Efficacy and Safety of Daratumumab Combined With All-Trans Retinoic Acid in Relapsed/Refractory Multiple Myeloma

Supplemental methods

Study population

In addition to the inclusion criteria for part A ~~and part B~~ listed in materials and methods, patients were required to have a World Health Organization (WHO) performance status of 0-2, an adequate bone marrow reserve defined by platelet count ≥75 x 10^9/L and absolute neutrophil count ≥1.0 x 10^9/L, a creatinine clearance ≥20 ml/min and serum hepatic aminotransferases and bilirubin levels <3-fold the upper limit of normal (ULN) ~~were required~~. Patients had to agree to use contraception in this trial. Exclusion criteria included clinically relevant active comorbid medical (e.g. significant pulmonary or cardiac disease) or psychiatric conditions, history of malignancy within the last 5 years (except squamous cell and basal cell carcinomas of the skin and carcinoma in situ of the cervix, or malignancy that is considered cured with minimal risk of recurrence within 5 years), or plasma cell dyscrasias other than MM.

Supportive care

All patients received prophylaxis for infusion-related reactions, which consisted of 20 mg dexamethasone, 1000 mg acetaminophen and 2 mg clemastine, administered 1 hour before start of the daratumumab infusion. Montelukast, a leukotriene antagonist, was given prior to the first 2 infusions. Patients ≤75 years also received 20 mg dexamethasone as post-infusion prophylaxis. Furthermore, infection prophylaxis with cotrimoxazol (480 mg once daily, or equivalent) and valaciclovir (500mg twice daily) was recommended.

Dose limiting toxicity assessment

Part B of the phase 1 study used a 3+3 dose escalation approach with each cohort starting with 3 patients. If no dose-limiting toxicity (DLT) occurred during the first treatment cycle in three evaluable patients, enrollment commenced at the next higher dose level to a maximum planned dose of 45 mg/m^2. If one of three patients experienced a DLT, the cohort was expanded to six evaluable patients. If no additional DLT occurred, patient enrollment to the next dose level proceeded. If a second patient in a cohort had a DLT, study treatment in that

cohort was to be stopped. In the highest predefined dose level of ATRA (45 mg/m^2), six patients had to be evaluated for DLT. DLT evaluation was performed according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.03[1]. A DLT was defined as any grade 3 or 4 non-hematologic toxicity, except for inadequately treated nausea or vomiting; or death whatever the cause, except death due to myeloma; any of which had to be unrelated to tumor progression and must occur before day 28 of cycle 1 in part B of the study.

Dose modification

Daratumumab administration was withheld if patients experienced grade 4 hematological toxicity (platelet count <25 x 10^9/L or neutrophil count <0.5 x 10^9/L), grade 3 thrombocytopenia in combination with bleeding, or any grade 3 non-hematological toxicity, with the exception of grade 3 nausea or grade 3 vomiting that responded to antiemetic treatment, grade 3 diarrhea that responded to antidiarrheal treatment, or grade 3 fatigue or asthenia that lasted for <7 days after the last administration of daratumumab. Daratumumab treatment was withheld until the toxicity had resolved to grade ≤2. Dose modifications of daratumumab were not permitted. If daratumumab administration was delayed >3 days during weekly infusions, >7 days during biweekly infusions or >21 days during 4-weekly infusions, that dose would be considered a missed dose, and administration would be resumed at the next planned dosing date. A missed dose of daratumumab was not made up. If a patient experienced a DLT during cycle 1 in dose level 1 in part B, the patient would be taken off treatment. In dose levels 2 or 3, dose reduction of ATRA by one level for further treatment cycles was permitted. In phase 2, dose reductions of ATRA would be performed according to schedule (Table S2). For ATRA-related grade 3 or 4 toxicities, ATRA treatment should be interrupted and resumed at the next lower dose level once the toxicity had resolved to grade ≤2.

Immune monitoring

Peripheral blood samples, from patients treated in both phase 1 and phase 2 of the study, were obtained at baseline prior to initiation of daratumumab treatment (part A, C1D1), after 1 cycle of daratumumab monotherapy (part A, C2D1), before initiation of ATRA treatment in part B (part B, C1D1), before re-initiation of daratumumab treatment in part B (part B, C1D3),

after 1 cycle of daratumumab and ATRA treatment (part B, C2D3) and at the time of disease progression (part B, EOT).

Peripheral blood mononuclear cells (PBMNCs) were isolated by Ficoll-Hypaque density-gradient centrifugation, and analyzed within 24 hours after the sample was obtained. In these samples, we assessed frequency of immune cell subsets by staining 1 x 10^6 nucleated cells with CD45 KO (Beckman Coulter) to assess lymphocyte subsets, CD14 PerCP for monocytes, CD19 APC-H7 for B-cells, CD3 V450 (all BD Biosciences) for T-cells, CD56 PC7 (Beckman Coulter) for natural killer (NK)-cells and CD16 PE (BD Biosciences) for activated NK-cells. To assess CD38 expression on immune cell subsets, we used HuMax-003 FITC (Janssen Pharmaceuticals), which binds to a CD38 epitope distinct from the epitope recognized by daratumumab[2].

