fluorescent dyes is less studied. A study led by Cho et al. used the antibody daratumumab with a NIR fluorophore called DARA-NIRDye800 [117]. Their

results showed that DARA-NIRDye800 is an interesting tool for non-invasive preclinical evaluation of CD38 expression in MM. Another study used this conjugate to create a bifunctional tool with mertansine, also called DM1—a tubulin inhibitor used to create an antibody–drug conjugate [118]. In this way, NIRDye800 monitors the release and activity of DM1. In addition, other sdAb have been coupled to fluorophores to detect CD38-expressing tumors. RID-Alexa680-MU1067 and Alexa680-JK36 were developed to monitor lymphoma development in tumor-bearing mice [119–121]. Even up to 24 to 48 h after administration, imaging revealed a high level of residual signal with minimal background interference and good clearance. JK36 has also been conjugated to Alexa Fluor 488 to allow measurement of plasma cells [122]. Chromobodies have also been used to assess CD38 expression using fluorescent proteins [95].

Radioligand therapy

Among the various therapeutic strategies, radioligand therapy (RLT) stands out as a promising approach that offers precise targeting of malignant cells while minimizing exposure to healthy tissue. RLT involves the use of radionuclides that are directed to the cancer cells in order to exert cytotoxic effects. Radionuclides are unstable atoms with excess of nuclear energy, which attempt, during radioactive decay, to reduce their energy by emission of α, β^- and Auger electrons. These high linear energy transfer (LET) particles are ionizing radiation capable of damaging cellular DNA, disrupting its replication process and ultimately causing cell death [96]. The damage is induced by direct and indirect effects of radiation. Ionizing radiations cause damage primarily by removing electrons from atoms, resulting in the breaking of covalent bonds of molecules such as critical DNA. They are responsible for single- and double-strand breaks of the DNA, with the latter being considered as the predominant cause of cell death after ionizing radiation exposure. Additionally, radiation-induced damage is amplified by the generation of reactive oxygen species (ROS), chemically reactive entities capable of oxidizing proteins and lipids and inducing single-strand DNA breaks (indirect effects). Although cells have mechanisms to repair such errors, increased levels of ROS combined with impaired repair mechanisms can lead to cell death [123]. Moreover, non-targeted effects such as the abscopal (RIAE) and bystander effects (RIBE) can induce biological consequences, such as adaptive response, genomic instability and genetic susceptibility, to neighboring cells through signaling factors released by irradiated cells [124, 125]. Alpha particles consist of two protons and two neutrons and carry a positive charge, while β^- particles are small-mass, negatively charged particles. Due to their higher LET, α-particles cause higher tissue damage within a short range of up to 100 µm [126]. β^- particles have a lower LET and a longer range of several millimeters, which increases the likelihood of inducing damage in adjacent healthy tissues. In the context of mAbs, radionuclides with a longer half-life emitters offer practical advantages in terms of pharmacokinetics, allowing for prolonged exposure and enhanced therapeutic efficacy [127]. Overall, RLT holds great promise as a targeted therapeutic strategy, offering precision targeting of cancer cells and efficacy in the treatment of cancer while minimizing adverse effects on healthy tissues.

Anti-CD38 agents for RLT

Currently, in the field of RLT, efforts have been made to radiolabel various anti-CD38 mAbs with therapeutic radionuclides. The efficacy of RLT in other hematologic disorders led to development of a CD38-based RLT approach initiated by J. Green and O.W. Press in 2014 [128]. Their pioneering work used both classical radioimmunotherapy (RIT) and a pre-targeted radioimmunotherapy (PRIT), both directed against CD38, to deliver radiation specifically to MM cells. Their comparative analysis demonstrated the advantages of PRIT over RIT. They used three different constructs: the OKT10-Ab (anti-CD38 Ab) and the two antibody-streptavidin (SA) OKT10-CC (synthetic chemical conjugate) and OKT10-FP (a single-chain variable fragment (scFv) of OKT10-Ab). In the conventional RIT approach, the OKT10-Ab was directly radiolabeled with yttrium-90 (^{90}Y) or Indium-111 (^{111}In). Conversely, PRIT was performed using an antibodies-SA constructs combined with a ^{90}Y-DOTA-biotin or ^{111}In-DOTA-biotin. In this pre-targeting, the Abs-SA constructs were first delivered to localise to tumor sites, followed by the introduction of radioactive biotin, which recognizes SA. While RIT effectively controlled the disease, radioactivity could be observed in healthy organs. In contrast, the pre-targeting system significantly improved the tumor-to-normal organ ratio of absorbed dose, reducing radiation exposure to healthy tissues. In fact, for RIT the ratio was < 1:1, while for the OKT10-CC and OKT10-FP ratios were 9:1 and 638:1 respectively. This improved biodistribution not only delayed tumor development, but also completely eradicated cancer cells with minimal toxicity in xenograft models [128]. Subsequently, a second pre-targeting method emerged with the development of bispecific Ab fusion proteins [129]. These constructs coupled an scFv of an anti-CD38 mAb with an scFv conjugated to an ^{90}Y-DOTA. This bispecific Ab showed enhanced anti-tumor activity, reduced risk of immunogenicity and lacked endogenous biotin interference compared to the SA-biotin system. In addition, the feasibility of PRIT as

a CD38-based therapy was confirmed in murine models with excellent tumor-to-normal organ ratios of absorbed dose [129]. Building on these studies, other anti-CD38 mAbs have been investigated for RLT, using both α- and β^--particle emitters. Specifically, daratumumab has been radiolabeled with various radionuclides to address resistance and to improve efficacy. In β^-radiation studies, daratumumab radiolabeled with lutetium-177 (^{177}Lu) using a DTPA chelator, demonstrated tumor growth reduction and necrosis induction in preclinical lymphoma models [130]. In addition, daratumumab has been conjugated to DOTA for radiolabeling with the alpha-emitter actinium-225 (^{225}Ac), showing potent CD38-positive tumor cell-killing in vitro and enhanced anti-tumor activity (~30-fold) in vivo without significant side effects [131]. The potential of α-radioimmunotherapy (α-RIT) has been extensively investigated by conjugating daratumumab with lead-212 (^{212}Pb) in both in vitro and in vivo models [132]. This innovative radioconjugate showed remarkable efficacy, significantly inhibiting the proliferation of human myeloma RPMI8226 cell lines, in stark contrast to the minimal effects observed with the ^{212}Pb isotypic control or cold antibodies. Notably, in vivo studies showed that mice treated with 0.3 MBq of the radioconjugate had a median survival of 55 days compared to 11 days in the control group, indicating a significant inhibition of tumor growth compared to controls [132]. Comparing the efficacy of radiolabeled daratumumab in MM patients, ^{225}Ac-based therapy showed superior tumor growth delay and reduced systemic toxicity compared to ^{177}Lu-based therapy [133]. This highlights the potential advantages of α-RIT over β^-RIT for the treatment of MM. In addition, promising results for RLT based on α-emitter radionuclides have also been obtained by radiolabeling other anti-CD38 mAbs. For example, the OKT10 mAb radiolabeled with the alpha emitter astatine-211 (^{211}As) was investigated for the treatment of minimal residual disease (MRD) in a disseminated disease model of MM [134]. This therapeutic approach demonstrated increased median long-term survival with sustained remission in 50–80% of mice with marginal toxicity at a single dose (24–45 μCi; 0.9–1.7 MBq). Importantly, the OKT10 mAb targets a different epitope to daratumumab, which may allow it to be effective in patients receiving daratumumab, potentially mitigating resistance [134].

