Chapter

The Modern Age of Monoclonal Antibodies: The Revolution of Daratumumab

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Abstract

CD38 is a transmembrane glycoprotein expressed on the surface of different cell lines with several functions (receptor, adhesion molecule, ectoenzyme). Based on its high expression in multiple myeloma cells, CD38 is one of the main molecules used in the target therapy age. Daratumumab is the first fully human monoclonal antibody tested in clinical trials, showing efficacy in relapsed/refractory multiple myeloma patients, especially in combination with immunomodulants and/or proteasome inhibitors. The synergic effect concerns multiple myeloma cells as well as the microenvironment (NK cells, macrophage, regulatory B/T cells and CD8+ effector cells). Therefore, the anti-multiple myeloma activity of Daratumumab greatly depends on the immune system: this is the reason why several ongoing clinical trial are testing its efficacy in the naïve patients, with a more effective immune system.

Keywords: daratumumab, monoclonal antibody, anti-CD38, multiple myeloma

1. Introduction: mechanism of action

Daratumumab is the first fully IgG_1K-human monoclonal antibody targeting CD38.CD38, also known as cyclic ADP ribose hydrolase, is a transmembrane glycoprotein expressed on the surface of hematopoietic and non-hematopoietic cell lines.

This protein plays different functions, both on the external and on the inner surface of cells. As a receptor, it takes part into the inflammatory response, stimulating the production of a great variety of cytokines through the interaction with CD31, on the surface of T cells. As enzyme, it is involved in the metabolism of nicotinamide adenine dinucleotide (NAD+), leading to the synthesis of cyclic ADP ribose (cADPR) which regulates cellular calcium trafficking [1].

In the context of bone niche, CD38 expression is very high on the surface of plasma cells. Pioneering studies have shown that this glycoprotein plays a key-role in the oncogenesis of multiple myeloma: increased intracellular levels of NAD+ seem to be associated with a less susceptibility to apoptosis [2] and the synthesis of cADPR favours the escape of tumour cells from the immune system [3]. In vitro, CD38 seems also to be associated with the formation of nanotubes that transfer mitochondria from the stromal cells to myeloma cells, boosting myeloma cell proliferation and survival [4].

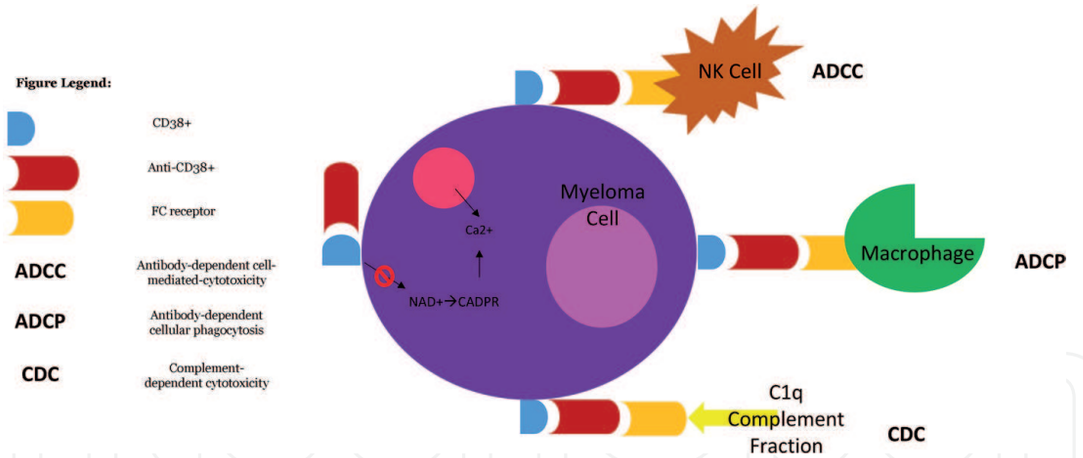

Figure 1.
Mechanism of action of daratumumab. Daratumumab binds CD38, killing myeloma cells via Fc-dependent immune effector mechanisms: CDC, ADCC and ADCP. Daratumumab also inhibits enzymatic activity of CD38, downregulating intracellular Ca^{2+} trafficking.

Daratumumab binds CD38, killing tumour cells via Fc-dependent immune effector mechanisms including complement-dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP) [5]. The complement activation seems to be the most effective mechanism used by Daratumumab [6]: the Fc tail of the drug binds the activating factor C1q leading both to ultimate activation of membrane attack complex and to deposition of C3b on the surface of multiple myeloma plasma cells. The activation of membrane attack complex causes osmotic lysis of cells while the deposition of complement factors attracts phagocytic cells. The recruitment of immune effector cells is also boosted by the release of circulating factors such as C3 and C5a (**Figure 1**).