Flow cytometry was performed using a 7-laser BD FACSCelesta (Becton Dickinson). Fluorescent labeled beads (CS&T beads, Becton Dickinson) were used daily to monitor the performance of the flow cytometer and verify optical path and stream flow. This procedure enables controlled standardized results and allows the determination of long-term drifts and incidental changes within the flow cytometer. No changes were observed which affected the results. Compensation beads were used to determine spectral overlap, compensation was automatically calculated using Diva software. Flow cytometry data were analyzed using FCS express software version 6 (DeNovo software).

References

1. NCI. Common Terminology Criteria for Adverse Events (CTCAE) Version 4.03. Updated 14-06-2010. 2018. https://www.eortc.be/services/doc/ctc/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf

2. Krejcik J, Frerichs KA, Nijhof IS, et al. Monocytes and Granulocytes Reduce CD38 Expression Levels on Myeloma Cells in Patients Treated with Daratumumab. *Clin Cancer Res*. Dec 15 2017;23(24):7498-7511. doi:10.1158/1078-0432.CCR-17-2027

Supplemental Tables

Supplemental Table 1. Daratumumab and ATRA treatment schedule for part B phase 1 and 2

Agent	Dose per day	Route	Days
Daratumumab	16 mg/kg	Intravenous	Cycles 1 and 2: Days 3, 10, 17 and 24 (QW) Cycles 3 to 6: Days 3 and 17 (Q2W) Cycles 7 and up: Day 3 (Q4W)
ATRA	15, 30 or 45 mg/m^2; divided in two doses*	Oral	Cycles 1 and 2: Days 1, 2, 3; 8, 9, 10; 15, 16, 17 and 22, 23, 24 Cycles 3 to 6: days 1, 2, 3 and 15, 16, 17 Cycles 7 and up: Days 1, 2 and 3

Cycles will be given every 28 days. The next cycle will start at day 29 of the previous cycle.
*During phase 2 ATRA will be administered at the MTD, or if no MTD is reached, at the dose of 45mg/m^2/day, divided in two doses.

Supplemental Table 2. Dose reduction levels for ATRA in part B phase 2

Dose reduction levels	**ATRA (mg/m2/day)**
Dose level 3	45 mg/m^2/day, divided in 2 doses
Dose reduction level 2	30 mg/m^2/day, divided in 2 doses
Dose reduction level 1	15 mg/m^2/day, divided in 2 doses
Dose reduction level -1*	10 mg/m^2/day, divided in 2 doses
Dose reduction level -2*	5 mg/m^2/day, divided in 2 doses

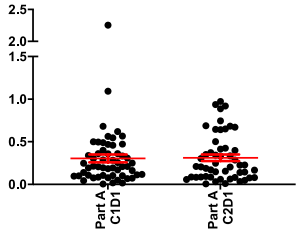
*ATRA doses are reduced according to the dose levels in part B phase 1. If further dose reductions are required, ATRA will be reduced to 10 mg/m^2/day (dose reduction level -1) or 5 mg/m^2/day (dose reduction level -2).

Supplemental Table 3. Adverse events during part B phase 1

	Part B dose level 1 n = 3		Part B dose level 2 n = 3		Part B dose level 3 n = 8	
Events	**Grade 2** n (%)	**Grade 3-4** n (%)	**Grade 2** n (%)	**Grade 3-4** n (%)	**Grade 2** n (%)	**Grade 3-4** n (%)
Infusion related reaction	-	-	-	-	-	-
Hematologic						
- Anemia	-	3 (100)†	1 (33)†	-	2 (25)†	2 (25)†
- Thrombocytopenia	-	2 (67)‡	-	-	-	2 (25)‡
- Neutropenia	1 (33)§	-	1 (33)§	-	-	1 (13)§
Infections						
- Upper respiratory tract	1 (33)	-	1 (33)	-	1 (13)	-
- Pneumonia	-	1 (33)	-	-	-	2 (25)
- Skin infection	-	-	-	-	1 (13)	-
Cardiac disorders						
- Atrial fibrillation	1 (33)	-	-	-	-	-
Ear and labyrinth disorders						
- Vertigo	-	-	-	-	-	1 (13)
Gastrointestinal disorders						
- Pyrosis	-	-	1 (33)	-	-	-
General and admission site disorders						
- Edema limbs	-	-	-	-	1 (13)	-
- Fatigue	-	-	-	-	1 (13)	-
- Fever	-	-	-	-	1 (13)	-
- Non-cardiac chest pain	-	-	-	-	-	2 (25)
Investigations						
- ASAT increased	-	-	-	-	1 (13)	-
- AF increased	-	-	-	-	-	1 (13)
- yGT increased	-	-	-	-	-	1 (13)
Musculoskeletal and connective tissue disorders						
- Myalgia	1 (33)	-	-	-	-	-
Nervous system disorders						
- Headache	*	-	1 (33)	-	-	2 (25)
- Neuralgia	-	-	1 (33)	-	-	-
- Peripheral sensory neuropathy	-	-	-	-	-	1 (13)
Psychiatric disorders						
- Delirium	-	-	-	-	1 (13)	-
Renal and urinary disorders						
- Acute kidney injury	-	1 (33)	-	-	-	1 (13)
- Chronic kidney injury	-	1 (33)	-	-	-	-
Respiratory, thoracic and mediastinal disorders						
- Dyspnea	-	-	-	-	1 (13)	-
Thromboembolic event	-	-	-	1 (33)	-	-