Anti-CD38 SdAbs-based RLT

In the field of sdAbs, the sdAb#2F8 was selected by our group as a promising theranostic agent for the monitoring and treatment of MM [108]. SdAb#2F8 has favorable properties for RLT, such as high affinity for the CD38 antigen and lack of competition with daratumumab, making it a promising theranostic agent. sdAb 2F8 can be radiolabeled with ^{99m}Tc, ^{111}In and ^{177}Lu to perform biodistribution studies and evaluate therapeutic efficacy in murine xenograft models. As a radiotherapeutic agent, the sdAb 2F8 radiolabeled with the β^-emitter ^{177}Lu, ^{177}Lu-DTPA-2F8 showed dose-dependent tumor regression and survival in myeloma bearing mice [108]. Similar results were obtained when the experiment was repeated with low radioactive injected activity. The aim was to mimic as closely as possible a clinical approach of fractionated dosing to decrease the toxicity to healthy tissues. Repeating the experiment in these conditions resulted in an improvement in mice survival [108]. The compelling results of these studies highlight the potential of RLT targeting CD38 in MM and achieved durable therapeutic responses. RLT holds promise as a highly effective treatment modality for MM by exploiting the precise targeting capabilities of radionuclides and their potent cytotoxic effects on malignant cells. These results not only demonstrate the efficacy of RLT in inhibiting tumor growth and prolonging survival, but also highlight its ability to induce durable responses.

Future perspective

One of the major benefits of using theranostic agents targeting CD38 is the potential for creating personalized therapy for patients. These radiopharmaceuticals provide a unique opportunity to conduct detailed, quantitative whole-body PET/CT scans, offering accurate information on tumor characteristics and location. This could help in selecting patients who could benefit from a specific, adapted therapy. Generally, these new strategies should be directed towards diseases with significant unmet clinical needs, including cases that have relapsed or are resistant to current treatments, as well as rare (orphan) diseases. For example, alpha therapy shows potential in eradicating minimal residual disease (MRD), which is crucial before stem cell transplants to improve patient outcomes. Ongoing research is also focusing on identifying new targets, investigating the effectiveness of combination therapies, and exploring the use of targeted radiotherapy on tumor stem cells. Moreover, these theranostic radiopharmaceuticals can serve as supportive treatments alongside standard anti-CD38 based therapies. Many blood cancers, such as aggressive B- and T-cell lymphomas, as well as acute lymphoblastic and myeloid leukemia, still exhibit poor outcomes with rapid progression after first-line treatments. Despite their extensive use in diagnostics, research to move these radiopharmaceuticals into clinical practice has been limited in recent years. The main challenges include overcoming financial and regulatory issues, especially for agents targeting hematological malignancies. The costs associated with mAbs, and antibody fragments are very high and

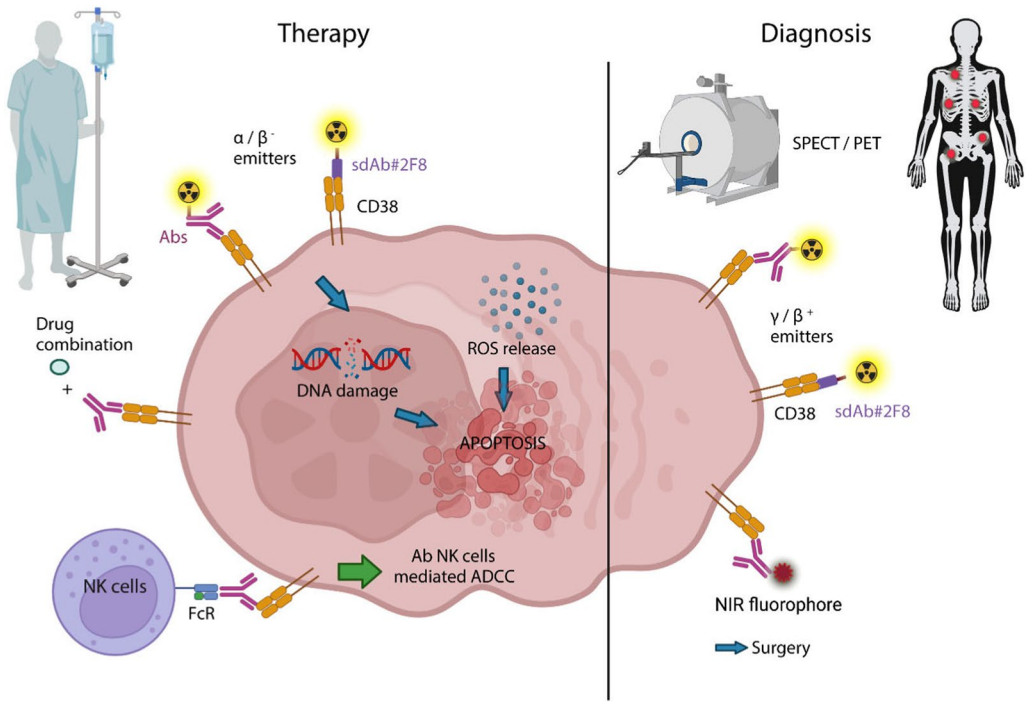

Fig. 5 Visual summary of theranostic potential of anti-CD38 agents. Created with BioRender.com

require significant industrial investments. In addition, obtaining certain therapeutic radionuclides like Actinium-225 and Astatine-211 is difficult due to the limited number of facilities producing these radionuclides. Using sdAbs and scaffold proteins as radiopharmaceuticals has shown significant advantages, though bone marrow and kidney toxicity remain concerns. These issues might be overcome by targeting more specifically CD38 by employing pre-targeting systems. In the end, resistance phenomenon can emerge due to the loss of antigen expression; however, dual-antigen targeting is considered a promising approach. In any case, the potential for RLT to demonstrate greater efficacy compared to current standard treatments, while maintaining manageable toxicity, could support the recommendation of these novel approaches for patients [96].