The anti-tumour activity of Daratumumab does not depend only on the direct action on plasma cells but also on the interaction with other lymphoid and myeloid cells with a weak expression of CD38: NK cells, B and T regulatory cells and CD8+ effector cells. Krejcik et al. have demonstrated that bone marrow and peripheral blood from patients on treatment with Daratumumab present low levels of regulatory cells and high levels of NK and CD8+ effector cells. This monoclonal antibody may interfere with the immunosuppressive microenvironment in the multiple myeloma bone niche, in favour of major susceptibility for the plasma cells to the NK and CD8+ cells toxicity [7].

2. Pharmacokinetics

Daratumumab is usually administered at the dosage of 16 mg/kg weekly for 8 weeks then every 2 weeks for 16 weeks and every 4 weeks thereafter until progression of disease. The administration on a mg/kg basis is due to the observation that distribution and clearance of daratumumab depends on bodyweight. It seems to be not influenced by age, gender, race, mild renal and liver impairment. To our knowledge, the extra-liver metabolism of daratumumab is the reason for the absence of interactions with other drugs.

The efficacy and safety of this schedule have been demonstrated by two studies involving patients with relapsed/refractory multiple myeloma (RRMM) treated with the anti-CD38 monoclonal antibody as single agent: GEN501 and SIRIUS.

GEN501 was a phase I/II, open-label, multicenter study. In the dose-escalation part, sequential cohorts of patients received intravenous doses of daratumumab ranging from 0.005 to 24 mg/kg, administered over 6–8 h. In the dose-expansion

study, in three of the enrolled cohorts, daratumumab was administered based on the findings from the previous part at 8 mg/kg weekly for 8 weeks, every 2 weeks for 16 weeks, and every 4 weeks until disease progression [8].

SIRIUS was a phase II study with two parts. In the first part, the patients were randomized to receive daratumumab 8 mg/kg every 4 weeks or 16 mg/kg weekly for 8 weeks, then every 2 weeks for 16 weeks and every 4 weeks thereafter. In the second part, all patients received daratumumab 16 mg/kg, according to the findings from the first part [9].

Intravenous administration of Daratumumab is associated with several side effects, included infusion-related reactions (see below). Therefore, this formulation requires a very slow infusion rate which may represent a disadvantage for the patient. Sever trials are evaluating the subcutaneous administration as an alternative. In the phase 1b PAVO study, the subcutaneous formulation of the monoclonal antibody was administered in patients with RRMM in combination with the recombinant human hyaluronidase PH20 enzyme (rHuPH20) to depolymerize hyaluronan in the subcutaneous space and increase the absorption rate [10]. This formulation at the dosage of 1800 mg was well tolerated and allowed to obtain similar concentrations and responses to the intravenous administration. Non-inferiority of subcutaneous daratumumab to intravenous formulation has been confirmed by preliminary results of the ongoing phase III trial COLUMBA [11]: enrolled patients with RRMM are randomized to receive either intravenous daratumumab 16 mg/kg or subcutaneous daratumumab 1800 mg. According to these studies, the approval of this formulation by the regulatory bodies is on the agenda.

3. Daratumumab in relapsed/refractory multiple myeloma

Approval of daratumumab by regulatory bodies was made possible thanks to clinical trials evaluating its use in RRMM. Patients with RRMM still represent the patients best benefitting from this monoclonal antibody, both as single agent and in combination with other agents (**Table 1a**).

3.1 Daratumumab in relapsed/refractory multiple myeloma as single agent

GEN501 and SIRIUS are the two main trials who led to approval of monotherapy with daratumumab. Both studies enrolled patients with RRMM: patients in GEN501 had relapsed after or were refractory to ≥2 prior lines of therapy, including inhibitors of proteasome (PIs), immunomodulatory drugs (IMiDs), chemotherapy and autologous stem cell transplantation (ASCT); patients in SIRIUS had relapsed after ≥3 lines of therapy, including a PI and a IMiDs or were double refractory to the most recently received PI and IMiDs. The primary endpoint of GEN501 was evaluation of safety while SIRIUS was designed to first evaluate overall response rate (ORR). Data regarding 148 patients from pooled analysis of the two trials confirmed how daratumumab, at the dosage of 16 mg/kg, is effective and safe in a population of heavily pretreated patients [12]. With a median number of 12 infusions, the ORR was 31.1%. At the time of the analysis, after a median follow-up of 20.7 months, the progression free survival (PFS) was 4 months, with a 12-month PFS rate of 22%. Stratifying the patients by the response according to International Myeloma Working Group, the PFS and the overall survival (OS) went out to be 15 months and not reached respectively for responders, 3 months and 18.5 months for patients with a stable disease or minimal response, 0.9 months and 3.7 months for non-responders. The median duration of response was 7.6 months and it deepened and improved in patients continuing daratumumab.

