Development and manufacture of novel locally produced anti-BCMA CAR T cells for the treatment of relapsed/refractory multiple myeloma: results from a phase I clinical trial

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Abstract

Anti-B-cell maturation antigen (BCMA) chimeric antigen receptor T-cell (CAR T) therapy shows remarkable efficacy in patients with relapsed and/or refractory (R/R) multiple myeloma (MM). HBI0101, a novel second generation optimized anti-BCMA CAR T-cell therapy, was developed in an academic setting. We conducted a phase I dose-escalation study of HBI0101 (cohort 1: $150x10^6$ CAR T cells, n=6; cohort 2: $450x10^6$ CAR T cells, n=7; cohort 3: $800x10^6$ CAR T cells, n=7) in 20 heavily pre-treated R/R MM patients. Grade 1-2 cytokine release syndrome (CRS) was reported in 18 patients (90%). Neither grade 3-4 CRS nor neurotoxicity of any grade were observed. No dose-limiting toxicities were observed in any cohort. The overall response rate (ORR), (stringent) complete response (CR/sCR), and very good partial response rates were 75%, 50%, and 25%, respectively. Response rates were dose-dependent with 85% ORR, 71% CR, and 57% minimal residual disease negativity in the high-dose cohort 3. Across all cohorts, the median overall survival (OS) was 308 days (range 25-466+), with an estimated OS of 55% as of June 27th (data cut-off). The median progression-free survival was 160 days, with 6 subjects remaining progression free at the time of data cut-off. Our findings demonstrate the manageable safety profile and efficacy of HBI0101. These encouraging data support the decentralization of CAR T production in an academic setting, ensuring sufficient CAR T supply to satisfy the increasing local demand. Clinicaltrials.gov NCT04720313.

Introduction

Therapy for multiple myeloma (MM) currently involves novel agents such as proteasome inhibitors (PI) and immune modulators (IMiD) as well as anti-CD38 antibodies that improve patient outcome.[1] Achievement of deep hematologic response is well correlated with better progression-free survival (PFS) and overall survival (OS) in MM patients.[2] However, despite major advances in therapy, the vast majority of patients will develop resistance to PI, IMiD, and anti-CD38 antibodies, and these individuals pose a major treatment challenge. Advanced-line therapies with second- and third-generation IMiD and PI, or with other newer agents, induce overall response rates (ORR) of 25-40% with limited PFS.[3-5] Patients resistant to five agents (penta-refractory) have an extremely dismal prognosis with a median PFS and OS of 3.88 and 5.97 months, respectively.[6] Newer agents, such as anti-B-cell maturation antigen (BCMA)-conjugated antibodies (e.g., belantamab mafodotin) and selective inhibitor of nuclear export (e.g., selinexor), have recently been registered as advanced-line treatments, yet they rarely lead to deep or prolonged responses.[7]

Adoptive transfer of genetically engineered chimeric antigen receptor T (CAR T) cells targeted to BCMA can induce durable and complete responses in patients with MM

with favorable safety and efficacy profiles, leading to deep and unprecedented durable responses in heavily pretreated patients.[8-12] Results have opened a new era for the treatment of MM in relapsed and refractory (R/R) patients. Idecabtagene vicleucel (Ide-cel) and Ciltacabtagene autoleucel (Cilta-cel) are two anti-BCMA CAR T-based therapies recently approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) that induce deep responses (over 85% ORR rates) in heavily pretreated patients.[9,10] However, economic, logistical, and manufacturing constraints significantly limit access to this "living-drug", and thus represent a major drawback of this approach. Given this, there is an urgent need to develop locally manufactured CAR T-cell treatments. For this purpose, we designed and developed a novel academic anti-BCMA CAR construct, named HBI0101.[13] Based on the favorable pre-clinical results showing marked *in vitro* and *in vivo* efficacy of HBI0101-engineered T cells, we initiated a phase I clinical trial for the treatment of R/R MM patients. Here we present the safety and efficacy outcomes of the first 20 R/R MM patients enrolled in this study. The question as to how to broaden the application of CAR T-based immunotherapy to patients lacking access to the commercial CAR T platform will also be discussed. Beyond the unprecedented clinical results achieved for the first time in Israel, our experience testifies to the feasibility of a decentralized approach for developing, manufacturing, and delivering such treatments, even with modest resources.

Methods

Details of the CAR structure, the generation of a clinical grade HBI0101 retroviral bank, the production of HBI0101 cells (including Quality Control and sterility testing), and patients' follow-up are provided in the *Online Supplementary Appendix*.

Study design

A single-center phase I clinical trial is being conducted at the Department of Bone Marrow Transplantation and Cancer Immunotherapy, Hadassah Medical Center (HMO). The aim is to explore the safety and efficacy of the HBI0101 CAR T-cell product that we manufactured in-house. Enrolled R/R MM patients had previously undergone at least three lines of treatment including a PI, an IMiD, and an anti-CD38 antibody. Details of the complete study protocol, eligibility criteria, and study design are to be found in the *Online Supplementary Table S1*, *Online Supplementary Figures S2* and *S3*. This study was authorized by the HMO institutional review board and by the Israeli Ministry of Health. The study was registered at clinicaltrials.gov (NCT04720313).

The phase I part of the trial was initiated in February 2021. Enrolled patients underwent lymphopheresis, and collected cells were delivered to the Good Manufacturing Practice (GMP) facility for further stimulation, transduction, and expansion (*Online Supplementary Figure S4*). Bridging with local radiotherapy was allowed according to the physician's discretion. Patients' lymphodepletion before HBI0101 infusion was achieved by the administration of fludarabine 25 mg/m^2 and cyclophosphamide 250 mg/m^2 on days -5 to -3 (*Online Supplementary Figure S2*). Patients with creatinine clearance <30 mL/min received 90 mg/m^2 bendamustine on days -4 and -3. Fresh HBI0101 cells were administered at escalating doses of 150- (cohort 1), 450- (cohort 2), and 800x10^6 (cohort 3) CAR+ cells. In accordance with the study protocol, patients remained hospitalized for at least 10 days post infusion. During hospitalization, patients were followed daily for adverse events (AE), and according to a pre-defined schedule for safety and efficacy. (See *Online Supplementary Figure S2* for details.)

Multiple myeloma clinical monitoring

Response to HBI0101 was evaluated at a pre-defined time schedule according to International Myeloma Working Group (IMWG) criteria.[14] Bone marrow biopsies and computed tomography / positron emission tomography (CT/PET) were used to assess response in patients with non-secretory disease.[14] Minimal residual disease (MRD) was evaluated by flow cytometry, in accordance with the Euroflow standards.[15] Patients were categorized as "no response" and "response" (no response: stable disease (SD) / progressive disease (PD); response: ≥ very good partial response [VGPR]). An analysis of patients' data grouped according to PD/SD, VGPR, and complete response (CR) is also provided in *Online Supplementary Figure S7*.

Primary and secondary end points

The primary end points of the present study were safety and the determination of the maximum tolerated dose (MTD) of HBI0101. Hematologic and non-hematologic adverse events were graded according to National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), version 5.00. Cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) adverse events were graded according to the 2019 American Society for Transplantation and Cellular Therapy (ASTCT) criteria.[16] Secondary end points included ORR, PFS, and OS.

Results

Patients' and disease characteristics

Between January 24, 2021, and December 23, 2021, 22 patients were screened, enrolled, and leukapheresed. For

three patients, apheresis material was cryopreserved; two patients left the study (one patient achieved CR, one patient died) before CAR T-cell manufacture (*Online Supplementary Figure S5*). HBI0101 drug products (DP) were successfully generated from fresh (n=19) and cryopreserved (n=1) raw materials; no production failure occurred, and these were infused back to patients. Three patients received bridging therapy during the manufacturing period. Two patients were treated with localized radiotherapy for pain control, and one patient received high-dose dexamethasone and plasmapheresis due to hyperviscosity. The majority of patients (n=18) underwent lymphodepletion with fludarabine-cyclophosphamide; two patients received bendamustine due to renal impairment (*Online Supplementary Figure S5*). All the patients were infused with fresh DP after ten days of production. The median duration of hospitalization post-CAR T infusion was 17 days (range 11-166), with the majority of the patients discharged after a maximum of 25 days, except for Patient 2 (P2) who had prolonged neutropenia and thrombocytopenia, and thus remained hospitalized for 166 days.