Conclusions

As CD38 is overexpressed by many cancer cells, particularly in hematological cancers, it is an important target for treatment. This glycoprotein has been extensively studied, particularly in MM, leading to the development of several drugs based on monoclonal antibodies targeting CD38, such as daratumumab and isatuximab. Both mAbs are commercially available and currently used in frontline therapy. However, resistance and relapse in patients have sparked interest in novel approaches using sdAbs derived from camelid species, which have favorable properties that can potentially overcome the limitations of traditional monoclonal antibodies. Their small size guarantees tissue penetration, rapid clearance and a high affinity for the antigen, thus allowing for a targeted therapy with a minimal impact on healthy tissues. The potential of CD38-targeting sdAbs has been explored through the creation of different structures, such as chimeric antibodies, biparatopic antibodies, heavy chain-only antibodies, and half-life-extended nano-BiKEs, which show promise for the treatment of MM. In nuclear medicine, the development and application of anti-CD38 conjugates with radionuclides represent significant advances in the diagnosis and treatment of MM, offering more precise, personalized and effective therapeutic strategies. In terms of diagnosis, radiolabeled anti-CD38 Abs offer insights into tumor biology and treatment monitoring compared to traditional methods. At the same time, radiolabeled sdAbs, such as sdAb#2F8 (developed by our laboratory) have shown rapid clearance, high tumor targeting ratios and possible use as theranostic agents. In RLT, the emergence of α-radioimmunotherapy (α-RIT) using α-emitters offers further opportunities for durable responses and potentially curative outcomes in MM. Approaches using radiolabeled anti-CD38 conjugates have resulted in high tumor-to-normal organ ratios and reduced toxicity and sdAb#2F8 labeled with therapeutic radionuclides confirmed its potential as

theranostic tools (Fig. 5). Despite all these advances, challenges remain in the production and use of these therapeutic probes. Nevertheless, ongoing research and clinical trials continue to refine these tools and demonstrate their potential to improve personalized treatment strategies and patient outcomes in MM and other CD38-positive diseases.

Abbreviations

ADCC	Antibody-dependent cell-mediated cytotoxicity
ADCP	Antibody-dependent cellular phagocytosis
ADO	Adenosine
ADPRP	ADP-ribose 2′-phosphate
AITL	Angioimmunoblastic T-cell lymphoma
ALL	Acute lymphoblastic leukemia
AML	Acute myeloblastic leukemia
BCR	B cell receptor
BiKEs	Bispecific Killer-cell Engagers
CADPR	Cyclic adenosine diphosphate (ADP)–ribose
CAFs	Cancer-associated fibroblasts
CDC	Complement-dependent cytotoxicity
CLL	Chronic lymphocytic leukemia
DLBCL	Diffuse large B-cell lymphoma
FcRn	Neonatal Fc receptor
hcAbs	Heavy chain antibodies
HGBL	High-grade B-cell lymphoma
HL	Hodgkin's lymphoma
IMiDs	Immunomodulatory drugs
LET	High linear energy transfer
mAbs	Monoclonal antibodies
MAC	Membrane attack complex
MAF	Metastasis-associated fibroblasts
MCL	Mantle cell lymphoma
MDSC	Myeloid-derived suppressor cells
MM	Multiple myeloma
MRD	Minimal residual disease
NAADP	Nicotinamide adenine dinucleotide phosphate
NAD	Nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate
NK	Natural killer
NSCLC	Non-small cell lung cancer
ORR	Overall response rate
PET	Positron emission tomography
PI	Proteasome inhibitors
PRIT	Pre-targeted radioimmunotherapy
PTCL	Peripheral T-cell lymphoma
RIT	Classical radioimmunotherapy and a
RLT	Radioligand therapy
RRMM	Relapsed/refractory MM
sdAbs	Single domain antibodies
SIRP α	Signal regulatory protein α
SPECT	Single photon emission computed tomography
SYK	Spleen tyrosine kinase
Tcons	T constitutive cells
TME	Tumor microenvironment
Tregs	Regulatory T cells
WM	Waldenström's macroglobulinemia

Supplementary Information

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Supplementary Material 1.

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Author contributions
All authors contributed to the conception and design of this study. VB: design, writing and reviewing. JB and NW: writing and reviewing. MP, RH and MD: reviewing. JC and GM: conceptualization, supervision, reviewing, and editing. All the authors contributed to the manuscript and approved the submitted version.

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References

1. Reinherz EL, Kung PC, Goldstein G, Levey RH, Schlossman SF. Discrete stages of human intrathymic differentiation: analysis of normal thymocytes and leukemic lymphoblasts of T-cell lineage. Proc Natl Acad Sci U S A. 1980;77(3):1588–92.
2. Graeff R, Liu Q, Kriksunov IA, Hao Q, Hon CL. Acidic residues at the active sites of CD38 and ADP-ribosylt cyclase determine nicotinic acid adenine dinucleotide phosphate (NAADP) synthesis and hydrolysis activities. J Biol Chem. 2006. https://doi.org/10.1074/jbc.M604370200.
3. Monaco G, Lee B, Xu W, Mustafah S, Hwang YY, Carré C, et al. RNA-Seq signatures normalized by mRNA abundance allow absolute deconvolution of human immune cell types. Cell Rep. 2019. https://doi.org/10.1016/j.celrep.2019.01.041.
4. Schmiedel BJ, Singh D, Madrigal A, Valdovino-Gonzalez AG, White BM, Zapardiel-Gonzalo J, et al. Impact of genetic polymorphisms on human immune cell gene expression. Cell. 2018. https://doi.org/10.1016/j.cell.2018.10.022.
5. Szlasa W, Czarny J, Sauer N, Rakoczy K, Szymańska N, Stecko J, et al. Targeting CD38 in neoplasms and non-cancer diseases. Cancers (Basel). 2022. https://doi.org/10.3390/cancers14174169.
6. Hoppe RT, Advani RH, Ai WZ, Ambinder RF, Armand P, Bello CM, et al. Hodgkin Lymphoma, Version 2.2020, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2020;18(6):755–81.
7. Di Gaetano R, Gasparetto V, Padoan A, Callegari B, Candiotto L, Sanzari MC, et al. Flow cytometry CD4 (+)CD26 (-)CD38 (+) lymphocyte subset in the microenvironment of Hodgkin lymphoma-affected lymph nodes. Ann Hematol. 2014;93(8):1319–26.
8. Hummel M, Bentink S, Berger H, Klapper W, Wessendorf S, Barth TFE, et al. A biologic definition of Burkitt's lymphoma from transcriptional and genomic profiling. N Engl J Med. 2006;354(23):2419–30. https://doi.org/10.1056/NEJMoa055351.
9. Atousa Maleki Adam C, Seegmiller NUNJK, Chen W. Bright CD38 expression is an indicator of MYC rearrangement. Leuk Lymphoma. 2009;50(6):1054–7. https://doi.org/10.1080/10428190902930470.
10. Liu Y, Bian T, Zhang Y, Zheng Y, Zhang J, Zhou X, et al. A combination of LMO2 negative and CD38 positive is useful for the diagnosis of Burkitt lymphoma. Diagn Pathol. 2019;14(1):100.
11. Ott G, Rosenwald A, Campo E. Understanding MYC-driven aggressive B-cell lymphomas: pathogenesis and classification. Blood. 2013;122(24):3884–91. https://doi.org/10.1182/blood-2013-05-498329.
12. Rosenthal A, Younes A. High grade B-cell lymphoma with rearrangements of MYC and BCL2 and/or BCL6: double hit and triple hit lymphomas and double expressing lymphoma. Blood Rev. 2017;31(2):37–42.