Trial	Phase	Therapy	Primary outcome
(a) RRMM			
GEN501	1/2	IV daratumumab single agent	evaluation of safety
SIRIUS	2	IV daratumumab single agent	ORR
PAVO	1B	SC daratumumab	Maximum ctrough N° of patients with AEs
COLUMBA	3	IV daratumumab *vs* SC daratumumab	ORR Maximum ctrough
NCT01615029	1 / 2	DARA-Rd	ORR
CASTOR	3	DARA-Vd *vs* Vd	PFS
POLLUX	3	DARA-Rd *vs* Rd	PFS
(b) NDMM			
ALCYONE	3	DARA-VMP *vs* VMP	PFS
MAIA	3	DARA-Rd *vs* DARA-Rd	PFS
CASSIOPEIA	3	DARA-VTd *vs* VTd	sCR after consolidation PFS
GRIFFIN	2	DARA-RVd *vs* RVd	sCR after consolidation
PERSEUS	3	DARA-RVd *vs* RVd	PFS

RRMM: Relapsed/Refractory Multiple Myeloma, IV: intravenous, SC: subcutaneous, Rd.: lenalidomide-dexamethasone, Vd: bortezomib-dexamethasone, ORR: Overall Response Rate, Maximum CTrough: Maximum Concentration Trough, AEs: Advese Events, PFS: Progression Free Survival, NDMM: Newly Diagnosed Multiple Myeloma, VMP: bortezomib-melphalan-dexamethasone, VTd: bortezomib-thalidomide-dexamethasone, RVd: lenalidomide-botrezomib-dexamethasone, sCR: stringent Complete Response.

Table 1.
Overview of main trials using Daratumumab in (a) RRMM, (b) NDMM.

3.1.1 Daratumumab in relapsed/refractory multiple myeloma in combination therapies: with IMiDs

Efficacy of daratumumab seems to be strengthened by other drugs used for multiple myeloma, given the synergic action on the immune system. As said before, the anti-CD38 may stimulate NK and T-cells, restoring "tumor suppressive immunological surveillance". Also IMiDs could increase the amount of regulatory cells in the bone niche, through inhibition of some transcriptional factors (Ikaros and Aialos) and the subsequent production of interleukin 2 [13]. Furthermore, some studies show that the main target of daratumumab is upregulated by action of IMiDs [14]. NCT01615029 was the first trial exploring the applicability of these laboratory observations, investigating efficacy of daratumumab in combination with lenalidomide and dexamethasone (Rd) [15]. It was a phase 1/2 study addressed to patients with relapsed multiple myeloma: phase 1 was a dose-escalation study in which the dose of 16 mg/kg for daratumumab was again determined; phase 2 was a dose-expansion study using the recommended dose of the first part. The three drugs were administered in cycle of 28 days: daratumumab was given according to the standard schedule, lenalidomide at 25 mg/day from days 1 to 21 of each cycle and dexamethasone at 40 mg/week. This combination revealed to be safe and very effective: the 18-months PFS rate was 72% and ORR was 81%, in this case too with an improvement of responses in time. To evaluate the advantage of adding daratumumab to a regimen with lenalidomide and dexamethasone, from 2014 to 2015, a phase III, randomized trial was carried out across Europe, Northern America and Asia [16]. The POLLUX trial enrolled 569 patients with multiple myeloma who had

received one or more previous lines of therapy: 286 were assigned to the daratumumab group (daratumumab plus lenalidomide and dexamethasone) and 283 to the control group (lenalidomide and dexamethasone). Also in this trial, each cycle was of 28 days, with daratumumab administered according to the usual schedule, lenalidomide at 25 mg/day from days 1 to 21 of each cycle and dexamethasone at a dose of 40 mg weekly. At 12 months, the PFS rate was 83.2% in the daratumumab group *vs* 60.1% in the control group. In a sub analysis, this extension of PFS in the experimental group went out to be independent from the number of previous lines of therapy and from the previous exposure to lenalidomide, even if the paucity of refractory patients to IMiDs enrolled in this trial may represent a bias. After a follow-up of 13.5 months, progression disease or death occurred in 53 patients in the daratumumab group *vs* 116 patients in the control arm, with a hazard ratio of 0.37 in favour of the first group. Also in this case, an improvement of deepness of molecular response was observed with continuation of therapy with the monoclonal antibody and it translated in a longer survival. Indeed, 22.4% of patients in the experimental group had results below the threshold for minimal residual disease (MRD), compared to 4.6% in the control group. Neutropenia, diarrhea and infusional reactions were the main adverse events reported in the experimental arm with a higher incidence than in the control group but, in spite of that, the rate of grade 3 and grade 4 infections was not so different. In conclusion, POLLUX trial confirmed the efficacy and safety of adding daratumumab to a regimen with IMiDs and high-dose steroid. Furthermore, the excellent results below the threshold for minimal residual disease suggest that minimal residual disease negativity could represent a goal also for RRMM patients.