Patients' characteristics are detailed in Table 1. The median age was 62 years (range 44-75), with a median time of 55 months from initial disease diagnosis (range 8-241) and a median number of 6 previous treatment lines (range 3-13). The majority of patients had undergone previous autologous bone marrow (BM) transplantation (85%), and all were refractory to PI, IMiD, anti-CD38 antibody (daratumumab), and their last treatment line. Seven of twenty patients (35%) were penta-refractory. Nine patients (45%) had previously received and were refractory to an anti-BCMA conjugated antibody (belantamab mafodotin) (Table 1). A detailed description of previous treat-

Table 1. Patients' and disease characteristics.

Variables	Total N=20	Cohort 1 N=6	Cohort 2 N=7	Cohort 3 N=7
Age in years, median (range)	62 (44-75)	58.5 (44-75)	62 (54-73)	62 (50-72)
Gender F/M, N	12/8	4/2	4/3	4/3
Time since diagnosis in months, median (range)	55 (8-241)	42 (20-123)	78 (44-241)	43 (8-104)
Extra-medullary disease, N (%)	6 (30)	3 (50)	1 (14)	2 (29)
LDH above normal, N (%)	8 (40)	3 (50)	2 (29)	2 (29)
ECOG performance status, N (%)				
0	7 (35)	3 (50)	2 (29)	2 (29)
1	4 (20)	1 (17)	2 (29)	1 (14)
2	9 (45)	2 (33)	3 (43)	4 (57)
Creatinine clearance <60 mL/min, N (%)	7 (35)	1 (17)	3 (43)	3 (43)
Ejection fraction <55%, N (%)	3 (15)	0	2 (29)	1 (14)
Involved heavy chain, N (%)				
IGG	4 (20)	1 (17)	1 (14)	2 (29)
IGA	5 (25)	1 (17)	2 (29)	2 (29)
Involved light chain, N (%)				
kappa	11 (55)	2 (33)	4 (57)	5 (72)
lambda	8 (40)	4 (67)	3 (43)	1 (14)
Non-secretory	1 (5)	0	0	1 (14)
BM involvement, N (%)	16 (80)	5 (83)	6 (86)	5 (72)
≥50%	6 (30)	3 (50)	2 (29)	1 (14)
PET positivity, N (%)	11 (55)	4 (67)	2 (29)	5 (72)
R-ISS, N (%)				
I	1 (5)	1 (17)	0	0
II	11 (55)	0	4 (57)	7 (100)
III	2 (10)	2 (33)	0	0
Cytogenetic abnormalities, N (%)				
High risk*	10 (50)	4 (67)	5 (72)	1 (14)
Standard risk	12 (60)	5 (83)	5 (72)	4 (57)
Unknown	1 (5)	1 (17)		
Previous auto HSCT, N (%)	17 (85)	5 (83)	7 (100)	5 (72)
>1	8 (40)	2 (33)	4 (57)	1 (14)
N of previous lines, median (range)	6 (3-13)	5 (4-7)	9 (3-13)	5 (3-7)

N: number; F: female; M: male; LDH: lactate dehydrogenase; ECOG: Eastern Cooperative Oncology Group; BM: bone marrow; PET: positron emission tomography; R-ISS: Revised International Staging System; HSCT: hematopoietic stem cell transplantation. *High-risk cytogenetic abnormalities included: del(17p), t(4;14), and t(14;16).

ment lines and refractory patients is available in *Online Supplementary Table S2*. Patients in cohort 1 had a higher rate of extramedullary disease and penta-refractory disease, high lactate dehydrogenase (LDH) levels, and extensive BM involvement (as defined by >50% plasma cells in BM biopsy), while patients in cohort 3 had a worse ECOG performance score and a lower incidence of cytogenetic high-risk disease (Table 1).

HBI0101 CAR T-cell manufacture

HBI0101 CAR T cells were locally produced at the GMP-accredited facility for advanced cellular therapy, at Hadassah Medical Center; cells were successfully manufactured for all the patients. Median lymphocyte count at the time of apheresis collection was $1.0x10^6$/mL (range 0.5-2.1), with no negative impact on the successful CAR T-cell manufacture. All final DP were released in compliance with the criteria specified in *Online Supplementary Table S3*. No significant differences in the production data or *in vitro* functionality of CAR T cells of the "response" and the "no response" groups (*Online Supplementary Table S4*) were observed, attesting to the robustness of the production process, despite the high variability in the materials that were available from the MM patients.

Safety

Hematologic toxicities

All patients developed a grade 3-4 neutropenia, two-thirds of whom had grade 3 febrile neutropenia (FN) (Table 2). The entire cohort developed grade 3-4 lymphopenia, with approximately 60% developing grade 3-4 thrombocytopenia and anemia. There was a higher incidence of thrombocytopenia and FN in cohort 3 compared to cohorts 1 and 2 (Table 2). The median duration of grade 3-4 neutropenia and grade 3-4 thrombocytopenia were 15 days (range 1-65) and 24 days (range 8-138), respectively (excluding 3 patients who progressed and died without recovery of these values). Less than one-third of patients had grade 3-4 cytopenia persisting for >28 days after CAR T-cell infusion (Table 2).

Cytokine release syndrome and immune effector cell-associated neurotoxicity syndrome

Ninety percent of patients developed CRS of any grade,

Table 2. Adverse events.

Adverse events, N (%)	Total All grades N=20	Total Grade 3-4	Cohort 1 Grade 3-4 N=6	Cohort 2 Grade 3-4 N=7	Cohort 3 Grade 3-4 N=7
Hematologic ≤28d					
Neutropenia	20 (100)	20 (100)	6 (100)	7 (100)	7 (100)
Thrombocytopenia	14 (70)	12 (60)	3 (50)	4 (57)	5 (71)
Anemia	19 (95)	13 (65)	3 (50)	6 (86)	4 (57)
Lymphopenia	20 (100)	20 (100)	6 (100)	7 (100)	7 (100)
Febrile neutropenia	13 (75)	13 (75)	3 (50)	4 (57)	6 (86)
Hematologic >28d					
Neutropenia	12 (60)	6 (30)	1 (17)	2 (29)	3 (43)
Thrombocytopenia	15 (75)	7 (35)	2 (34)	2 (29)	3 (43)
Anemia	14 (70)	0	0	0	0
Lymphopenia	15 (75)	6 (30)	3 (50)	1 (14)	2 (29)
Hypogammaglobulinemia	14 (70)	5 (25)	2 (34)	1 (14)	2 (29)
Other ≤28d					
Renal failure	3 (15)	0	0	0	0
Elevated liver enzymes	2 (10)	2 (10)	1 (17)	0	0
Sepsis	3 (15)	3 (15)	0	2 (28)	1 (14)
Infectious gastroenteritis	1 (5)	1 (5)	0	1 (14)	0
Atrial fibrillation	1 (5)	0	0	0	0
Pulmonary edema	1 (5)	1 (5)	0	1 (14)	0
Urinary tract infection	1 (5)	0	0	0	0
Deep vein thrombosis	2 (10)	0	0	0	0
Other >28d					
Atrial fibrillation	1 (5)	1 (5)	1 (17)	0	0
Pulmonary edema	1 (5)	1 (5)	1 (17)	0	0
Elevated liver enzymes	1 (5)	1 (5)	0	0	1 (14)
Pneumonia	1 (5)	0	0	0	0
Pulmonary embolism	1 (5)	1 (5)	1 (17)	0	0

N: number; d: day.

mostly at the day of CAR T infusion (median 0 days; range 0-21), with a median duration of 2 days (range 1-5); none developed grade 3 or over CRS (*Online Supplementary Table S4*). However, a higher rate of CRS grade 2 was noted in cohorts 3 and 2 as compared with cohort 1 (3/7, 4/7, 1/6, respectively) (*Online Supplementary Table S4*). Tocilizumab was used in 40% percent of patients with a median of one administered dose (range 1-4). No ICAN event was observed, and none of the patients required glucocorticoids.