13. Rodig SJ, Vergilio J-A, Shahsafaei A, Dorfman DM. Characteristic expression patterns of TCL1, CD38, and CD44 identify aggressive lymphomas harboring a MYC translocation. Am J Surg Pathol. 2008;32(1):113–22.
14. Alsuwaidan A, Pirruccello E, Jaso J, Koduru P, Garcia R, Krueger J, et al. Bright CD38 expression by flow cytometric analysis is a biomarker for double/triple hit lymphomas with a moderate sensitivity and high specificity. Cytometry B Clin Cytom. 2019;96(5):368–74.
15. Calabretta E, Carlo-Stella C. The many facets of CD38 in lymphoma: from tumor-microenvironment cell interactions to acquired resistance to immunotherapy. Cells. 2020. https://doi.org/10.3390/cells9040802.
16. Castillo JJ, Libby EN, Ansell SM, Palomba ML, Meid K, Flynn CA, et al. Multicenter phase 2 study of daratumumab monotherapy in patients with previously treated Waldenström macroglobulinemia. Blood Adv. 2020;4(20):5089–92. https://doi.org/10.1182/bloodadvances.2020003087.
17. Morice WG, Chen D, Kurtin PJ, Hanson CA, McPhail ED. Novel immunophenotypic features of marrow lymphoplasmacytic lymphoma and correlation with Waldenström's macroglobulinemia. Mod Pathol. 2009;22(6):807–16.
18. Vose J, Armitage J, Weisenburger D. International peripheral T-cell and natural killer/T-cell lymphoma study: pathology findings and clinical outcomes. J Clin Oncol. 2008;26(25):4124–30.
19. Swerdlow SH, Campo E, Pileri SA, Harris NL, Stein H, Siebert R, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood. 2016;127(20):2375–90.
20. Zaja F, Tabanelli V, Agostinelli C, Calleri A, Chiappella A, Varettoni M, et al. CD38, BCL-2, PD-1, and PD-1L expression in nodal peripheral T-cell lymphoma: possible biomarkers for novel targeted therapies? Am J Hematol. 2017. https://doi.org/10.1002/ajh.24571.
21. Wang L, Wang H, Li P, Lu Y, Xia Z, Huang H, et al. CD38 expression predicts poor prognosis and might be a potential therapy target in extranodal NK/T cell lymphoma, nasal type. Ann Hematol. 2015;94(8):1381–8.
22. Vakiti A, Mewawalla P. Acute myeloid leukemia. In Treasure Island (FL); 2023.
23. Piedra-Quintero ZL, Wilson Z, Nava P, Guerau-de-Arellano M. CD38: an immunomodulatory molecule in inflammation and autoimmunity. Front Immunol. 2020. https://doi.org/10.3389/fimmu.2020.597959.
24. Farber M, Chen Y, Arnold L, Möllmann M, Boog-Whiteside E, Lin Y-A, et al. Targeting CD38 in acute myeloid leukemia interferes with leukemia trafficking and induces phagocytosis. Sci Rep. 2021;11(1):22062.
25. Diorio C, Teachey DT. Harnessing immunotherapy for pediatric T-cell malignancies. Expert Rev Clin Immunol. 2020;16(4):361–71. https://doi.org/10.1080/1744666X.2020.1732819.
26. Bride KL, Vincent TL, Im S-Y, Aplenc R, Barrett DM, Carroll WL, et al. Preclinical efficacy of daratumumab in T-cell acute lymphoblastic leukemia. Blood. 2018;131(9):995–9. https://doi.org/10.1182/blood-2017-07-794214.
27. Ghia P, Ferreri AJM, Caligaris-Cappio F. Chronic lymphocytic leukemia. Crit Rev Oncol Hematol. 2007;64(3):234–46.
28. Patten PEM, Buggins AGS, Richards J, Wotherspoon A, Salisbury J, Mufti GJ, et al. CD38 expression in chronic lymphocytic leukemia is regulated by the tumor microenvironment. Blood. 2008;111(10):5173–81.
29. Burgler S. Role of CD38 expression in diagnosis and pathogenesis of chronic lymphocytic leukemia and its potential as therapeutic target. Crit Rev Immunol. 2015;35(5):417–32.
30. Bauvois B, Chapiro E, Quiney C, Maloum K, Susin SA, Nguyen-Khac F. The value of neutrophil gelatinase-associated lipocalin receptor as a novel partner of CD38 in chronic lymphocytic leukemia: from an adverse prognostic factor to a potential pharmacological target? Biomedicines. 2023. https://doi.org/10.3390/biomedicines11092335.
31. Vaisitti T, Aydin S, Rossi D, Cottino F, Bergui L, D'Arena G, et al. CD38 increases CXCL12-mediated signals and homing of chronic lymphocytic leukemia cells. Leukemia. 2010;24(5):958–69. https://doi.org/10.1038/leu.2010.36.
32. Benkisser-Petersen M, Buchner M, Dörffel A, Dühren-von-Minden M, Claus R, Kläsener K, et al. Spleen tyrosine kinase is involved in the CD38 signal transduction pathway in chronic lymphocytic leukemia. PLoS ONE. 2016;11(12): e0169159.
33. Paulus A, Malavasi F, Chanan-Khan A. CD38 as a multifaceted immunotherapeutic target in CLL. Leuk Lymphoma. 2022;63(10):2265–75.
34. Kumar SK, Rajkumar V, Kyle RA, van Duin M, Sonneveld P, Mateos M-V, et al. Multiple myeloma. Nat Rev Dis Prim. 2017;3:17046.
35. Heusschen R, Muller J, Duray E, Withofs N, Bolomsky A, Baron F, et al. Molecular mechanisms, current management and next generation therapy in myeloma bone disease. Leuk Lymphoma. 2018;59(1):14–28.
36. Kumar SK, Lee JH, Lahuerta JJ, Morgan G, Richardson PG, Crowley J, et al. Risk of progression and survival in multiple myeloma relapsing after therapy with IMiDs and bortezomib: a multicenter international myeloma working group study. Leukemia. 2012;26(1):149–57.
37. Lin P, Owens R, Tricot G, Wilson CS. Flow cytometric immunophenotypic analysis of 306 cases of multiple myeloma. Am J Clin Pathol. 2004;121(4):482–8.
38. Costa F, Dalla Palma B, Giuliani N. CD38 expression by myeloma cells and its role in the context of bone marrow microenvironment: modulation by therapeutic agents. Cells. 2019. https://doi.org/10.3390/cells8121632.
39. Kawano Y, Kushima S, Hata H, Matsuoka M. The role of CD38 in multiple myeloma cell biology. Blood. 2021;138(Supplement 1):1580. https://doi.org/10.1182/blood-2021-150884.
40. Morandi F, Marimpietri D, Horenstein AL, Bolzoni M, Toscani D, Costa F, et al. Microvesicles released from multiple myeloma cells are equipped with ectoenzymes belonging to canonical and non-canonical adenosinergic pathways and produce adenosine from ATP and NAD (). Oncoimmunology. 2018;7(8): e1458809.
41. Sun L, Iqbal J, Dolgilevich S, Yuen T, Wu X-B, Moonga BS, et al. Disordered osteoclast formation and function in a CD38 (ADP-ribosyl cyclase)-deficient mouse establishes an essential role for CD38 in bone resorption. FASEB J. 2003;17(3):369–75.
42. An G, Acharya C, Feng X, Wen K, Zhong M, Zhang L, et al. Osteoclasts promote immune suppressive microenvironment in multiple myeloma: therapeutic implication. Blood. 2016;128(12):1590–603.
43. Costa F, Toscani D, Chillemi A, Quarona V, Bolzoni M, Marchica V, et al. Expression of CD38 in myeloma bone niche: a rational basis for the use of anti-CD38 immunotherapy to inhibit osteoclast formation. Oncotarget. 2017;8(34):56598–611.
44. Krejcik J, Casneuf T, Nijhof IS, Verbist B, Bald J, Plesner T, et al. Daratumumab depletes CD38+ immune regulatory cells, promotes T-cell expansion, and skews T-cell repertoire in multiple myeloma. Blood. 2016;128(3):384–94.
45. Bu X, Kato J, Hong JA, Merino MJ, Schrump DS, Lund FE, et al. CD38 knockout suppresses tumorigenesis in mice and clonogenic growth of human lung cancer cells. Carcinogenesis. 2018;39(2).
46. Gao L, Liu Y, Du X, Ma S, Ge M, Tang H, et al. The intrinsic role and mechanism of tumor expressed-CD38 on lung adenocarcinoma progression. Cell Death Dis. 2021;12(7).
47. Chen L, Diao L, Yang Y, Yi X, Rodriguez BL, Li Y, et al. CD38-mediated immunosuppression as a mechanism of tumor cell escape from PD-1/PD-l1 blockade. Cancer Discov. 2018. https://doi.org/10.1158/2159-8290.CD-17-1033.
48. Bertolini G, Compagno M, Belisario DC, Bracci C, Genova T, Mussano F, et al. CD73/adenosine pathway involvement in the interaction of non-small cell lung cancer stem cells and bone cells in the pre-metastatic Niche. Int J Mol Sci. 2022. https://doi.org/10.3390/ijms23095126.
49. Heimes A-S, Riedel N, Almstedt K, Krajnak S, Schwab R, Stewen K, et al. Prognostic impact of CD38- and IgκC-positive tumor-infiltrating plasma cells in triple-negative breast cancer. Int J Mol Sci. 2023. https://doi.org/10.3390/ijms242015219.
50. Ben BB, Blacher E, Mantsur E, Schwartz H, Vaknine H, Erez N, et al. Stromal CD38 regulates outgrowth of primary melanoma and generation of spontaneous metastasis. Oncotarget. 2018. https://doi.org/10.18632/oncotarget.25737.
51. Morandi F, Morandi B, Horenstein AL, Chillemi A, Quarona V, Zaccarello G, et al. A non-canonical adenosinergic pathway led by CD38 in human melanoma cells induces suppression of T cell proliferation. Oncotarget. 2015. https://doi.org/10.18632/oncotarget.4693.
52. Walterskirchen N, Müller C, Ramos C, Zeindl S, Stang S, Herzog D, et al. Metastatic colorectal carcinoma-associated fibroblasts have immunosuppressive properties related to increased IGFBP2 expression. Cancer Lett. 2022. https://doi.org/10.1016/j.canlet.2022.215737.

