Daratumumab and its potential in the treatment of multiple myeloma: overview of the preclinical and clinical development

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***Abstract*:** Despite the recent major advancement in therapy for multiple myeloma, it remains an incurable disease. There remains an unmet need for novel therapies that target different mechanisms of action. Immunotherapy with monoclonal antibodies is a promising area of development and will expand our therapeutic armamentarium in the fight against myeloma. Daratumumab is a novel, high-affinity, therapeutic human monoclonal antibody against unique CD38 epitope with broad-spectrum killing activity. It has a favorable safety profile as monotherapy in patients with relapsed/refractory myeloma and also demonstrates significant single-agent activity. Abundant preclinical data supports its use in combination therapy and clinical studies on various exciting combinations are underway. This review focuses on the CD38 antigen and its targeting with daratumumab and provides an update on the results of recent clinical studies involving daratumumab.

Keywords: daratumumab, monoclonal, myeloma

Introduction

Multiple myeloma (MM) is a plasma cell malignancy that accounts for approximately 1% of all cancers [Siegel *et al.* 2012]. It has a high propensity for bone and kidney involvement causing morbidity from pathological fractures, bone pain, spinal cord compression and renal failure. In the past decade, intensive research into the biology of myeloma has exposed many new potential therapeutic targets, including histone deacetylation, the catalytic activity of proteasomes, signaling pathways of Akt, mammalian target of rapamycin, MEK, as well as cellular expression of CS-1, CD38, and CD40 [Arrigo *et al.* 1988; Ocio *et al.* 2014]. These discoveries have hailed the advent of the novel drugs in MM, which have displaced the routine use of conventional chemotherapy, such as VAD (vincristine–adriamycin–dexamethasone), from induction regimens. The introduction of the proteasome inhibitors and immunomodulatory agents into upfront and relapse therapies has in fact improved progression-free survival (PFS) and overall survival (OS) [Kumar *et al.* 2008; Mateos *et al.* 2010; Nair *et al.* 2010]. However, these responses are not uniform among MM patients. Eight-year OS of 75% is seen in younger patients with low international staging system (ISS) scores lacking high-risk cytogenetic features. Conversely, patients who have a combination of these risk factors have a median OS as low as 33 months [Avet-Loiseau *et al.* 2012]. Current treatments may mitigate, but are unable to completely circumvent, the inherent genomic instability and clonal heterogeneity that leads to recurrent relapses and ultimately progressive MM. In addition, in the era of novel agents, where MM patients are surviving longer, physicians are encountering more resistant and atypical relapses. Salvaging such patients becomes particularly challenging and their prognosis often remains dismal [Short *et al.* 2011; Gangatharan *et al.* 2012]. There remains an unmet need for novel therapies that target different mechanisms of action. In this respect, monoclonal antibodies (moAbs) against antigens that are regularly expressed on myeloma cells represents a novel way of overcoming resistant pathways related to current small molecules. The paradigm of this approach is the targeting of CD20 in B-cell non-Hodgkin lymphomas. Owing to the wide expression of this molecule on most

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B-cell lymphomas, the moAb rituximab has become a mainstay of treatment in these diseases.

Monoclonal antibody targets in multiple myeloma

Various antigens have been implicated as potential therapeutic targets in MM. These include those involved in cell signaling, cell adhesion, apoptosis, and interactions with the bone marrow microenvironment. Specifically, SLAMF7 (CS1), CD40, CD138, IL-6, CD74, CD162, PD1, β_2-microglobulin, GM-2, and CD38 receptor have been studied intensively for the development of moAb-based interventions.

SLAMF7 is a cell surface glycoprotein that is expressed almost exclusively on the surface of plasma cells in MM. Its exact role has not been fully defined but is likely involved in MM cell adhesion and tumor proliferation through interactions with bone marrow stromal cells (BMSCs) [Tai *et al.* 2009]. CD40 is a transmembrane protein that is highly expressed on B cells, dendritic cells and primary MM cells that is involved in cell proliferation and migration, as well as triggering the secretion of interleukin-6 (IL-6) and vascular endothelial growth factor [Urashima *et al.* 1996; Tai *et al.* 2002]. CD138 (syndecan-1) is frequently expressed on normal and MM plasma cells. Shedding of syndecan-1 has been shown to promote cell proliferation through positive regulation of the tumor microenvironment [Wijdenes *et al.* 1996; Dhodapkar *et al.* 1997].

