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LETTER TO THE EDITOR

The therapeutic human CD38 antibody daratumumab improves the anti-myeloma effect of newly emerging multi-drug therapies

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Multiple myeloma (MM) is an incurable malignancy of antibody-producing clonal plasma cells. The mean life expectancy of patients has remained at <3 years during the past few decades. The introduction of a new generation drug regimens including an immunomodulatory agent such as lenalidomide (LEN) or a proteasome inhibitor such as bortezomib (BORT) used alone or in combination with classical anti-MM drugs melphalan (MEL), dexamethasone (DEX) or prednisone (PRED) has significantly improved the overall survival of MM patients.[1,2] All treatment strategies, including multidrug regimens, however, are eventually hampered by the development of drug resistance.[3] Targeted immunotherapy, based on human antibodies against relevant tumor antigens has shown to be a feasible and highly promising approach in hematological malignancies that can be effectively combined with chemotherapy to further increase the potency of anti-tumor effects. For instance in several B-cell malignancies, clinically approved human antibodies against CD20 are now being successfully combined with fludarabine, cyclophosphamide or LEN.[4,5] Building on this knowledge and to achieve a similar goal in the MM setting, we recently generated daratumumab (DARA), a human CD38 antibody with broad-spectrum killing activity.[6] We have shown that DARA mediates strong lysis of MM cells via CDC (complement-dependent cytotoxicity) as well as ADCC (antibody-dependent cellular cytotoxicity), although the potency of autologous ADCC was donor-dependent. In our initial work to combine DARA with novel chemotherapeutics, we have demonstrated that DARA-mediated cellular lysis of MM cells is significantly improved by LEN, mainly because of the potent capacity of LEN to activate the effector cells of ADCC.[7] Current clinical practice, however, shows that the future of successful MM treatment lies in the use of drug combination regimens. It appears essential to identify regimens in which individual components synergize to obtain the greatest achievable effects. Therefore, we now explored the potential clinical benefit of combining targeted DARA therapy with newly emerging multi-drug chemotherapy regimens. To this end, we used a recently developed *ex vivo* flow cytometry-based assay platform,[7] which enables us to enumerate and subsequently deduce the drug/antibody-mediated lysis of primary $CD138^+$ MM cells directly in bone marrow samples from the MM patients. The assays are performed with bone marrow mononuclear cells (BM-MNC), thus without the need for separating malignant cells from autologous effector cells and tumor-supporting accessory cells, such as stromal cells. With this *ex vivo* assay system, we first addressed the benefits of combining DARA with both LEN and BORT, since not only LEN but also BORT may enhance the therapeutic efficacy of DARA by sensitizing tumor cells for antibody-mediated lysis. In a series of experiments, we incubated BM-MNC from 16 MM patients, containing 2–20% malignant plasma cells, either with medium alone or with combinations of LEN, BORT and DARA at carefully selected individual concentrations inducing half-maximal lysis of MM cells. An antibody against an irrelevant antigen (Keyhole Limpet Hemocyanin (KLH)) was used as an isotype control. After 48 h, we harvested the cells, labeled them with a monoclonal CD138 antibody and enumerated the surviving $CD138^+$ MM cells using single-platform flow cytometry, to assess the percentage of MM cell lysis in each sample (Figure 1a) relative to that obtained with the control antibody KLH, which induced negligible MM cell lysis (data not shown). LEN and BORT alone or in combination caused low to moderate lysis of MM cells (mean lysis 10%, 18% and 25%, respectively). Addition of DARA significantly increased the MM cell lysis by more than twofold in all combinations ($P=0.001$). The highest MM cell lysis was observed with the triple LEN-BORT-DARA combination. Notably, combination with DARA seemed to improve MM cell lysis especially in the samples that poorly responded to LEN and BORT (MM cell lysis <30%; Figure 1a). To evaluate this, we analyzed the data of LEN/BORT high and low responders separately (Figures 1b and c). A significant improvement of MM cell lysis by DARA was observed in the LEN/BORT low-responder subset particularly (Figure 1c) and the effect was synergistic. This group included cells from five patients who had been treated in the clinic with LEN and/or BORT without success (Figure 1d).

Interestingly, the synergy between DARA and LEN/BORT treatment was also apparent for cells from the five LEN/BORT-resistant patients (Figure 1d, as illustrated by the fact that observed levels of MM cell lysis with DARA/LEN/BORT treatment were significantly higher than the expected levels of MM cell lysis, calculated on the assumption that there was no treatment interaction). Although we have only been able to evaluate a small number of samples from resistant patients to date, this remarkable synergy suggests the maintenance of anti-tumorigenic properties of LEN and BORT, despite the development of drug resistance. Taken together, these results indicate the potential clinical benefits of combining DARA with these two novel anti-MM agents and warrant further investigation even in patients who are low responders or have become resistant to the latter drugs.