53. Karakasheva TA, Waldron TJ, Eruslanov E, Kim SB, Lee JS, O'Brien S, et al. CD38-expressing myeloid-derived suppressor cells promote tumor growth in a murine model of esophageal cancer. Cancer Res. 2015. https://doi.org/10.1158/0008-5472.CAN-14-3639.
54. Karakasheva TA, Dominguez GA, Hashimoto A, Lin EW, Chiu C, Sasser K, et al. CD38+ M-MDSC expansion characterizes a subset of advanced colorectal cancer patients. JCI Insight. 2018. https://doi.org/10.1172/jci.insight.97022.
55. Levy A, Blacher E, Vaknine H, Lund FE, Stein R, Mayo L. CD38 deficiency in the tumor microenvironment attenuates glioma progression and modulates features of tumor-associated microglia/macrophages. Neuro Oncol. 2012. https://doi.org/10.1093/neuonc/nos121.
56. van de Donk NWCJ, Richardson PG, Malavasi F. CD38 antibodies in multiple myeloma: back to the future. Blood. 2018;131(1):13–29.
57. Touzeau C, Moreau P. Daratumumab for the treatment of multiple myeloma. Expert Opin Biol Therapy. 2017;17(7):887–93.
58. Moreno L, Perez C, Zabaleta A, Manrique I, Alignani D, Ajona D, et al. The mechanism of action of the anti-CD38 monoclonal antibody isatuximab in multiple myeloma. Clin Cancer Res. 2019;25(10):3176–87. https://doi.org/10.1158/1078-0432.CCR-18-1597.
59. Raab MS, Engelhardt M, Blank A, Goldschmidt H, Agis H, Blau IW, et al. MOR202, a novel anti-CD38 monoclonal antibody, in patients with relapsed or refractory multiple myeloma: a first-in-human, multicentre, phase 1–2a trial. Lancet Haematol. 2020;7(5):e381–94.
60. Fedyk ER, Zhao L, Koch A, Smithson G, Estevam J, Chen G, et al. Safety, tolerability, pharmacokinetics and pharmacodynamics of the anti-CD38 cytolytic antibody TAK-079 in healthy subjects. Br J Clin Pharmacol. 2020;86(7):1314–25.
61. Zhang G, Guo C, Wang Y, Zhang X, Liu S, Qu W, et al. FTL004, an anti-CD38 mAb with negligible RBC binding and enhanced pro-apoptotic activity, is a novel candidate for treatments of multiple myeloma and non-Hodgkin lymphoma. J Hematol Oncol. 2022;15:177.
62. Kassem S, Diallo BK, El-Murr N, Carrié N, Tang A, Fournier A, et al. SAR442085, a novel anti-CD38 antibody with enhanced antitumor activity against multiple myeloma. Blood. 2022;139(8):1160–76.
63. Ugamraj HS, Dang K, Ouisse L-H, Buelow B, Chini EN, Castello G, et al. TNB-738, a biparatopic antibody, boosts intracellular NAD+ by inhibiting CD38 ecto-enzyme activity. MAbs. 2022;14(1):2095949.
64. Chehab S, Panjic EH, Gleason C, Lonial S, Nooka AK. Daratumumab and its use in the treatment of relapsed and/or refractory multiple myeloma. Future Oncol. 2018;14(30):3111–21.
65. Ulaner GA, Sobol NB, O'Donoghue JA, Kirov AS, Riedl CC, Min R, et al. CD38-targeted immuno-PET of multiple myeloma: from xenograft models to first-in-human imaging. Radiology. 2020;295(3):606–15.
66. Taylor RP, Lindorfer MA. Cytotoxic mechanisms of immunotherapy: harnessing complement in the action of anti-tumor monoclonal antibodies. Semin Immunol. 2016;28(3):309–16.
67. de Weers M, Tai Y-T, van der Veer MS, Bakker JM, Vink T, Jacobs DCH, et al. Daratumumab, a novel therapeutic human CD38 monoclonal antibody, induces killing of multiple myeloma and other hematological tumors. J Immunol. 2011;186(3):1840–8.
68. Mellor JD, Brown MP, Irving HR, Zalcberg JR, Dobrovic A. A critical review of the role of Fc gamma receptor polymorphisms in the response to monoclonal antibodies in cancer. J Hematol Oncol. 2013;6:1.
69. Overdijk MB, Verploegen S, Bögels M, Van Egmond M, Lammerts Van Bueren JJ, Mutis T, et al. Antibody-mediated phagocytosis contributes to the anti-tumor activity of the therapeutic antibody daratumumab in lymphoma and multiple myeloma. MAbs. 2015;7(2):311–21.
70. Overdijk MB, Jansen JHM, Nederend M, Lammerts van Bueren JJ, Groen RWJ, Parren PWHI, et al. The therapeutic CD38 monoclonal antibody daratumumab induces programmed cell death via Fcγ receptor-mediated cross-linking. J Immunol. 2016;197(3):807–13.
71. Lokhorst HM, Plesner T, Laubach JP, Nahi H, Gimsing P, Hansson M, et al. Targeting CD38 with daratumumab monotherapy in multiple myeloma. N Engl J Med. 2015;373(13):1207–19.
72. Lonial S, Weiss BM, Usmani SZ, Singhal S, Chari A, Bahlis NJ, et al. Daratumumab monotherapy in patients with treatment-refractory multiple myeloma (SIRIUS): an open-label, randomised, phase 2 trial. Lancet (London, England). 2016;387(10027):1551–60.