Non-hematologic toxicities

Non-hematologic toxicities were reported in 80% (n=16) of patients (Table 2). Bacteremia was documented in 15% of patients. Grade 3-4 reported toxicities were: sepsis (n=3), elevated liver enzymes (n=2), atrial fibrillation (n=1), infectious gastroenteritis (n=1), pulmonary edema which was secondary to cardiac causes with fluid overload and sepsis (n=2); all of these resolved. One patient developed grade 3 pulmonary embolism (PE) 10 months after CAR T therapy while in remission. PE developed due to the patient's immobilization following infection with pseudomonas pneumonia, which was successfully treated with anticoagulation therapy.

Five patients were re-hospitalized during the subsequent follow-up period due to COVID19 infection. The reasons for this were: observation (n=1), 9 months after infusion of cells; pulmonary embolism, pneumonia, and atrial fibrillation, treated with anticoagulation, antibiotics and cardioversion, 8 months after infusion of cells (n=1); infectious gastroenteritis due to salmonella and adenovirus in stool treated with antibiotics and fluids (n=1), 27 days after infusion of cells; fever treated with broad spectrum antibiotics and 2 doses of tocilizumab (n=1), 21 days after infusion of cells; nausea, vomiting, and dyspnea resolved with fluids and antiemetics (n=1), 19 days after infusion of cells (Table 2). No other severe adverse effects (SAE) were observed in any of the cohorts. One patient died within 30 days of infusion due to disease progression. There were no treatment-related mortalities.

Short-term efficacy

At a median follow-up of 136 days (80, 182, and 160 for cohorts 1, 2 and 3, respectively), ORR was 75% for the entire cohort with 50% (3/6), 85% (6/7), and 85% (6/7) responding patients in cohorts 1, 2, and 3, respectively (Figure 1A). Ten patients achieved (stringent) complete response (sCR/CR), with six patients achieving MRD negativity (four in cohort 3); five patients achieved VGPR (four in cohort 2), with two patients achieving MRD negativity (Figure 1A, B). All patients' best responses were achieved one month after CAR T infusion, except patients P7 and P11 who achieved VGPR at their first follow-up, and further deepened their response to sCR/CR and VGPR MRD-, respectively, a few months later (Figure 1B). The median PFS for the entire cohort was 160 days (range 14-326+); 80 days (range 23-248), 182 days (range 33-326+), and not reached (range 14-223+) for cohorts 1, 2, and 3, respectively (Figure 2A and B). Median OS was 308 days (range 25-466+), with an estimated OS of 55% as of data cut-off on June 27th. The median OS was 237 days (range 25-466+), 282 days (range 63-347+), and not reached in cohorts 1, 2, and 3, respectively (Figure 2D). All deaths (9/20, 45%) but one were attributed to disease progression.

The death of one patient (P7) was related to COVID19 infection. Free light chain (FLC) levels prior to and following CAR T-cell infusion in responders *versus* non-responders are shown in Figure 3A-C. In addition, soluble BCMA (sBCMA) levels, as a biomarker of responsiveness to anti-myeloma therapies,[9,10,17-19] declined rapidly in the serum of the responders, while serum levels were barely affected in the non-responding patients (Figure 3D). In addition, median LDH levels pre-lymphodepletion tended to be higher in the non-responding patients compared to responders (P=0.068). No correlation with response was seen for C-reactive protein peak level, fibrinogen levels, and the relative increase in ferritin levels during the first 14 days following CAR T infusion (*Online Supplementary Figure S6A-D*). No significant difference in terms of CRS was observed between the "response" *versus* "no response" groups (*data not shown*).

Evaluation and phenotypic characterization of bone marrow plasma cells

In order to assess patient's response to HBI0101 treatment at the cellular level, BM aspirates were analyzed by flow cytometry prior to (n=12) and one month following (n=11) CAR T administration (Figure 4A). Of these patients, three were non-responsive to HBI0101 therapy, and showed only a minor decline, or even an increase, in the percentage of BM plasma cells (BM-PC) (Figure 4B). In contrast, nine patients who did respond showed a significant reduction in the percentage of BM-PC (Figure 4B). In addition, we found that baseline BCMA and mean fluorescence intensity (MFI) on the surface of PC was significantly higher in patients in the “response” group, compared to patients in the “no response” group (Figure 4C and D; *Online Supplementary Figure S7A* and *B*). Interestingly, we found that the CD56 molecule was significantly expressed on the PC of HBI0101-responding patients (Figure 4E).

CAR T-cell kinetics

The pharmacokinetics of HBI0101 cells was assessed at serial time-points in the peripheral blood of MM patients following CAR T administration. Median time to HBI0101 peak concentration (C_{max}) was day 10 (range, 6-13) in the "response" group (sCR/CR; range, 6-13), and VGPR (range,