IL-6 is a key growth and survival factor for MM cells, as well as a major morbidity factor for MM patients that is produced mainly by BMSCs.

CD38 is a transmembrane protein that is highly expressed on malignant plasma cells. Three moAbs against CD38 (SAR650984, MOR03087, and daratumumab) are currently being investigated in clinical trials. This review will focus on the CD38 antigen and its targeting with the human moAb, daratumumab.

CD38 and potential for targeting

CD38 is a single-chain type II transmembrane molecule with a molecular weight of ~ 46 kDa. It has a short 20-amino acid (aa) N-terminal cytoplasmic tail and a long 256-aa extracellular domain [Ellis *et al.* 1995]. The functional molecule is a dimer, with its central portion hosting the catalytic site [Malavasi *et al.* 2011]. The CD38 molecule is widely expressed in multiple cell types of the immune system with differential expression according to age, being high in T and B cells in cord blood and low in mature B cells in adults.

While not a B-lineage specific marker, CD38 expression is tightly regulated during B-cell ontogenesis. It is present at high levels on bone marrow precursors, is downregulated in resting B-cells and is induced during naive B-cell activation and upon entering the germinal center. The expression then declines during centroblast and centrocyte differentiation before completely disappearing in memory B cells [Malavasi *et al.* 2008]. CD38 expression then becomes strongly expressed by terminally differentiated plasma cells. Apart from immune cells, the molecule has also been found in the brain, pancreatic acinar cells, smooth muscle and osteoclasts although expression in these tissues is in the cytosol or nucleus rather than the cell membrane.

CD38 serves not only as an antigen but also as an enzyme that catalyzes the metabolism of cyclic adenosine diphosphate ribose and nicotinic acid adenine dinucleotide phosphate, both of which regulate intracellular calcium stores [Lee, 2006]. When agonistic or nonagonistic moAbs interact with CD38, antigen ligation occurs and involves significant fractions of the surface molecule. Endocytosis and internalization follows. These are reproducible events that are independent of signal transduction [Chillemi *et al.* 2013]. MoAbs that specifically activate CD38 have been shown to induce rapid calcium fluxes that trigger phosphorylation of multiple intracellular substrates that eventually result in activation of the nuclear factor-κB complex. Therefore, CD38 represents an important immunotherapy target due to its high expression on malignant plasma cells and low expression on other normal lymphoid and myeloid cells as well as being an important modulator of intracellular signaling.

Preclinical development

Daratumumab is a human anti-CD38 IgG_1 (κ subclass) antibody. It was generated by immunization of transgenic mice possessing the human (not mouse) immunoglobulin gene (HuMAb-mouse) with recombinant CD38 protein and NIH 3T3 (expressing human CD38) cells until CD38-specific serum titer development. The antibodies generated were shown to have good

binding to Daudi (B-lymphoblast cell line) and fresh MM cells [De Weers *et al.* 2011].

Specifically, daratumumab binds to two β-strand-containing amino acids 233–246 and 267–280 of CD38 [De Weers *et al.* 2011]. The binding to this unique fine epitope cluster positions the antibody Fc in a way that facilitates optimal interaction for complement activation and allowed for efficient complement-dependent cytotoxicity (CDC) when tested with Daudi and fresh MM cells. Potent CDC activity was demonstrated initially against the Daudi cell line after incubation with daratumumab, pooled human serum (as the source of complement), and propidium iodine (PI; to demonstrate elevated membrane permeability). Complement-dependent lysis/ killing, determined by flow cytometric measurement of PI-positive cells, was induced with an EC_{50} of 0.16 μg/ml and maximal lysis of 56%. CDC activity was then shown in MM cell lines from samples obtained from 11 patients (of a 13-patient cohort) with median maximal lysis of 51%. It has been further demonstrated that the CDC mediated killing does not diminish in the presence of BMSCs, suggesting that daratumumab may induce CDC in the tumor-preserving bone marrow microenvironment as well. Similarly, antibody-dependent cellular cytotoxicity (ADCC) against Daudi and CD38-positive MM cell lines was exhibited after incubation with daratumumab and peripheral blood mononuclear cells (PBMCs) enriched with natural (NK) cells, and release of calcein-AM from target cell death. The lysis was dose-dependent (EC_{50}~0.01 μg/ml). ADCC killing was also shown in MM cells obtained from patients, occurring whether in the presence of effector cells derived from healthy donors or patients themselves (autologous PBMCs) [De Weers *et al.* 2011].