73. Nijhof IS, Casneuf T, van Velzen J, van Kessel B, Axel AE, Syed K, et al. CD38 expression and complement inhibitors affect response and resistance to daratumumab therapy in myeloma. Blood. 2016;128(7):959–70.
74. Landgren CO, Chari A, Cohen YC, Spencer A, Voorhees P, Estell JA, et al. Daratumumab monotherapy for patients with intermediate-risk or high-risk smoldering multiple myeloma: a randomized, open-label, multicenter, phase 2 study (CENTAURUS). Leukemia. 2020;34(7):1840–52.
75. Tang W, Zhang L, Zheng Y, Pan L, Niu T. Daratumumab-based immunotherapy vs lenalidomide, bortezomib and dexamethasone in transplant-ineligible newly diagnosed multiple myeloma: a systemic review. Front Oncol. 2024. https://doi.org/10.3389/fonc.2024.1286029.
76. Rajkumar SV. Multiple myeloma: 2024 update on diagnosis, risk-stratification, and management. Am J Hematol. 2024;99(9):1802–24.
77. Liu Z, Xu X, Liu H, Zhao X, Yang C, Fu R. Immune checkpoint inhibitors for multiple myeloma immunotherapy. Exp Hematol Oncol. 2023;12(1):99.
78. Saltarella I, Desantis V, Melaccio A, Solimando AG, Lamanuzzi A, Ria R, et al. Mechanisms of resistance to anti-CD38 daratumumab in multiple myeloma. Cells. 2020. https://doi.org/10.3390/cells9010167.
79. Richardson PG, Perrot A, San-Miguel JF, Beksac M, Spicka I, Leleu X, et al. Updates from ICARIA-MM, a phase 3 study of isatuximab (Isa) plus pomalidomide and low-dose dexamethasone (Pd) versus Pd in relapsed and refractory multiple myeloma (RRMM). J Clin Oncol. 2021;39(15_Suppl):8017. https://doi.org/10.1200/JCO.2021.39.15_suppl.8017.
80. Martin T, Dimopoulos M-A, Mikhael J, Yong K, Capra M, Facon T, et al. Isatuximab, carfilzomib, and dexamethasone in patients with relapsed multiple myeloma: updated results from IKEMA, a randomized Phase 3 study. Blood Cancer J. 2023;13(1):72. https://doi.org/10.1038/s41408-023-00797-8.
81. Deckert J, Wetzel M-C, Bartle LM, Skaletskaya A, Goldmacher VS, Vallée F, et al. SAR650984, a novel humanized CD38-targeting antibody, demonstrates potent antitumor activity in models of multiple myeloma and other CD38+ hematologic malignancies. Clin Cancer Res. 2014;20(17):4574–83.
82. Jiang H, Acharya C, An G, Zhong M, Feng X, Wang L, et al. SAR650984 directly induces multiple myeloma cell death via lysosomal-associated and apoptotic pathways, which is further enhanced by pomalidomide. Leukemia. 2016;30(2):399–408.
83. van de Donk NWCJ, Usmani SZ. CD38 antibodies in multiple myeloma: mechanisms of action and modes of resistance. Front Immunol. 2018;9:2134.
84. Feng X, Zhang L, Acharya C, An G, Wen K, Qiu L, et al. Targeting CD38 suppresses induction and function of T regulatory cells to mitigate immunosuppression in multiple myeloma. Clin Cancer Res. 2017;23(15):4290–300.
85. Jovčevska I, Muyldermans S. The therapeutic potential of nanobodies. BioDrugs. 2020;34(1):11–26.
86. Farajpour Z, Rahbarizadeh F, Kazemi B, Ahmadvand D. A nanobody directed to a functional epitope on VEGF, as a novel strategy for cancer treatment. Biochem Biophys Res Commun. 2014;446(1):132–6.
87. Muyldermans S. Nanobodies: natural single-domain antibodies. Annu Rev Biochem. 2013;82:775–97.
88. Bannas P, Hambach J, Koch-Nolte F. Nanobodies and nanobody-based human heavy chain antibodies as antitumor therapeutics. Front Immunol. 2017;8:1603.
89. Schriewer L, Schütze K, Petry K, Hambach J, Fumey W, Koenigsdorf J, et al. Nanobody-based CD38-specific heavy chain antibodies induce killing of multiple myeloma and other hematological malignancies. Theranostics. 2020;10(6):2645–58.
90. Bannas P, Koch-Nolte F. Perspectives for the development of CD38-specific heavy chain antibodies as therapeutics for multiple myeloma. Front Immunol. 2018;9:2559.
91. Baum N, Eggers M, Koenigsdorf J, Menzel S, Hambach J, Staehler T, et al. Mouse CD38-specific heavy chain antibodies inhibit CD38 GDPR-cyclase activity and mediate cytotoxicity against tumor cells. Front Immunol. 2021;12: 703574.
92. Baum N, Fliegert R, Bauche A, Hambach J, Menzel S, Haag F, et al. Daratumumab and nanobody-based heavy chain antibodies inhibit the ADPR cyclase but not the NAD (+) hydrolase activity of