After showing the potential benefits of combining DARA with LEN and BORT, our further investigation focused on two recently introduced and so far the most successful first-line combination therapies based on these two novel agents, namely the triple combinations of LEN, BORT, DEX, abbreviated as RVD, and of MEL, PRED, BORT, abbreviated as MPV. To assess the impact of combining DARA with these combination chemotherapies, we prepared cocktails of these agents, by mixing them at concentrations causing ±30% of the maximal lytic effect on various MM cell lines (data not shown). We then incubated BM-MNC of MM patients with serial dilutions of these cocktails alone or in the presence of DARA and assessed MM cell lysis. As expected, cocktails of RDV (Figure 2a) as well as MPV (Figure 2b) induced dose-dependent lysis of MM cells. Addition of DARA to both RDV and MPV significantly increased the treatment efficacy by almost doubling the lysis levels

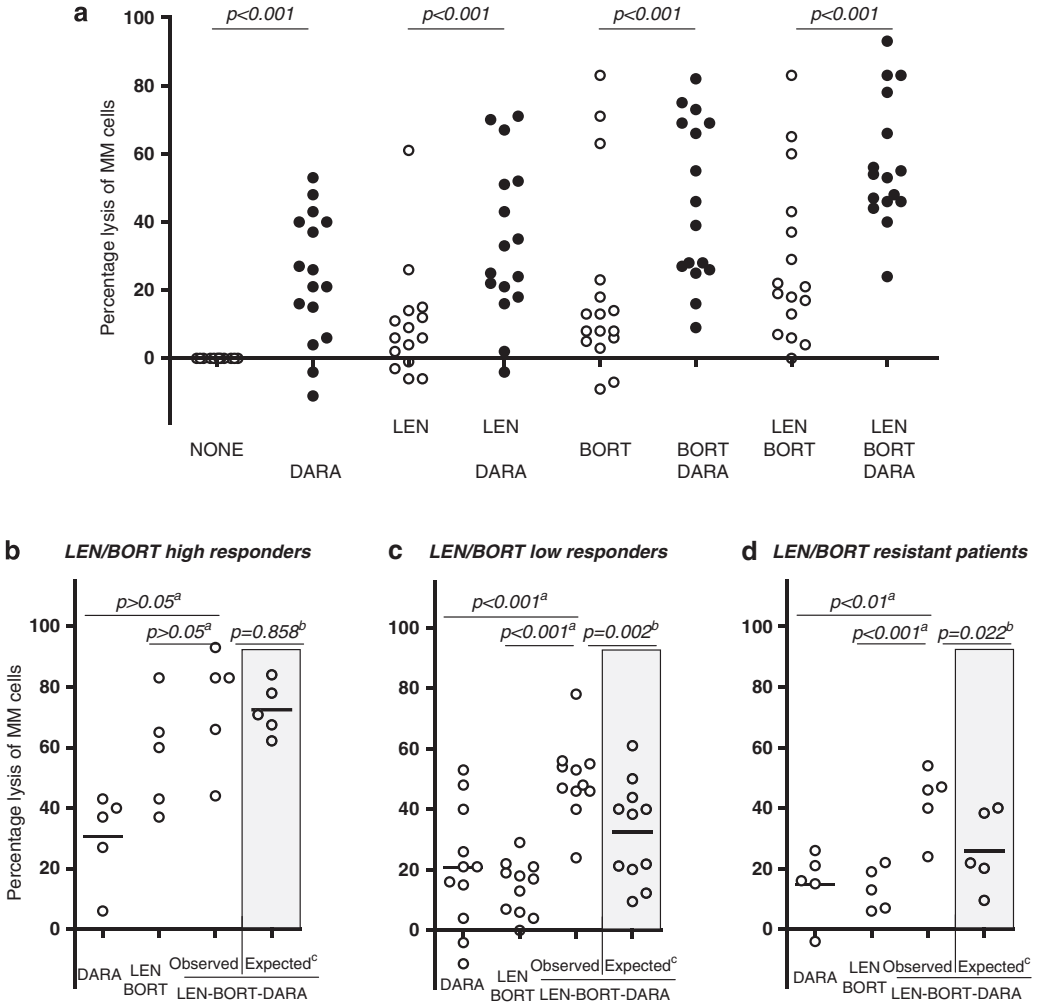

Figure 1 Addition of DARA to BORT-LEN significantly increases the MM cell lysis in BM-MNC of MM patients, particularly in LEN/BORT low responder or refractory patients. (**a**) BM-MNC from MM patients ($n=16$), containing 2–20% $CD138^+$ MM cells as detected by flow cytometry were incubated with LEN (3 μM), BORT (3 nM) and DARA (10 μg/ml) alone or in combination for 48 h in RPMI + 10% fetal bovine serum in 96-well u bottom plates in fully humidified incubators at 37 °C, 5% CO_2-air mixture. Surviving MM cells were enumerated by the single-platform fluorescence-activated cell sorting analysis of $CD138^+$ cells in the presence of Tru-Count beads (Beckman Coulter, Miami, FL, USA). Percentage lysis of MM cells in LEN, DARA and LEN + DARA-treated conditions were calculated using the MM survival of wells treated with the control KLH antibody alone. Differences between indicated groups were tested for significance in repeated measures analysis of variance (ANOVA), using Bonferonni's *post-hoc* multiple comparison tests with two-tailed 95% confidence intervals. In **b**–**d**, data are analyzed for low LEN/BORT responders, high LEN/BORT responders and LEN/BORT refractory patients, respectively. [a]*P*-values were calculated using a repeated measures ANOVA. [b]*P*-values were calculated by a paired *t*-test. [c]Expected values were calculated to test the null hypothesis that there is no synergism between DARA and LEN/BORT using the following formula: % expected lysis = 100−%survival after DARA × %survival after LEN/BORT.

especially at lower doses of the cocktails. These results illustrate that targeted immunotherapy of MM by DARA holds a significant potential to improve the clinical outcome of currently available novel combination therapies.