3.1.2 Daratumumab in relapsed/refractory multiple myeloma in combination therapies: with PIs

Some *in-vitro* studies have shown that not only IMiDs but also PIs interact with daratumumab in a synergic way, strengthening its effect. An assay performed by the Dutch group [14] evaluated the rate of lysis in samples of bone marrow mononuclear cells from 16 multiple myeloma patients incubated with medium containing either daratumumab, lenalidomide and bortezomib or just one drug. The rate of lysis went out to be higher in the samples with the addition of daratumumab, showing that not only lenalidomide but also bortezomib enhance the effect of this monoclonal antibody by sensitizing the cells to the antibody-mediated lysis. The "lysis effect" was even better in cells from patients who previously showed refractoriness to IMiDs or IPs, suggesting that immunomodulatory effects of daratumumab may restore host susceptibility to anti-myeloma agents. Based on a phase 1b trial in which daratumumab showed encouraging results in combination with PIs-based regimens in naive patients [17], a phase 3 trial randomized patients with relapsed and/or refractory multiple myeloma to a treatment with only bortezomib and dexamethasone or with the addition of daratumumab [18]. Of 498 patients, 251 were assigned to the daratumumab group and 247 to the control group. Each cycle had a duration of 21 days. Daratumumab was administered at the usual dosage of 16 mg/kg once per week during cycles 1 to 3, once every 3 weeks during cycles 4 to 8 and once every 4 weeks thereafter until toxicity or progression disease. Dexamethasone was given for a total dose of 160 mg per cycle and bortezomib was administered in the subcutaneous formulation at the dosage of 1.3 mg per square meter on days 1, 4, 8 and 11 of cycles 1 to 8. The 12-month rate of PFS was 60.7% in the experimental group and 26.9% in the control group. After a follow up of 7.4 months, progression disease or death occurred in 67 patients in the daratumumab group *vs* 122 in the control group. Given the results of the interim analysis, the trial was unblended

earlier and patients in the control group with a progression disease were offered daratumumab monotherapy. This may represent a bias in the interpretation of all the long-term results. Nevertheless, this trial showed how daratumumab could give an advantage also in combination with PIs-based regimens. The recorded responses are deep and durable. The main adverse events reported in the daratumumab group were thrombocytopenia and infusion-related reactions but none of them led to a treatment discontinuation higher than in the control group.

3.1.3 Daratumumab in relapsed/refractory multiple myeloma in combination therapies: the experience from the Multiple Myeloma GIMEMA Lazio Group

Fazio et al. performed a multicentre retrospective analysis of patients with relapsed/refractory multiple myeloma treated with IMiDs or IPs-based regimens containing daratumumab in the hospitals of the GIMEMA (Gruppo Italiano Malattie Ematologiche dell'Adulto) network in the Italian region of Lazio [19]. Of 188 patients, sixty-five performed at least one cycle of therapy and were evaluable for hematologic response. The ORR was 81.97%; with four patients (6.56%) achieving a stringent complete response (sCR), 20 (32.79%) patients a complete response (CR), 5 (8.2%) patients a non-complete response (NCR), 13 (21.31%) patients a very good partial response (VGPR) and 8 (13.11%) patients a partial response (PR). After a median follow-up of 8.8 (range 0.23–22.3) months, 50 (42.37%) patients were alive maintaining response, eight (13.11%) patients presented a progression disease and one (1.64%) patients died. The overall survival and progression-free survival were 86.3% (95% CI, 79.2–94) and 70.8% (95% CI, 61.2–82), respectively. The most common grade 3 or 4 hematologic treatment-emergent adverse events (TAEs) included neutropenia, anemia and thrombocytopenia. The most common non-hematologic TAEs, of any grade, were infections, peripheral sensory neuropathy (7.6%) and fatigue (7.6%). Among the cases of infection, 17 (26%) patients presented pneumonia, eight (12%) patients FUO and five (7.7%) patients viral reactivation. Our preliminary results confirm data from POLLUX and CASTOR trial, suggesting that treatment with daratumumab in combination with lenalidomide or bortezomib plus dexamethasone is a highly effective and well-tolerated regimen to be considered for multiple myeloma patients after first relapse.