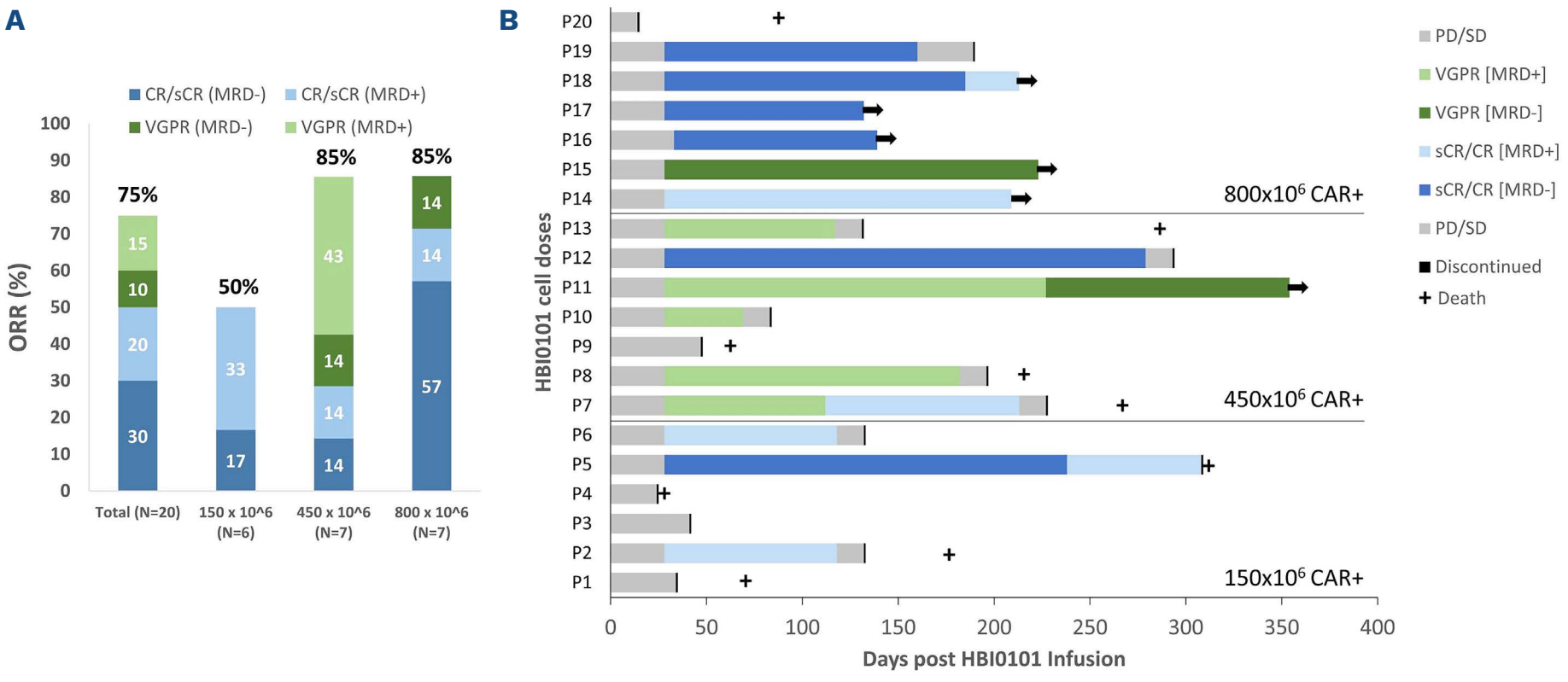

Figure 1. Objective responses in patients treated with HBI0101 CAR T cells. (A) Overall response rate (ORR). The best responses for each patient are shown according to dose (150-, 450- and 800x10^6 CAR+ cells/dose). Disease response was determined according to the International Myeloma Working Group (IMWG) consensus criteria.[14] Minimal residual disease (MRD) is defined as the number of multiple myeloma (MM) plasma cells detected in the bone marrow per 1x10^5 total nucleated cells. An MRD of 1x10^{-5} or less is considered MRD-negative (MRD-). (B) Response to HBI0101 treatment. Swimmer's plot of best responses among individual MM patients are shown according to cell dose (150- to 800x10^6 CAR+). Response assessment according to IMWG criteria. Grey: progressive disease (PD) / stable disease (SD); green: very good partial response (VGPR); light blue: stringent complete response/complete response with MRD positive (sCR/CR [MRD+]); blue: stringent complete response/complete response with MRD negative (sCR/CR [MRD-]).

10-13), and day 13 (range, 10-13) in the "no response" group (Figure 5D, *Online Supplementary Figure S7F*), with a rapid decline in HBI0101 cell proliferation within a month of post CAR T infusion. The area under the curve, as measure of overall CAR T-cell expansion within the first month of post CAR T infusion, was at borderline significance (*P*=0.0597) between the two groups (Figure 5B); this difference was more pronounced when patients were classified into SD/PD, VGPR and sCR/CR subgroups (*Online Supplementary Figure S7C* and *D*). In line with this observation, C_{max} values, as a measure of maximal CAR T-cell expansion, were found to differ significantly between the two groups: 60,655 HBI0101 cells/mL blood (range 4,945-493,152) in the “response” group *versus* 3,740 HBI0101 cells/mL blood (range 1,117-21,857) in the “no response” group (Figure 5C). *Online Supplementary Figure S7E* further shows that increased C_{max} values correlate with depth of response to HBI0101. It is noteworthy that, while a significant difference in the levels of sBCMA was observed between cohort 1 and cohorts 2 and 3 (*P*<0.0004), no significant difference in terms of HBI0101 cell kinetics in the peripheral blood was found between these cohorts (*Online Supplementary Figure S8A* and *B*). In the “response” group, the decline observed in sBCMA levels is concomitant with HBI0101 CAR T expansion in the peripheral blood in contrast to the "no response" group (Figure 5E and F), suggesting that the decrease in sBCMA is associated with HBI0101 CAR T-cell anti-myeloma activity, which results in the eradication of PC and subsequent sBCMA clearance.

Cytokine profiling analysis to predict response to HBI0101-therapy

To better understand and potentially predict patients' responsiveness to HBI0101 therapy, we determined the cytokine "signature' which can be associated with the HBI0101 CAR T-cell anti-myeloma function *in vivo*. To this end, sera were collected from MM patients at baseline (day -10) and at T_{max} (day of C_{max} of each patient) and analyzed for cytokine secretion by multiplex array. The multiplex panel included 25 pro- and anti-inflammatory chemokines/cytokines. (A detailed panel is provided in the *Online Supplementary Appendix*.) Among the 25 biomarkers analyzed, the cytokines that were differently expressed in the different groups (at baseline and T_{max}) are described in the heat-map representation in *Online Supplementary Figure S9A*. Of these, six cytokines (IL-1ra, IL-10, CCL4, IL-15, G-CSF, IL-6) were significantly differentially expressed between the different groups (*Online Supplementary Figure S8B*). More specifically, at Tmax , IL-10, CCL4, and IL-15 were found at higher levels in the "no response" group, while G-CSF and IL-6 were found at higher levels in the "response" group (*Online Supplementary Table S5, Online Supplementary Figure S9A*). At T_{max} , IL-1ra was significantly upregulated in the "no response" group but not in the "re-

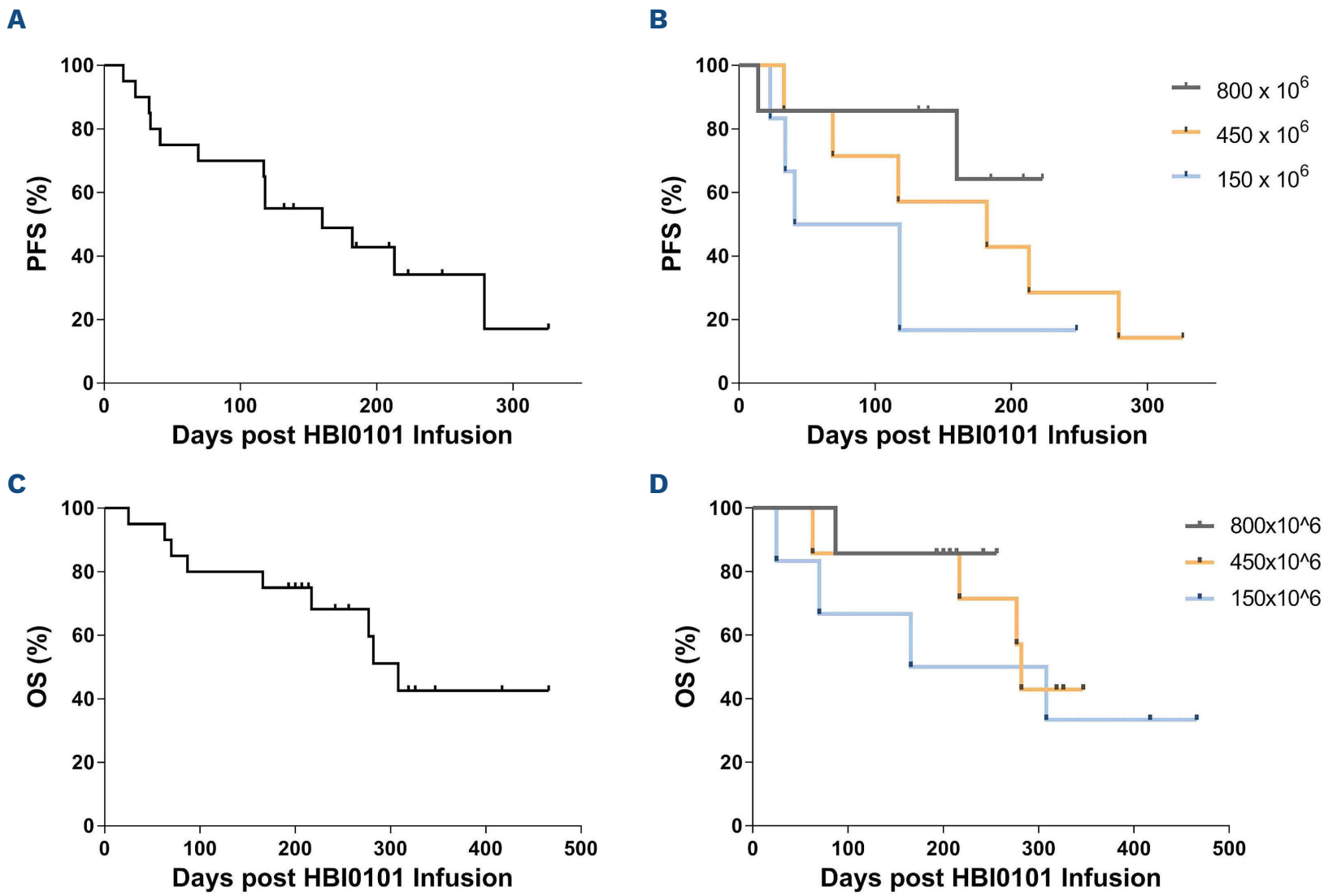

Figure 2. Survival of patients with relapsed / refractory multiple myeloma administered with HBI0101 CAR T cells. Kaplan-Meier analysis of progression-free survival (PFS) in all the patients (A) or grouped according to dose (B), overall survival (OS) in all patients (C) or grouped according to dose (D).

sponse" group. A significant increase at T_{max} in the "response" *versus* "no response" patient groups was also seen for IFN-γ (*Online Supplementary Figure S9A*). No statistical difference was observed in the level of TNF-α or IL-2 in either group.