CD38-expressing multiple myeloma cells. Cancers (Basel). 2020. https://doi.org/10.3390/cancers13010076.
93. Schütze K, Petry K, Hambach J, Schuster N, Fumey W, Schriewer L, et al. CD38-specific biparatopic heavy chain antibodies display potent complement-dependent cytotoxicity against multiple myeloma cells. Front Immunol. 2018;9:2553.
94. Hambach J, Fumey W, Stähler T, Gebhardt AJ, Adam G, Weisel K, et al. Half-life extended nanobody-based CD38-specific bispecific killercell engagers induce killing of multiple myeloma cells. Front Immunol. 2022;13: 838406.
95. Li T, Qi S, Unger M, Hou YN, Deng QW, Liu J, et al. Immuno-targeting the multifunctional CD38 using nanobody. Sci Rep. 2016;6:27055.
96. Caers J, Duray E, Vrancken L, Marcion G, Bocuzzi V, De Veirman K, et al. Radiotheranostic agents in hematological malignancies. Front Immunol. 2022;13: 911080.
97. Zheleznyak A, Tang R, Duncan K, Manion B, Liang K, Xu B, et al. Development of new CD38 targeted peptides for cancer imaging. Mol Imaging Biol. 2024. https://doi.org/10.1007/s11307-024-01901-5.
98. Salvador J-P, Vilaplana L, Marco M-P. Nanobody: outstanding features for diagnostic and therapeutic applications. Anal Bioanal Chem. 2019;411(9):1703–13.
99. Wang C, Chen Y, Hou YN, Liu Q, Zhang D, Zhao H, et al. ImmunoPET imaging of multiple myeloma with [68Ga]Ga-NOTA-Nb1053. Eur J Nucl Med Mol Imaging. 2021;48(9):2749–60.
100. Crișan G, Moldovean-Cioroianu NS, Timaru D-G, Andrieș G, Căinap C, Chiș V. Radiopharmaceuticals for PET and SPECT imaging: a literature review over the last decade. Int J Mol Sci. 2022. https://doi.org/10.3390/ijms23095023.
101. Ghai A, Maji D, Cho N, Chanswangphuwana C, Rettig M, Shen D, et al. Preclinical development of CD38-targeted [(89)Zr]Zr-DFO-daratumumab for imaging multiple myeloma. J Nucl Med. 2018;59(2):216–22.
102. Caserta E, Chea J, Minnix M, Poku EK, Viola D, Vonderfecht S, et al. Copper 64-labeled daratumumab as a PET/CT imaging tracer for multiple myeloma. Blood. 2018;131(7):741–5.
103. Krishnan A, Adhikarla V, Poku EK, Palmer J, Chaudhry A, Biglang-Awa VE, et al. Identifying CD38+ cells in patients with multiple myeloma: first-in-human imaging using copper-64-labeled daratumumab. Blood Adv. 2020;4(20):5194–202.
104. Zamagni E, Patriarca F, Nanni C, Zannetti B, Englaro E, Pezzi A, et al. Prognostic relevance of 18-F FDG PET/CT in newly diagnosed multiple myeloma patients treated with up-front autologous transplantation. Blood. 2011;118(23):5989–95.
105. Steinmeyer DE, McCormick EL. The art of antibody process development. Drug Discov Today. 2008;13(13):613–8.
106. Suzuki T, Ishii-Watabe A, Tada M, Kobayashi T, Kanayasu-Toyoda T, Kawanishi T, et al. Importance of neonatal FcR in regulating the serum half-life of therapeutic proteins containing the Fc domain of human IgG1: a comparative study of the affinity of monoclonal antibodies and Fc-fusion proteins to human neonatal FcR. J Immunol. 2010;184(4):1968–76.
107. Chakravarty R, Goel S, Cai W. Nanobody: the "magic bullet" for molecular imaging? Theranostics. 2014;4:386–98.
108. Duray E, Lejeune M, Baron F, Beguin Y, Devoogdt N, Krasniqi A, et al. A non-internalised CD38-binding radiolabelled single-domain antibody fragment to monitor and treat multiple myeloma. J Hematol Oncol. 2021;14(1):183.
109. Wei W, Zhang D, Wang C, Zhang Y, An S, Chen Y, et al. Annotating CD38 expression in multiple myeloma with [(18)F]F-Nb1053. Mol Pharm. 2022;19(10):3502–10.
110. Shi L, Chen B, Liu T, Li L, Hu B, Li C, et al. (99m)Tc-CD3813: a nanobody-based single photon emission computed tomography radiotracer with clinical potential for myeloma imaging and evaluation of CD38 expression. Mol Pharm. 2022;19(7):2583–94.
111. Li X, Wang Y, Yang Q, Song L, Kang L, Hu Z, et al. Microarray-based CD38 peptide probe screening for multiple myeloma imaging. Mol Pharm. 2024;21(1):245–54.
112. Akbarian M, Khani A, Eghbalpour S, Uversky VN. Bioactive peptides: synthesis, sources, applications, and proposed mechanisms of action. Int J Mol Sci. 2022. https://doi.org/10.3390/ijms23031445.
113. Choi J-S, Joo SH. Recent trends in cyclic peptides as therapeutic agents and biochemical tools. Biomol Ther (Seoul). 2020;28(1):18–24.
114. Sharma AK, Gupta K, Mishra A, Lofland G, Marsh I, Kumar D, et al. CD38-specific Gallium-68 labeled peptide radiotracer enables pharmacodynamic monitoring in multiple myeloma with PET. Adv Sci. 2024;11(16): e2308617.