3.1.4 Daratumumab in relapsed/refractory multiple myeloma in combination therapies: with other novel agents

In the setting of heavily pretreated myeloma patients, daratumumab has shown good results also in association with novel drugs belonging to the last generations of IMiDs and IPs. Both combination of daratumumab with pomalidomide and dexamethasone and with carfilzomib and dexamethasone allowed to obtain deep and durable responses with a tolerable toxicity profile [20, 21]. Therefore, it seems reasonable to use daratumumab in combination with triplets or quadruplets in RRMM to obtain the best response.

3.2 Daratumumab in relapsed/refractory multiple myeloma in combination therapy: after or before allogenic hematopoietic cell-transplantation for young patients?

Despite improvements in the MM outcome and in the depth and response duration following subsequent lines of therapy, MM remains an incurable disease. It is reasonable to consider allogenic (allo) hematopoietic cell transplantation (HCT) as a treatment strategy for young patients with high-risk disease and an available

donor. Allo-HCT is potentially effective by virtue of a graft-versus-myeloma (GvM) effect but currently, there is little available data regarding this treatment [22]. Given the action of daratumumab on the microenvironment, it could be used both to control the graft-versus-host disease and to improve the GvM effect. In the review by Nikolaenko et al., 34 patients treated with daratumumab after aploidentical HCT were evaluated [23]. The ORR after the treatment with the monoclonal antibody was 41%, only five cases of acute GVHD were reported and no cases of chronic GVHD, showing the efficacy of this strategy on a population of high-risk heavily pretreated patients. Based on this little data, we may speculate that the modification of microenvironment induced by daratumumab could be used to "plow the land" for the transplant. To our knowledge, none is known about the use of anti-CD38 as a bridge to the transplant. We recently reported the case of a young patients with relapsed myeloma after the standard induction therapy and a tandem ASCT who underwent 11 cycles of rescue therapy with daratumumab in combination with lenalidomide and dexamethasone, followed by haploidentical transplant. Thanks to this treatment, he achieved a partial response and is now on consolidation with Daratumumab-Rd regimen [24].

4. Daratumumab in untreated newly diagnosed multiple myeloma

More recently, the use of daratumumab has been also explored in the setting of newly diagnosed multiple myeloma (NDMM) patients, showing encouraging results both in the population of transplant eligible patients and in that of transplant ineligible patients. The first results about daratumumab in NDMM patients proceed from a phase 1b study evaluating tolerability and safety of this monoclonal antibody in combination with myeloma backbone regimens: bortezomib-dexamethasone (VD), bortezomib-thalidomide-dexamethasone (VTD), bortezomib-melphalan-dexamethasone (VMP), pomalidomide-dexamethasone (PD) [25]. NDMM patients were included in all the arms except the PD one: in the VD and VTD arms the patients were enrolled irrespective of the transplant eligibility, while all patients in the VMP arm were transplant ineligible. In all the four arms, daratumumab was well tolerated and safe (**Table 1b**).

4.1 Daratumumab in untreated newly diagnosed multiple myeloma: transplant ineligible patients

ALCYONE and MAIA are the two main trials which evaluated the efficacy of adding daratumumab in the standard treatment of untreated patients with multiple myeloma ineligible to transplant. ALCYONE enrolled 706 naive patients randomized to receive VMP alone or with daratumumab [26]. Each cycle had a duration of 42 days. In the control group, all the patients received up to nine cycles of subcutaneous bortezomib, administered at the dosage of 1.3 mg per square meter of body-surface area (twice weekly on weeks 1, 2, 4, and 5 of cycle 1 and once weekly on weeks 1, 2, 4, and 5 of cycles 2 through 9), oral melphalan (9 mg per square meter, once daily on days 1 through 4 of each cycle), and oral prednisone (60 mg per square meter, once daily on days 1 through 4 of each cycle). In the experimental group, intravenous daratumumab at the usual dose of 16 mg/kg was administered with oral or intravenous dexamethasone at a dose of 20 mg once weekly in cycle 1, every 3 weeks in cycles 2 through 9, and every 4 weeks thereafter until disease progression or toxicity. Dexamethasone at a dose of 20 mg was substituted for prednisone on day 1 of each cycle. At 12 months, the PFS was 86.7% in the daratumumab group *vs* 76.0% in the control group. At the clinical data cut-off,