Effect of exposure to previous anti-BCMA antibody on HBI0101 therapy

In this study, 9/20 patients (2/6 from cohort 1, 5/7 from cohort 2, and 2/7 from cohort 3) had received belantamab mafodotin prior to HBI0101 therapy. Patients exposed to belantamab (belantamab(+)) tended to have a shorter PFS and a higher progression rate (7/9, 78%) in comparison with the non-exposed (belantamab(-)) patients (6/11, 55%) (Figure 6A). Moreover, OS was lower in belantamab(+) patients (3/9, 33%) than that of belantamab(-) patients (8/11, 73%) (Figure 6B). ORR was higher in the belantamab(-) group in comparison with the belantamab(+) group (91% *vs*. 55%, respectively) (Figure 6C). Notably, when we looked at depth of response, we found that belantamab(-) patients achieved significantly deeper responses than belantamab(+) patients (sCR/CR, P=0.002; VGPR, P=0.02; by unpaired t test). However, there was no significant difference in the levels of BCMA expression on PC (determined by MFI) or in the percentage of BCMA-positive PC between the two groups.

Discussion

Access to CAR T therapy for MM patients is still limited. There is a need to explore available, safe and effective methods to deliver this promising technology. We recently presented an optimized novel anti-BCMA CAR molecule and showed that modifications in the transmembrane (TM)/hinge of this CAR construct impact CAR performance both *in vitro* and *in vivo*.[13] Here we share the safety and efficacy outcomes of the first 20 R/R MM patients enrolled on a phase I academically designed trial on locally manufactured anti-BCMA CAR T cells.

Our safety results are in line with those of approved BCMA-targeted CAR T therapies.[9,10] We observed grade 3-4 neutropenia in the entire cohort, and anemia and thrombocytopenia in less than two-thirds of patients, with a median time to recovery of 3 weeks. Two-thirds of patients experienced FN, with a higher rate in cohort 3. This reversible cytopenia appears to be dose-dependent rather than being driven only by the lymphodepletion-related

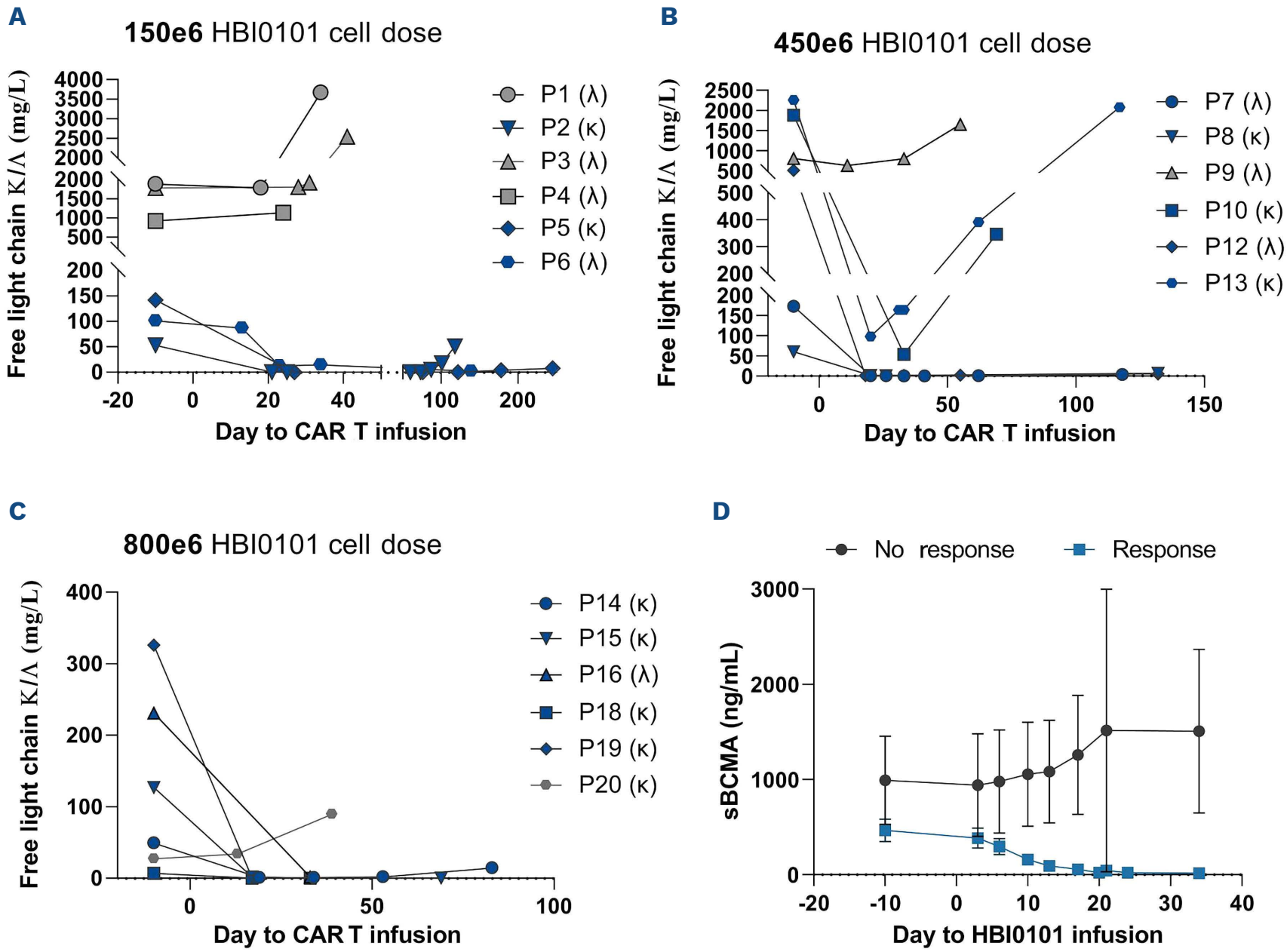

Figure 3. Multiple myeloma disease monitoring. Free light chain levels were determined at the indicated time points prior to and following HBI0101 infusion in cohort 1 (N=6) (A), cohort 2 (N=6) (B), and cohort 3 (N=6) (C). Normal range for Kappa light chain: 6.7-22.4 mg/L, and Lambda light chain: 8.3-27 mg/L. (D) Soluble BCMA (sBCMA) levels prior to and following CAR T infusion were determined by ELISA in the "response" (blue squares) *versus* "no response" (black dots) groups.

chemotherapy. However, this AE was not associated with cytopenia-related complications.

CRS was observed at the rates reported in previous studies. We observed a high proportion of any grade manageable CRS without grade 3-4 CRS. This was also reflected by the median of one tocilizumab dose administered, and only in 40% of patients. In addition, there were no ICAN events in the entire cohort.