*Dose level 1, headache CTC 1 (n=1);
†Erythrocyte transfusions were administered to 1 patient (33%) treated at dose level 1, to 1 patient (33%) treated at dose level 2, and to 3 patients (38%) treated at dose level 3;
‡Thrombocyte transfusions were administered to 1 patient (33%) treated at dose level 1, and to 2 patients treated at dose level 3, while no patients treated at dose level 2 required thrombocyte transfusions;
§G-CSF was administered to 2 patients treated at dose level 3, no patients treated at dose level 1 or 2 required G-CSF.

Supplemental figures



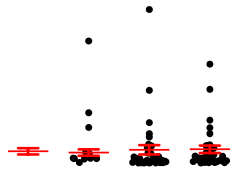


Figure S1. Absolute numbers of immune cell subsets in peripheral blood during treatment with daratumumab monotherapy followed by daratumumab with ATRA. (A) Absolute numbers of monocytes, B-cells, T-cells, NK-cells and activated NK-cells ($CD16^{+}$) in sequential peripheral blood samples obtained before initiation of daratumumab monotherapy (C1D1 of part A; n=55), and after 1 cycle of daratumumab monotherapy (C2D1 of part A; n=51). (B) Absolute numbers of these immune cell subsets in sequential peripheral blood samples obtained before start of ATRA treatment (C1D1 of part B; n=51), before first dose of daratumumab after initiation of ATRA (C1D3 of part B; n=42), after 1 cycle of daratumumab and ATRA treatment (C2D3 of part B; n=36) and at disease progression during daratumumab and ATRA treatment (EOT, part B; n=33). Dots represent individual cell numbers, error bars represent mean and SEM. Differences between indicated groups were calculated using Wilcoxon matched-pairs rank test.

NK, natural killer; PB, peripheral blood; ATRA, all-trans retinoic acid; SEM, standard error of mean; *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001; ns, not significant.

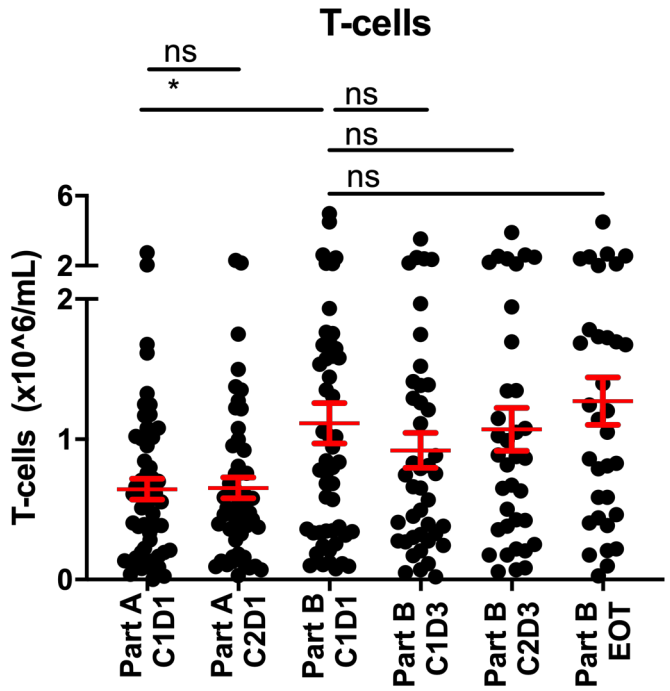


Figure S2. Absolute numbers of T-cells in peripheral blood during treatment with daratumumab monotherapy followed by daratumumab with ATRA. Absolute numbers of T-cells in sequential peripheral blood samples obtained before initiation of daratumumab monotherapy (C1D1 of part A; n=55), after 1 cycle of daratumumab monotherapy (C2D1 of part A; n=51), before start of ATRA treatment (C1D1 of part B; n=51), before first dose of daratumumab after initiation of ATRA (C1D3 of part B; n=42), after 1 cycle of daratumumab and ATRA treatment (C2D3 of part B; n=36) and at disease progression during daratumumab and ATRA treatment (EOT, part B; n=33). Dots represent individual T-cell counts, error bars represent mean and SEM. Differences between indicated groups were calculated using Wilcoxon matched-pairs rank

test.

PB, peripheral blood; ATRA, all-trans retinoic acid; SEM, standard error of mean; *P<0.05; ns, not significant.