In a series of experiments using MM cell lines, purified MM cells, mononuclear cell suspensions isolated from MM and lenalidomide-treated patients, it was shown that daratumumab and lenalidomide together significantly increased lysis of MM cells compared with either of these agents alone. The effect was demonstrated to be mainly due to the ability of lenalidomide to activate effector cells of ADCC, since lenalidomide did not appear to interfere with CDC [Van Der Veer *et al.* 2011a]. This is consistent with studies carried out using lenalidomide and another antibody, elotuzumab that targets SLAMF7 on the surface of MM tumor cells.

The main antimyeloma effect of daratumumab is attributed to its prominent ADCC and CDC activities (Figure 1). However, other mechanisms have also shown to be important. *In vitro* studies using CD38-positive MM tumor cells as well as patient-derived MM cell lines incubated with Fc gamma receptor I-expressing cells lacking ADCC activity showed significant apoptosis in the presence of daratumumab. This suggests that an additional mode of action is the induction of apoptosis via FcR-mediated crosslinking [Marco Jansen *et al.* 2012]. Further experiments using a Burkitt's lymphoma (Daudi) cell line mixed with human macrophages in the presence of daratumumab showed daratumumab-specific antibody-dependent cellular phagocytosis (ADCP) that resulted in a 50% reduction in tumor cells. Dose-dependent daratumumab-specific phagocytosis was also observed with patient-derived MM cell lines transduced with CD38. Life-cell imaging documented that single macrophages could engage multiple target cells and was able to engulf up to six tumor cells sequentially in a 30-minute period, suggesting that ADCP might be a very potent mechanism of action of daratumumab *in vivo* [Overdijk *et al.* 2012]. Other mechanisms of action include activation of caspase-dependent MM cell death [Kong *et al.* 2011].

Pharmacokinetics and clinical trials

A phase I/II dose escalation study of daratumumab was carried out in 32 patients with relapsed/refractory MM to establish its safety profile and also considered pharmacokinetics and maximum tolerated dose among secondary objectives [Plesner *et al.* 2012]. The study utilized a 3+3 dose-escalation design (GEN501) with daratumumab administered over a 9-week period consisting of two pre- and seven full doses, ranging from 0.005 to 24 mg/kg. Peak plasma levels were reached after the first full dose and rapid clearance of the drug was observed at low doses, indicating a target-mediated clearance. The pre-dose (2 mg/kg) achieved trough levels that were far below expected values. However, doses of 4 mg/kg or greater resulted in adequate and sustained trough levels and abrogated the effect of target-mediated clearance. The elimination half-life as predicted using a two-compartment pharmacokinetic model was 21 days. The difference in tumor load amongst patients may have accounted for the high interpatient variability that was observed.

The most common adverse reactions were infusion-related events (IREs) such as fever, cough, nausea, dizziness, and bronchospasm. A total of

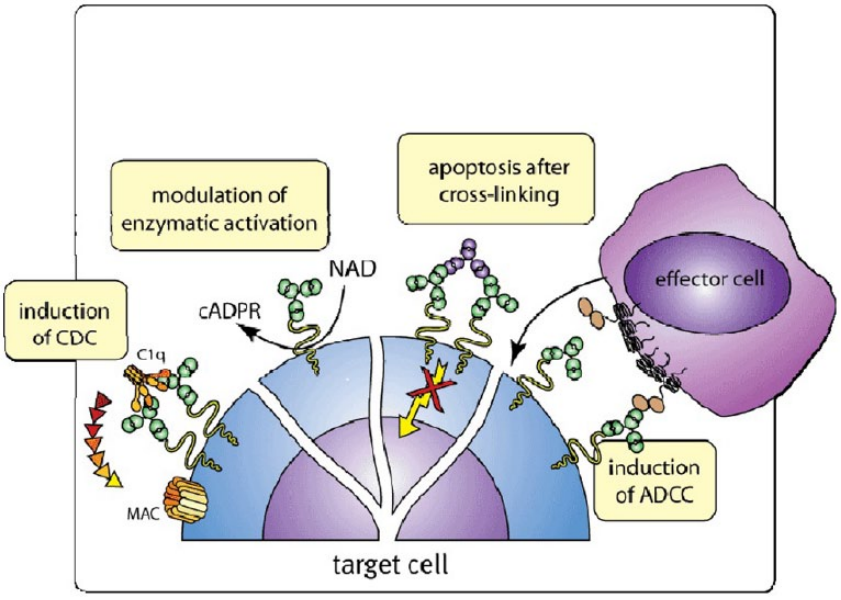

Figure 1. Mechanisms of action of daratumumab (Courtesy of Dr Torben Plesner).