The short-term efficacy data reported here corroborates the results reported in the literature,[8,10-12] with higher CAR T doses correlating with higher and deeper responses (ORR of 85% for cell doses 450- and 800x10^6). Indeed, responses ≥VGPR were observed in more than two-thirds of patients, with half of these achieving sCR/CR. The OS and PFS in this dose-escalating phase I study reflect the poor clinical condition of the subjects, six of whom also had a low dose of HBI0101 CAR T cells. One limitation of our study is the relatively short follow-up (median 136 days); these objectives will, therefore, be re-evaluated in our dose-expansion phase Ib-II study (administering a cell dose of 800x10^6).

There is still no complete picture of the response to anti-BCMA CAR T cells following anti-BCMA antibody. In our study, comparing patients who had received prior belantamab mafodotin therapy to those who had not revealed a trend towards better PFS and OS in those patients who had not been exposed to this treatment. In addition, responses were significantly deeper in patients who had not received belantamab. Some groups have re-targeted BCMA using belantamab after anti-BCMA CAR T-cell therapy.[20-22] Our observation suggests that sequential treatment of MM patients with anti-BCMA.CAR T cells following anti-BCMA antibody may impair, but does not exclude, response to CAR T therapy. Interestingly, the majority of patients pre-treated with belantamab were in cohort 2 (55%, 5/9). Although this cohort was treated with a higher dosage of HBI0101 cells compared to cohort 1, the rate of sCR/CR in this cohort was lower. This observation raises the question as to whether the depth of response in cohort 2 was hampered by a higher proportion of patients previously being exposed to belantamab. Since belanta-

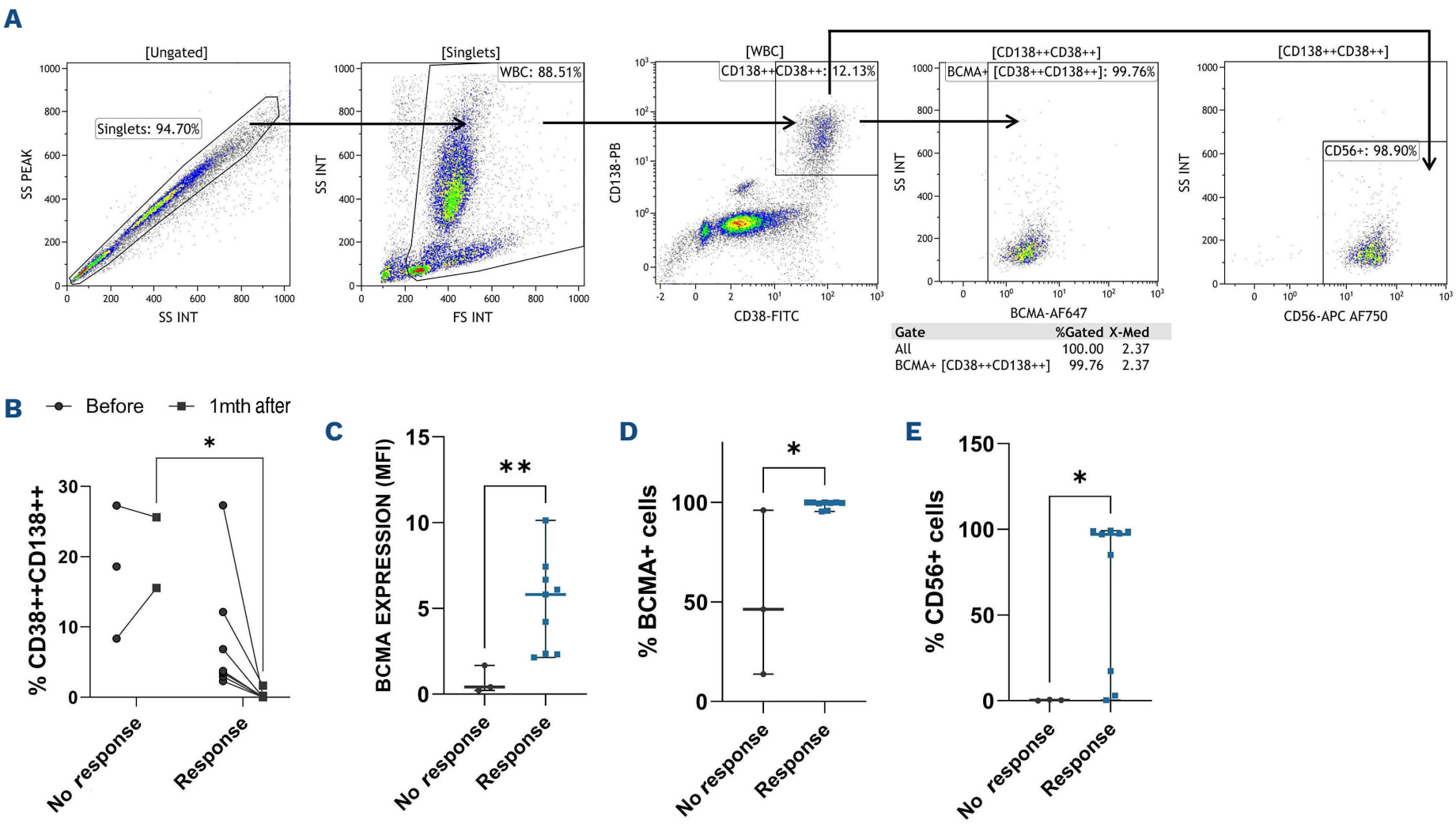

Figure 4. Efficacy of HBI0101 CAR T-based therapy in eliminating CD38++CD138++ malignant plasma cells. (A) Flow cytometric gating strategy for the assessment of anti-B-cell maturation antigen (BCMA) and CD56 expression on multiple myeloma plasma cells (MM-PC) (indicated by arrows). Samples were gated on CD38++CD138++. (B) Bone marrow (BM) samples prior to and one month (mth) following HBI0101-CAR T infusion were assessed for the presence of PC (as % of CD38++CD138++ cells) by flow cytometry by gating on white blood cells (WBC), as illustrated in (A). (C-E) Analysis of BCMA and CD56 expression on MM-PC, by "response" (blue squares) *versus* "no response" (black dots) group. (C, D) BCMA expression levels in MM patients (N=12). The mean fluorescence intensity (MFI) (C) and the percent of BCMA-positive PC (D) were determined by flow cytometry. (E) Percent of CD56-positive PC in "response" (N=9) *versus* "no response" (N=3) groups.

mab consists of a humanized IgG1 anti-BCMA monoclonal antibody originally generated from the murine CA8 clone,[23] it is unlikely that anti-drug antibodies (ADA) with a cross-reactivity to the 11D5-3-derived scFv of the HBI0101 CAR molecule[13,24] could cause the resistance to HBI0101 therapy following exposure to belantamab. In addition, there was no difference in terms of BCMA expression on PC in patients receiving belantamab in comparison with those who had not, suggesting that the reduced depth of response was not associated with a downregulation of the BCMA protein at the PC surface. It is, therefore, evident that additional factors contribute to resistance to anti-BCMA CAR T therapy; thus, unraveling mechanisms of resistance remains of great importance.

Additional biomarkers that predict responsiveness to HBI0101 CAR T therapy will enable a more accurate prediction and understanding of clinical outcome. CD56 is a membrane glycoprotein of the immunoglobulin superfamily that is expressed on clonal PC in 60-80% of MM patients.[25] Studies assessing the relationship between MM prognosis and the expression of CD56 have given contradictory results.[26] While in some studies CD56 expression on MM patient PC has been associated with a good prognosis,[27] others reported a negative or no effect on patient survival.[25,28,29] To the best of our knowledge, there is no study in the literature reporting on the prognostic value of CD56 expression on PC in the context of anti-BCMA CAR T-based therapies. In our study, about 70% of the responsive patients display CD56 expression at the PC surface, while none of the patients that have failed HBI0101 therapy expressed CD56 at the PC surface. Being an adhesion molecule, this implies that the PC-BM microenvironment interaction has a significant role to play. These observations suggest that CD56 may serve as a biomarker to predict a patient's response to HBI0101 therapy. Further studies of surface marker expression are required to establish and validate these findings.