115. Woo Y, Chaurasiya S, O'Leary M, Han E, Fong Y. Fluorescent imaging for cancer therapy and cancer gene therapy. Mol Therapy Oncolytics. 2021. https://doi.org/10.1016/j.omto.2021.06.007.
116. Kang H, Kang MW, Kashiwagi S, Choi HS. NIR fluorescence imaging and treatment for cancer immunotherapy. J ImmunoTherapy Cancer. 2022. https://doi.org/10.1136/jitc-2022-004936.
117. Cho N, Ko S, Shokeen M. Preclinical development of near-infrared-labeled CD38-targeted daratumumab for optical imaging of CD38 in multiple myeloma. Mol Imaging Biol. 2021. https://doi.org/10.1007/s11307-020-01542-4.
118. Cho N, Ko S, Shokeen M. Tissue biodistribution and tumor targeting of near-infrared labelled anti-CD38 antibody-drug conjugate in preclinical multiple myeloma. Oncotarget. 2021. https://doi.org/10.18632/oncotarget.28074.
119. Fumey W, Koenigsdorf J, Kunick V, Menzel S, Schütze K, Unger M, et al. Nanobodies effectively modulate the enzymatic activity of CD38 and allow specific imaging of CD38+ tumors in mouse models in vivo. Sci Rep. 2017. https://doi.org/10.1038/s41598-017-14112-6.
120. Pape LJ, Hambach J, Gebhardt AJ, Rissiek B, Stähler T, Tode N, et al. CD38-specific nanobodies allow in vivo imaging of multiple myeloma under daratumumab therapy. Front Immunol. 2022;13:1010270.
121. Fang C, Li T, Li Y, Xu GJ, Deng QW, Chen YJ, et al. Correction: CD38 produces nicotinic acid adenine dinucleotide phosphate in the lysosome. (J Biol Chem. 2018;293:8151–8160. https://doi.org/10.1074/jbc.RA118.002113). J Biol Chem. 2019; 294.
122. ElMaraashly AH, Tario J, Hillengass J, Qian Y-W. Camelid-derived CD38 antibody successfully circumvents epitope blockade by the therapeutic anti-CD38 antibody daratumumab. Am J Clin Pathol. 2023;160(5):524–32.
123. Borrego-Soto G, Ortiz-López R, Rojas-Martínez A. Ionizing radiation-induced DNA injury and damage detection in patients with breast cancer. Genet Mol Biol. 2015;38(4):420–32.
124. Daguenet E, Louati S, Wozny A-S, Vial N, Gras M, Guy J-B, et al. Radiation-induced bystander and abscopal effects: important lessons from preclinical models. Br J Cancer. 2020;123(3):339–48. https://doi.org/10.1038/s41416-020-0942-3.
125. Hu S, Shao C. Research progress of radiation induced bystander and abscopal effects in normal tissue. Radiat Med Prot. 2020;1(2):69–74.
126. D'Huyvetter M, Xavier C, Caveliers V, Lahoutte T, Muyldermans S, Devoogdt N. Radiolabeled nanobodies as theranostic tools in targeted radionuclide therapy of cancer. Expert Opin Drug Deliv. 2014;11(12):1939–54.
127. Hatcher-Lamarre JL, Sanders VA, Rahman M, Cutler CS, Francesconi LC. Alpha emitting nuclides for targeted therapy. Nucl Med Biol. 2021;92:228–40.
128. Green DJ, Orgun NN, Jones JC, Hylarides MD, Pagel JM, Hamlin DK, et al. A preclinical model of CD38-pretargeted radioimmunotherapy for plasma cell malignancies. Cancer Res. 2014;74(4):1179–89.
129. Green DJ, O'Steen S, Lin Y, Comstock ML, Kenoyer AL, Hamlin DK, et al. CD38-bispecific antibody pretargeted radioimmunotherapy for multiple myeloma and other B-cell malignancies. Blood. 2018;131(6):611–20.
130. Kang L, Jiang D, Ehlerding E, Barnhart T, Ferreira C, Wang R, et al. Safety and dosimetry evaluation of radioimmunotherapy using 177Lu-labeled antibodies in lymphoma. J Nucl Med. 2019;60(supplement 1):1004.
131. Dawicki W, Allen KJH, Jiao R, Malo ME, Helal M, Berger MS, et al. Daratumumab-225Actinium conjugate demonstrates greatly enhanced antitumor activity against experimental multiple myeloma tumors. Oncoimmunology. 2019;8(8):1607673.
132. Quelven I, Monteil J, Sage M, Saidi A, Mounier J, Bayout A, et al. (212)Pb α-radioimmunotherapy targeting CD38 in multiple myeloma: a preclinical study. J Nucl Med. 2020;61(7):1058–65.
133. Minnix M, Adhikarla V, Caserta E, Poku E, Rockne R, Shively JE, et al. Comparison of CD38-targeted α- versus β-radionuclide therapy of disseminated multiple myeloma in an animal model. J Nucl Med. 2021;62(6):795–801.
134. O'Steen S, Comstock ML, Orozco JJ, Hamlin DK, Wilbur DS, Jones JC, et al. The α-emitter astatine-211 targeted to CD38 can eradicate multiple myeloma in a disseminated disease model. Blood. 2019;134(15):1247–56.

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