9% occurred during predosing and 26% during the first full infusion with a gradual decrease with subsequent infusions. The onset of IREs was within 3–4 hours of infusion. Prophylactic steroids were administered to reduce the incidence of IREs (up to a maximum dose equivalent of 27 mg of dexamethasone per week). There were six serious adverse events (SAEs) related to daratumumab. In the 0.1 mg/kg cohort, there was one case of grade 3 anemia, one case of grade 4 thrombocytopenia, and one case of grade 2 cytokine release syndrome; in the 1 mg/kg cohort, there was one case of grade 3 elevated aspartate aminotransferase; in the 2 mg/kg group, one case of grade 3 bronchospasm; with 24 mg/kg, one case of grade 2 bronchospasm. All patients recovered from their SAEs with treatment and the maximum tolerated dose has not yet been reached. There was a dose-dependent decrease in peripheral-blood NK cells that was noted, with full recovery after treatment [Lokhorst *et al.* 2013].

In this phase I/II study, daratumumab displayed a dose-dependent efficacy with greater decreases in paraprotein levels seen in patients at higher doses. A high area under the curve correlated with prolonged PFS. Biochemical responses were accompanied by clearance of MM cells from the bone marrow. Four patients achieved a partial response (PR), six had a minimal response, while five had stable disease by International Myeloma Working Group criteria.

Building upon initial results, the GEN501 expansion served to evaluate safety and efficacy of 2 doses of daratumumab (8 mg/kg, at three different infusion times, or 16 mg/kg once a week for 8 weeks, followed by 8 doses twice monthly and monthly dosing up to 24 months) [Lokhorst *et al.* 2014]. The phase II part of the trial has recruited 50 patients so far; 30 patients received the 8 mg/kg dosing and 20 the 16 mg/kg dosing. Fever, fatigue, upper respiratory tract infection, dyspnea, cough and diarrhea were the most common adverse events and were reported in more than 20% of patients. Mild (grade 1 and 2) IREs occurred in 27% of patients in the 16 mg/kg group and 20% of the 8 mg/kg group, and there were no reports of severe IREs. There were two cases of SAEs between the two groups: one case of thrombocytopenia and one case of lymphopenia. Omission of the predose did not have an impact on the incidence and severity of IREs [Lokhorst *et al.* 2014]. Investigators did not observe any dose-related increases in side effects. Although this phase I/II study did not include a quality of life analysis with daratumumab therapy, it is encouraging to note that, except for IREs, there were no major side effects and the drug was very well tolerated.

Of the 30 patients in 8 mg/kg dosing groups, 10% responded, and all were partial responses (PRs). The overall response rate (RR) was much higher in the 16 mg/kg dosing group where 35% of the 20 patients responded, with 10% achieving a complete response (CR), 5% a very good partial response (VGPR), and

20% a PR. The median PFS was 15 months in the 8 mg/kg dosing groups, compared with 23 months in the 16 mg/kg dosing group. The dose of 16 mg/kg achieves deeper responses and is presumed to be target-saturating and therefore, is the dose that has been used in subsequent trials. More mature results of the study will inform if extended exposure with daratumumab monotherapy for up to 24 months in responding patients will further prolong the PFS.

Ongoing clinical trials

The early results of daratumumab used as a single agent are impressive. Considerable responses have been observed in a cohort of heavily pretreated patients with relapsed/refractory MM. However, in order to harness the full potential of the antimyeloma effect of daratumumab, the identification of synergistic drug combinations that target various mechanisms to overcome drug resistance will be vital. As such, daratumumab will be explored as a component of multidrug chemotherapy regimens and these include combinations with bortezomib and lenalidomide. This is predicated on convincing preclinical data showing the potential and synergism of these combinations. The potential role of cytotoxicity induced by the anti-CD38 antibody and the activation of effector cells with the immunomodulatory effects of lenalidomide could make this a very attractive antimyeloma combination therapy. Indeed, *ex vivo* analyses evaluating daratumumab in combination with lenalidomide, bortezomib and dexamethasone showed enhanced lysis of MM cells in bone marrow aspirates derived from 22 patients of whom 9 were refractory to lenalidomide and 6 were refractory to lenalidomide and bortezomib. Apart from lenalidomide-induced activation of effector cells, increased direct killing of MM cells was also observed [Nijhof *et al.* 2012]. Bortezomib may enhance the therapeutic effect of daratumumab by sensitizing tumor cells for antibody-mediated lysis. A more than two-fold increase in MM cell lysis has been observed with the bortezomib–daratumumab combination [Van Der Veer *et al.* 2011b].