A better understanding of the cytokine profile is important to identify those patients that would benefit most from HBI0101 treatment. Multiplex analysis of pro- and anti-inflammatory biomarkers in the peripheral blood at the day of CAR T peak revealed lower levels of IL-10, IL-1RA, CCL-4 and IL-15 and higher levels of IFN-γ, IL-6 and G-CSF cytokines in the response group. This suggests that production

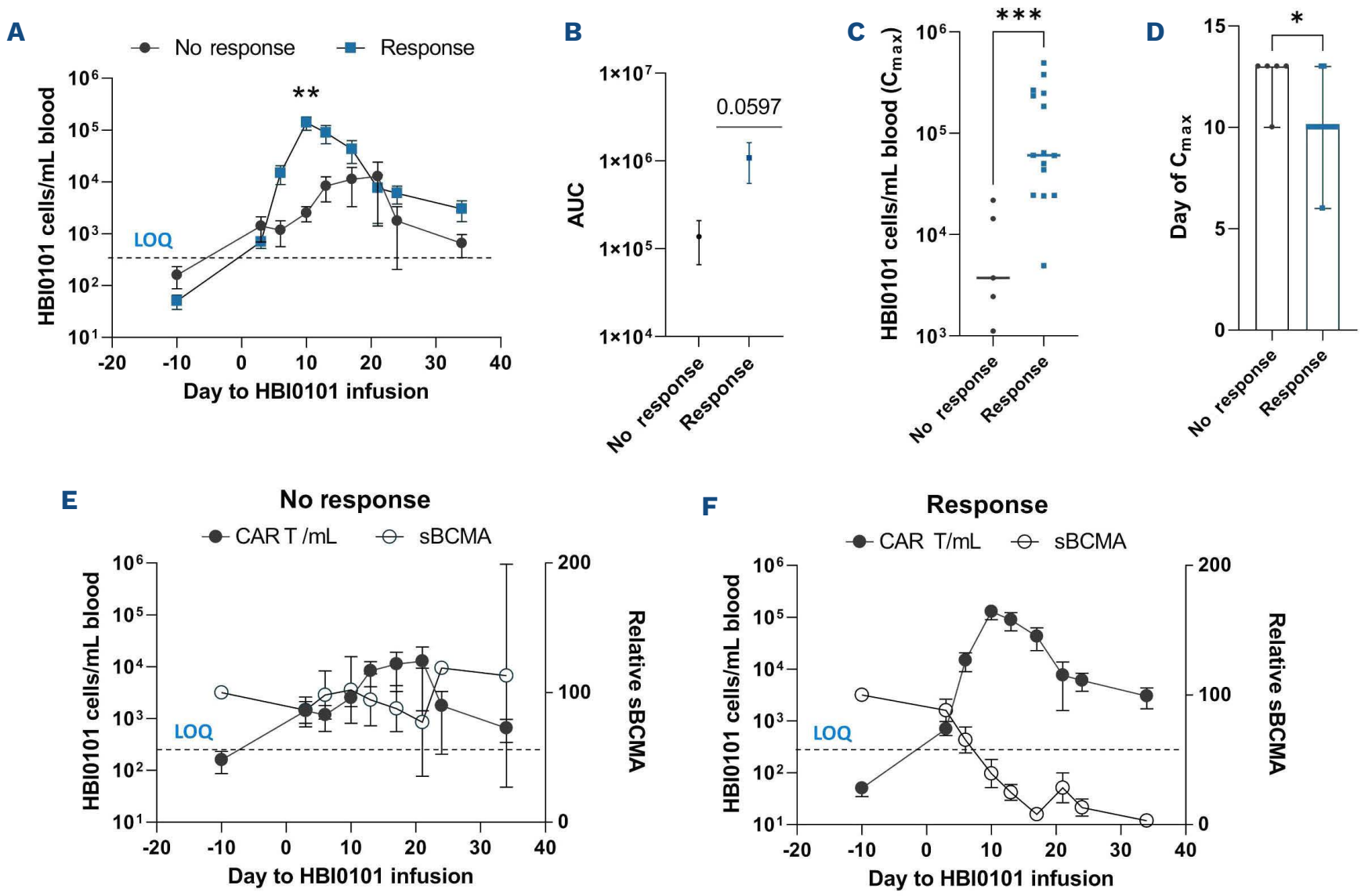

Figure 5. Soluble anti-B-cell maturation antigen clearance and HBI0101 CAR T-cell *in vivo* kinetics. (A) The median number of HBI0101-CAR T cells per 1 mL blood in the "response" *versus* "no response" group was determined by quantification of CAR transgene levels by qRT-PCR method following CAR T infusion at the indicated times and further adjusted to the copy numbers per transduced cell at the day of CAR T infusion. The limit of quantitation (LOQ) was 500 CAR T/mL blood. (B) HBI0101 CAR T-cell overall expansion in the first month of CAR T therapy. Area under the curve (AUC) as a measure of CAR T-cell overall expansion was calculated with Prism software (GraphPad). (C) HBI0101 cells *in vivo* median concentration at peak (C_{max}) in "response" (blue squares) *versus* "no response" (black dots) groups. (D) Median time to C_{max} (T_{max}) in "response" (blue squares) *versus* "no response" (black dots) groups. Upper and lower bars I represent the maximal and minimal values, respectively. (E, F) Soluble anti-B-cell maturation antigen (sBCMA) levels prior to and following HBI0101 infusion determined by ELISA and further normalized to sBCMA concentration at baseline (right y-axis; empty circles) *versus* HBI0101-cell expansion indicated by the CAR T/mL (left y-axis; filled circles) in the "no response" group (E), and in the "response" group (F). **P<0.01, by unpaired t test.

of pro-inflammatory cytokines by T and innate immune cells supports response to HBI0101 therapy, while IL-10 and IL-1RA anti-inflammatory cytokines may hamper that response. If this observation proves correct in a larger cohort of patients, modulation of the cytokine milieu towards a pro-inflammatory supportive environment for CAR T cells should be considered. Overall, our data suggest that responsiveness to HBI0101 therapy is associated with an early and significant expansion of CAR T cell in the peripheral blood with an upregulation of pro-inflammatory cytokines and with at least minimal expression of BCMA on the PC surface.

Although two BCMA-targeted CAR treatments have been approved by the FDA and the EMA for the treatment of R/R MM, a wave of new anti-BCMA CAR-based clinical trials[8,11,12] attests to the growing worldwide demand for such treatments, access to which, unfortunately, is still limited due to logistical and financial constraints.

Our results represent the first time an Israeli academic institution has completed the full CAR T-product cycle: development of a new CAR T treatment, proof-of-concept *in vitro* and *in vivo*,[13] approval by regulatory authorities, local production in a GMP facility, and delivery to patients (*Online Supplementary Figure S10*). The main advantages of such an approach over the commercial products are the availability of the DP and the shortened vein-to-vein delivery. Moreover, the significance of these results extends far beyond the clinical dimension. Affordable, locally produced CAR T cells represent a major step in broadening access to this cutting edge advanced cellular therapy. This achievement was made possible through the successive achievements of several key milestones (*Online Supplementary Figure S9*). It is acknowledged that accreditation from the Joint Accreditation Committee ISCT-Europe and EBMT (JACIE) forms a good basis for the qualification of