Recent studies have indicated that combination of multiple drugs are superior over single- or double-agent combinations.[2] Addition of new drugs to the available regimens can mediate their clinical benefit because of the induction of a higher rate of initial complete responses, which in turn improves the relapse free and overall survival.[8] Contingent on the premise that the combined agents have non-overlapping and synergistic mechanisms of actions, immediate and effective targeting of tumors with multiple agents appears a successful strategy to improve the clinical outcome of MM therapy. Indeed, such a strategy is in full agreement with the emerging concept that the genetic signature of MM, and consequently, the individual patient's susceptibility to a specific agent will be highly heterogeneous and this eventually may lead to drug resistance. Nevertheless, the complete response rate of the best chemotherapeutic combinations is currently $<50\%$, and all current combination therapies eventually induce drug resistance.[2,9] In this respect, DARA, with its immediate and effective cell-mediated cytotoxic effects against MM cells, and the observed remarkable synergy with LEN/BORT even in LEN/BORT refractory patients, may potentially improve the achievement of first CR in MM when combined with these agents either alone or in multidrug chemotherapy regimens. In conclusion, our study, in which we demonstrate the potential benefits of combining DARA-mediated targeted therapy with newly emerging chemotherapy options, warrants the evaluation of this approach in MM in clinical phase I/II trials.

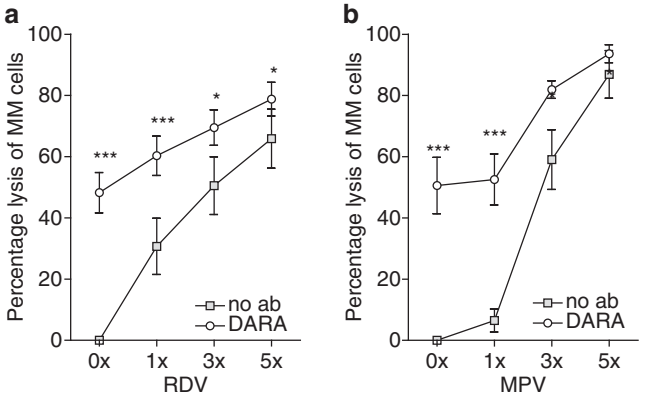

Figure 2 DARA increases response against MM in triple chemotherapy treatments. BM-MNC form patients ($n=7$) were incubated with increasing dilutions of a cocktail of (**a**) LEN, BORT and DEX (1 × dilution contains: 1 μM LEN, 1 nM BORT and 1 μM DEX) or with a cocktail of (**b**) MEL, PRED, BORT (1 × dilution contains 1 μM MEL, 1 μM PRED and 1 nM BORT) in the presence or absence of DARA (10 μg/ml) for 48 h. Surviving MM cells were enumerated by fluorescence-activated cell sorting analysis of CD138+ cells. *P*-values were calculated by a paired *t*-test. $^{*}P<0.05$, $^{***}P<0.001$.

Conflict of interest

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MS van der Veer[1], M de Weers[2], B van Kessel[1], JM Bakker[2], S Wittebol[3], PWHI Parren[2], HM Lokhorst[4] and T Mutis[1]
[1]Department of Clinical Chemistry and Hematology, University Medical Center Utrecht, Utrecht, Netherlands; [2]Genmab, Utrecht, Netherlands; [3]Department of Internal Medicine, Meander Medical Center, Amersfoort, Netherlands and [4]Department of Hematology, University Medical Center Utrecht, Utrecht, Netherlands
E-mail: t.mutis@umcutrecht.nl

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