The ongoing phase I/II Gen503 study combining daratumumab with lenalidomide and dexamethasone in relapsed, refractory MM seeks to establish the safety and evaluate the efficacy and pharmacokinetics of the combination [Janssen Research & Development, LLC, 2015a]. In this study, daratumumab is dosed at 2–16 mg/kg and given weekly for 8 weeks, then fortnightly for 16 weeks and then monthly until disease progression or unmanageable toxicity arises, for a maximum of 24 months. Lenalidomide and dexamethasone are dosed as per the conventional lenalidomide and low-dose dexamethasone (Rd) regimen. The most frequent adverse events reported from the preliminary results of the 12 study patients were neutropenia and diarrhea, of which more than 40% of patients were involved. One patient was withdrawn from the study due to recurrent grade 1 QT prolongation and hypokalemia. The addition of lenalidomide and dexamethasone did not appear to affect the pharmacokinetics profile of daratumumab. Of the 11 patients, 8 achieved PR or better with 5 achieving VGPR [Plesner *et al.* 2014]. A major phase III study (MMY3004) is currently comparing daratumumab, lenalidomide, and dexamethasone (DRd) with Rd in subjects with relapsed or refractory MM. Daratumumab is dosed at 16 mg/kg in this study [Plesner *et al.* 2012].

Similarly, another ongoing phase I/II combination study aims to evaluate the safety, tolerability, and dose of daratumumab when administered in combination with the various backbone standard treatments for MM [Janssen Research & Development, LLC, 2015b]. Newly diagnosed patients will receive daratumumab 16 mg/kg with bortezomib–dexamethasone (VD), bortezomib–melphalan–prednisone (VMP), or bortezomib–thalidomide–dexamethasone (VTD), while patients who are relapsed/refractory after two or more lines will receive combination with pomalidomide–dexamethasone. Data from 17 patients in the newly diagnosed cohorts (VD, $N=5$; VMP, $N=6$; VTD, $N=6$) has recently been reported after a median treatment duration of 44 days (range 1–113). No additional toxicity was attributed to daratumumab apart from infusional reactions. The most common adverse events were hematologic in nature with three episodes of grade $\geqslant 3$ neutropenia and one episode of grade 3 anemia. These were likely related to the backbone therapy. Other common (three or more occurrences) grade 1 and 2 adverse events were peripheral sensory neuropathy, headache, asthenia, pyrexia, and constipation. The authors concluded that the addition of daratumumab was safe and did not result in significant additional toxicity, and that patient enrollment would continue [Moreau *et al.* 2014]. Plans for phase III randomized controlled studies to study the efficacy of addition of daratumumab to bortezomib-based combinations (bortezomib–dexamethasone in R/R MM and bortezomib–melphalan–prednisolone in untreated MM) are also underway [US National Library of Medicine, 2014a, 2004b].

Future direction and conclusion

Daratumumab is a novel, high-affinity, therapeutic human monoclonal antibody against the unique CD38 epitope. From the preliminary data of the phase I/I Gen501 and the Gen503 studies, it has demonstrated a favorable safety profile as monotherapy and in combination with Rd among patients with RR MM. Initial efficacy analysis has also shown significant single agent activity in a group of heavily pretreated patients. Abundant preclinical data supports its use in combination therapy and the efficacy data of the combination trials with standard MM backbone therapy will be eagerly awaited. There are also plans to explore daratumumab in smoldering MM and as a maintenance agent. This is will be analogous to the role of rituximab in low tumor burden follicular lymphoma and in maintenance therapy. The tolerability of the daratumumab is advantageous in this regard.

Despite the recent advances in therapy, MM remains an incurable disease, and novel approaches that induce long-term tumor regression with little cross-resistance with existing drugs are always needed to expand our therapeutic armamentarium against MM. In this regard, daratumumab is a promising addition with multiple effector mechanisms including, complement activation, recruitment of Fc-bearing cells, induction of apoptosis and cell-mediated phagocytosis, as well as modulation of cellular enzymatic activities associated with calcium signaling that culminate in malignant plasma cell killing. The eagerly anticipated results of further clinical studies in the future will hopefully fill the emergent need for improved treatment strategies in MM.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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