an event of disease progression or death had occurred in 88 (25.1%) patients in the daratumumab group *vs* 143 (40.2%) patients in the control group, with a hazard-ratio of 0.50 in favour of the first group. The superiority was even confirmed in the older patients, in those with a poor performance status and worse stage. It seemed to be also independent from impairment of renal and liver function which were quite frequent in the enrolled population. In spite of this general advantage given adding daratumumab, a prespecified subgroup analysis of progression-free survival showed that the D-VMP combination is not so effective in the overcome of the bad prognosis given by the high-risk cytogenetics (defined by t (4;14), t (14;16), del17p). The main adverse effect was represented by infections of the respiratory tract but they were not a cause of discontinuation of treatment. MAIA compared Rd. to daratumumab-Rd [27]. The trial enrolled 737 naïve patients: in cycles of 28 days, all of them received oral lenalidomide 25 mg on days 1 through 21 and oral dexamethasone 40 mg per week, until disease progression or toxicity. In the experimental group, daratumumab was added at a dose of 16 mg/kg once weekly during cycles 1 and 2, every 2 weeks during cycles 3 through 6, and every 4 weeks thereafter. At the median follow-up of 28 months, PFS was not reached in the daratumumab group and was 31.9 months in the control group. Disease progression or death occurred in 97 patients in the experimental group *vs* 143 in the control group, with a hazard-ratio of 0.56. Also in this trial, the benefit was maintained in older patients with worse performance status but not in the patients with high-risk cytogenetics. Pneumonia was recorded as the most frequent side effect in the experimental group but it did not influence the general outcome. Based on the exciting results of ALCYONE and MAIA, several ongoing trials throughout the world aim to evaluate the benefit of adding both subcutaneous and intravenous daratumumab to the different combinations of drugs used for the induction of multiple myeloma in naïve unfit patients (NCT03993912, NCT03742297, NCT03652064, NCT03217812, NCT04052880, NCT04009109, NCT03695744, NCT02918331). Some of these are designed to study possibility of combining the monoclonal antibody with the newest generations of IMiDs and IPs: NCT4009109 is a phase II trial with two arms based on induction with lenalidomide, ixazomib, daratumumab and dexamethasone; maintenance in arm 1 is with the only lenalidomide, in the arm 2 it is with lenalidomide, ixazomib and daratumumab. Ixazomib is a last-generation IPs which recently received the approval to be used in combination with lenalidomide and steroid in RRMM. The interim analysis of this phase II trial showed an overall response rate (ORR) of 70%, with good molecular response [28].

4.2 Daratumumab in untreated newly diagnosed multiple myeloma: transplant eligible patients

The excellent results achieved in the population of unfit NDMM patients led to evaluate the efficacy of daratumumab also in the population of NDMM transplant eligible patients. CASSIOPEIA trial is the first largest study going in this direction: it enrolled 1085 patients across Europe, randomly assigned to the control arm with the use of VTD triplet or to the experimental arm adding daratumumab [29]. All patients received up to four 28-day, pre-transplant induction cycles and two 28-day, post-transplant consolidation cycles of subcutaneous bortezomib (administered according to the usual schedule), oral thalidomide (100 mg daily in all cycles), and oral or intravenous dexamethasone. Daratumumab was administered intravenously at a dose of 16 mg/kg of bodyweight once weekly in induction cycles 1 and 2 and once every 2 weeks during induction cycles 3 and 4 and consolidation. At 100 days post-transplant, the rate of sCR was higher in the daratumumab group than in the control group (29% *vs* 20%) and this superiority was maintained in older patients,

but not in patients with a higher stage disease and a higher risk cytogenetics. Also in this trial the main adverse events were represented by infections but none of them represented a cause of treatment discontinuation. Surprisingly, daratumumab went out to be associated with a reduction of the amount of collected stem cells CD34+ and the subsequent use of plerixafor, even if this aspect did not translate into a worse performance of the transplant. Recently, Voorhees et al. published the results of another study evaluating the use of daratumumab as first line in transplant eligible patients, the GRIFFIN trial [30]. In this phase II randomized trial, 207 enrolled patients received four 21-day induction cycles and two 21-day consolidation cycles of oral lenalidomide (25 mg daily on days 1–14), subcutaneous bortezomib (1.3 mg/m2 on days 1, 4, 8, and 11), and oral dexamethasone (VRD), followed by maintenance with lenalidomide until toxicity or progression disease. Patients in the experimental group received daratumumab (16 mg/kg) on days 1, 8, and 15 of cycles 1 through 4 and day 1 of consolidation cycles and of maintenance cycles. After the end of post-transplant consolidation, the primary end-point of sCR was achieved in 42 patients in the experimental group *vs* 31 patients in the control group. Also the secondary end-points of overall response rate and rate of VGPR or better resulted higher in the daratumumab group. These good results deepened over time. The observed benefit was maintained also in the older population but not again in patients with a higher disease stage and with high-risk cytogenetics. As usually observed, also in this trial the experimental arm recorded a high rate of not statistically significant infections. Several ongoing trials aim to evaluate the use of daratumumab as first-line in transplant eligible NDMM patients: among these, PERSEUS is a promising ongoing phase III trial evaluating efficacy of daratumumab plus VRD *vs* VRD in terms of PFS, utilizing subcutaneous daratumumab to minimize toxicity. There are also few ongoing trials evaluating induction with daratumumab irrespective of transplant eligibility and some of them are based on MRD-driven therapies (MASTER trial). The results of all these studies are awaited.

5. Daratumumab in other plasma cell neoplasms

Given the promising results in the treatment of multiple myeloma with daratumumab, its use is being investigating also in the treatment of other plasma cell neoplasms, especially immunoglobulin light chain (AL) amyloidosis and smouldering myeloma (SMM).

5.1 Daratumumab in amyloidosis

AL amyloidosis is due to the production of misfolded immunoglobulin light chain by an aberrant plasma-cells clone. This pathologic protein deposits in a variety of organs, usually heart and kidney, causing serious dysfunction. In spite of good results showed by treatment of this disease with PIs and IMiDs [31, 32], there is still a significant proportion of patients that do not respond to these agents. Based on a variety of reports showing safety and efficacy of daratumumab in patients with relapsed/refractory AL amyloidosis [33–36], some perspective trials have been recently conducted. NCT028441033 is a phase II study led at Boston Medical Center and aimed to evaluate safety and tolerability of daratumumab in a cohort of 25 participants with relapsed/refractory AL amyloidosis. The preliminary results were encouraging, with only infusion reactions being reported as main side effect [37]. The ORR is instead the primary outcome of a multi-center phase II study across France and Italy (NCT02816476): it enrolled 35 patients with AL amyloidosis not in VGPR or better after previous treatment. The preliminary results showed an ORR of 59% with 44% of patients achieving

at least a VGPR [38]. These good results are confirmed by a report with the collaboration of our group [39]: 59 patients out of 72 with relapsed/refractory AL amyloidosis achieved a hematologic response after eight infusions of daratumumab, single agent or combined with bortezomib and lenalidomide, and the quality of this response improved with the continuation of therapy. The demonstration of the efficacy of daratumumab in the treatment of AL amyloidosis provided the rationale for exploring its use earlier in the disease course. Hossein Taghizadeh MA et al. presented the case of two patients with advanced cardiac involvement who achieved a normalization of light chain levels within one cycle of therapy with the anti-CD38, without any serious adverse events in spite of the cardiac dysfunction [40]. A phase III trial comparing cyclophosphamide, bortezomib and dexamethasone with or without daratumumab in the first-line treatment of AL amyloidosis has recently completed the enrolment and the results are awaited (NCT03201965).

5.2 Daratumumab in SMM

Smouldering myeloma is defined by a medullar infiltration of clonal plasmacells ≥10% in the absence of symptoms. According to the Mayo Clinic criteria, M-protein >2 g/dl, medullar infiltration ≥20% and free-light chain ratio > 20 define risk categories. Patients with one, two and three of these criteria are considered to be at low, intermediate and high risk with 5-year progression of 23% in the low risk, 47% in the intermediate risk and 82% in the high risk [41]. However, in spite of the important risk of transformation into symptomatic disease, current guidelines recommend "watch and wait" even in people with high and intermediate risk smouldering myeloma. Since the earlier intervention may delay progression, different studies are evaluating the use of new drugs in this subset of patients. Daratumumab could be the perfect drug, given the efficacy and the tolerability showed in other subsets. Based on the good results of the CENTAURUS trial, a phase II study for patients with intermediate and high risk smouldering multiple myeloma, randomly assigned, in a 1:1:1 ratio, to receive one of three different schedules of daratumumab [42], a phase III trial has been designed (NCT03301220). In this study, patients with high-risk smouldering myeloma are randomized either to receive subcutaneous daratumumab or to be just monitored. Daratumumab is administered according to the usual schedule, until 39 cycles or up to 36 months or until confirmed disease progression or unacceptable toxicity. This study recently completed the enrolment and the results are still awaited but all the most recent findings suggest that the anti-CD38 could be used with safety and efficacy also in smouldering myeloma.

6. The dark side of daratumumab: adverse events

All pivotal studies leading to approval of daratumumab for the treatment of relapsed-refractory or newly diagnosed multiple myeloma showed a slight major susceptibility to infections in the studied populations. This risk seems to be due to the neutropenia and to the impairment of cellular immunity which is a direct consequence of targeting CD38 [43]. In the study by Nahi et al., nine patients out of 23 treated with daratumumab had viral and/or bacterial complications, mainly involving the respiratory tract. In these patients, assessment of circulating lymphocytes indicated a selective depletion of NK cells and viral reactivation after Daratumumab treatment. This finding is in line with data emerging from all the trials using anti-CD38-based regimens and suggest the necessity of screening for cytomegalovirus, Epstein–Barr virus and viral hepatitis before starting the treatment, therefore an adequate antiviral and antibacterial prophylaxis in the treated

population. In the consensus document by ESCMID Study Group for Infections in Compromised Hosts (ESGICH), based on the pooled analysis of the two trials GEN501 an SIRIUS, daratumumab is associated also with an increased risk of varicella-zoster virus (VZV) infections, especially in the presence of combination therapy with protease inhibitors and/or corticosteroids [44]. Anti-herpesvirus prophylaxis with (val)acyclovir should be administered to VZV-seropositive patients at least 1 week before starting daratumumab therapy and for at least 12 weeks after its discontinuation. The consensus document also recommends seasonal-influenza vaccination. In the review of the drug conducted under the EMA's accelerated assessment program for drugs that are of major interest for public health, also thrombocytopenia and anemia are reported as the most common side effects, besides neutropenia [45]. In this same report, half of all patients experienced infusion-related reactions, mainly occurring at the first infusion. These reactions usually presented with nasal congestion, cough, throat irritation, chills, vomiting and nausea. Serious adverse reactions with bronchospasm, dyspnea, laryngeal edema, pulmonary edema and hypoxia have been also reported but in a few cases. Based on this phenomenon, EMA gave indication to premedicate every infusion with antihistamines, antipyretics and corticosteroids. Furthermore, oral corticorsteroids should be taken by all patients on the first and second day after all infusions. Patients on therapy with Daratumumab may present with positive indirect and direct Coombs test, due to the CD38 expression also on the red blood cells. This interference could complicate the safe provision of blood products to people on treatment with this drug. Chapuy et al. demonstrated that this "laboratory side effect" might be solved by incubating red blood cells with dithiothreitol (DTT) or trypsin [46]. These reagents remove the CD38 on the surface of red blood cells, easing routine compatibility testing. Evaluation of disease response in patients with multiple myeloma on treatment with daratumumab could also be complicated by this antibody. Given its proteic nature (IgG1), the drug can be confused with the endogenous monoclonal component during the interpretation of serum immunofixation electrophoresis (IFE). McCudden et al. proposed a daratumumab-specific immunofixation electrophoresis reflex assay (DIRA) using a mouse anti-daratumumab antibody in order to discriminate between endogenous myeloma protein and daratumumab [47]. Both Castor and Pollux trials showed a slight increase of rates of secondary primary cancers in the experimental arms, within 6 months after the initiation of trials [16, 18]. Most of the cases were non-melanocytes related cutaneous tumours and occurred in patients already treated with IMiDs and alkylating agents. Further studies and longer follow-up are needed to clarify the potential carcinogenicity of Daratumumab. Another concern, regarding the use of daratumumab, is due to the expression of CD38 on the surface of CD34+ hematopoietic progenitor cells. This could theoretically translate into a delay in stem cells collection for eligible patients to ASCT on treatment with the monoclonal antibody. Xun Ma et al. conducted an assay in which specimens of mobilized peripheral blood CD34 + cells from myeloma patients were evaluated to determine percentage of CD38 expression and later incubated with daratumumab and complement-rich human serum. First, CD38 is minimally expressed on CD34+ cells, compared to the control cell lines used. Furthermore, CDC did not occur, showing that, in vitro, daratumumab is not toxic to mobilized CD34 + progenitor cells from myeloma patients [48].

7. Conclusions

Daratumumab has showed proven efficacy and tolerability both in patients with RRMM and with NDMM, as confirmed in all the studies conducted during the last

years. A deep and durable response with easy-to-control side effects was obtained using this monoclonal antibody. The revolutionary power of this new drug could be also extended to patients with other plasma cell neoplasms, such as AL amyloidosis and SMM. Given the specific mechanisms of action of daratumumab targeting both clonal plasma-cells and bone-niche microenvironment, further studies are warranted to better understand the correct timing to introduce this monoclonal antibody in the context of a sequential therapy. On a side, the immune-mediated plasma-cell killing, induced by daratumumab in the early phase of treatment, acts as a debulking for the disease; on the other side, the restoration of the immune system may boost other metabolic effects of the monoclonal antibody, in a later phase of therapy, when the control of the disease is better [49]. Based on these hypothesis, the retreatment with daratumumab after a wash-out period may seem reasonable. Therefore, the anti-CD38 is a revolutionary weapon: understanding the best moment to use it in the battle against multiple myeloma is the great challenge of the future.

Conflict of interests

GL and FF have nothing to declare. MTP served as a consultant or on an advisory board for and received honoraria from Janssen-Cilag, Celgene, Bristol-Myers Squibb,Amgen, Takeda and Sanofi.

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