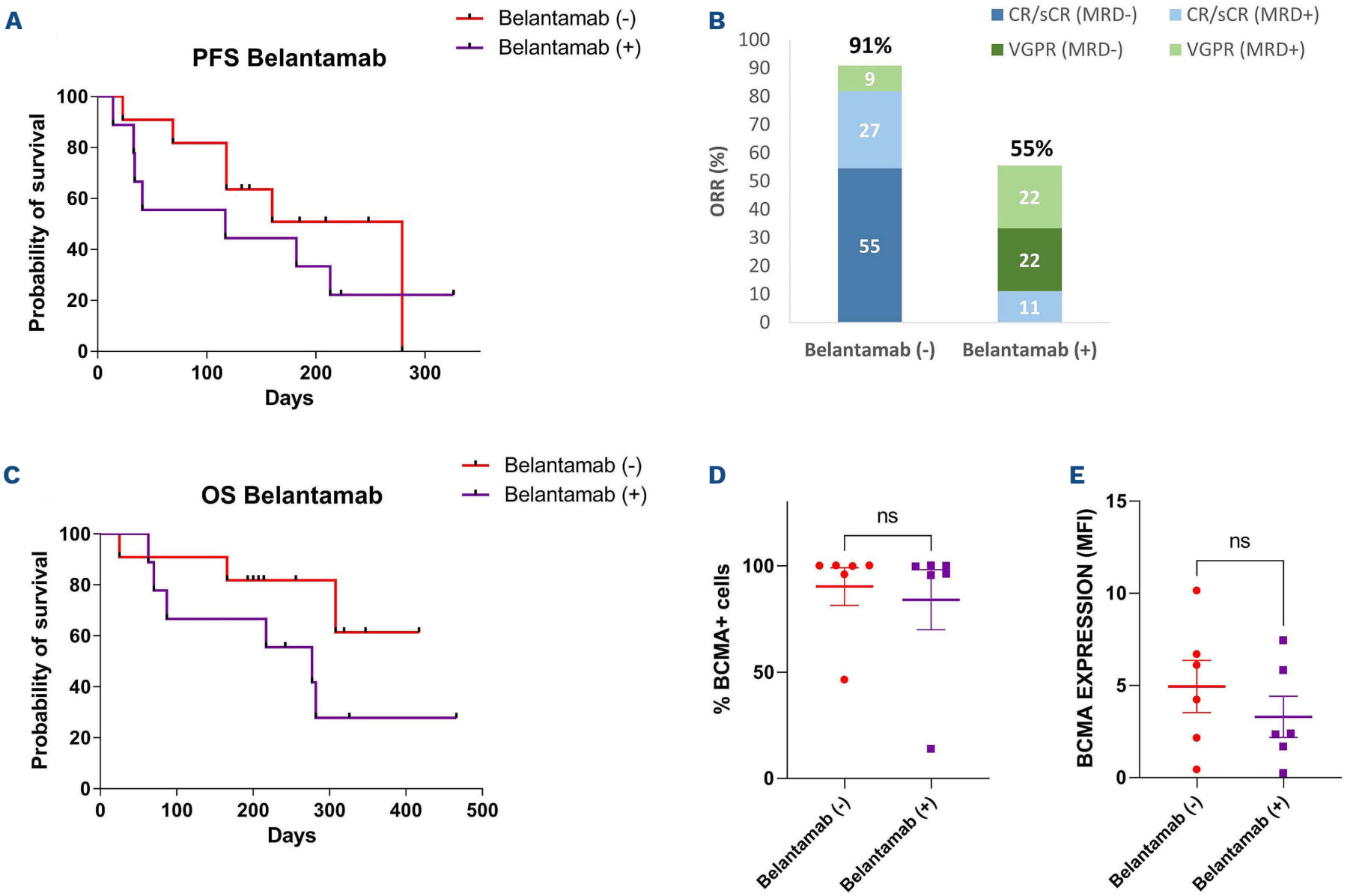

Figure 6. Effect of belantamab pre-treatment on multiple myeloma patients' response to HBI0101 therapy. Kaplan-Meier analysis of progression-free survival (PFS) (A) and overall survival (OS) (C) in "belantamab(+)" *versus* "belantamab(-)" group. (B, D) Effect of belantamab prior therapy on plasma cell anti-B-cell maturation antigen (BCMA) expression. Percent of BCMA-expressing bone marrow-plasma cell (BM-PC) (B) and BCMA expression mean fluorescence intensity (MFI) (D) on BM-PC were determined by flow cytometry prior to HBI0101 infusion and analyzed according to patient's pre-exposure to belantamab. Samples were gated on CD38++CD138++ cells. CR: complete response; sCR: stringent complete response; VGPR: very good partial response; MRD: minimal residual disease; ns: not significant.

CAR T-cell therapy centers.[30] Thus, in parallel to developing our own CAR T-based therapy, our center applied and was successfully accredited for FACT-JACIE. Another important milestone to support CAR T manufacture was the establishment of a GMP-infrastructure that meets both production and regulatory requirements. The entire process from the beginning of CAR development to the treatment of the first patients was completed within three years, with modest human resources, and without compromising on regulatory requirements or product quality (*Online Supplementary Figure S10*).

In the interest of making CAR T therapy readily accessible via a decentralized approach, some suggestions may be made. It is important to maintain an open dialogue with the regulatory authorities since promoting such innovative therapies is of mutual benefit. In addition, practitioners should explore how to incorporate newer non-viral gene-transfer technologies (e.g., electroporation of naked DNA,[31] transposon/transposase systems[32,33]) to help reduce costs. Finally, implementing an automated production process (e.g., CliniMACS Prodigy®) will further reduce costs and increase scalability.

Pharma companies currently operate the centralized manufacture of CAR T cells from autologous T cells. Until the promise of safe and efficient off-the-shelf allogeneic CAR T cells becomes a reality,[34] it will be essential to decentralize production. Such decentralization can be achieved by academic medical centers with an established translational R&D platform and GMP-grade facilities. This approach will lower costs, promote shorter vein-to-vein delivery times, and provide greater manageability.

In conclusion, we present the good safety profile and favorable results of a phase I clinical trial with locally produced anti-BCMA CAR T cells for highly refractory MM patients. Our experience testifies to the capability of an academic institution to provide the local population with safe, efficient and accessible state-of-the-art CAR T tech-

nology. A continuous Phase Ib clinical trial is ongoing and will provide further data to validate this approach. Implementation of in-house CAR T-based therapy is a complex multi-step process. Successful incorporation of CAR T cells into existing treatment paradigms requires the combined efforts of the scientific community, clinicians and government agencies in order to broaden access to this unique method of treatment for the benefit of the patients. We believe that this approach will help to make CAR T therapy more accessible and significantly advance the treatment of cancer.

Disclosures

No conflicts of interest to disclose.

Contributions

NA and SKE designed and performed the experiments, wrote the protocol, and were responsible for CAR T production and evaluation, patient follow-up, and contributed to manuscript preparation; SG and BA wrote the protocol, were responsible for CAR T treatment, and contributed to manuscript preparation; EZ, EL and AS performed CAR T treatment and evaluation, and contributed to manuscript preparation; MA wrote the protocol, and is responsible for CAR T production and evaluation, and statistical analyses; TDS and NZ performed CAR T production and evaluation, and patients' follow-up; MP performed CAR T evaluation and contributed to manuscript preparation; YC and IA performed CAR T evaluation and contributed to manuscript writing; CC designed the HBI0101 CAR construct, supervised the pre-clinical MM study, and contributed to manuscript preparation; PS designed and supervised the study, wrote the protocol, was responsible for CAR T treatment and evaluation, and contributed to manuscript preparation; MEG wrote the protocol, performed CAR T treatment and evaluation, and contributed to manuscript preparation.

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Data-sharing statement

For original data, please contact the corresponding authors.

References

1. Cowan AJ, Green DJ, Kwok M, et al. Diagnosis and management of multiple myeloma: a review. JAMA. 2022;327(5):464-477.
2. Gay F, Larocca A, Wijermans P, et al. Complete response correlates with long-term progression free and overall survival in elderly myeloma treated with novel agents: analysis of 1175 patients. Blood. 2011;117(11):3025-3031.
3. Gandhi UH, Cornell RF, Lakshman A, et al. Outcomes of patients with multiple myeloma refractory to CD38-targeted monoclonal antibody therapy. Leukemia. 2019;33(9):2266-2275.
4. Kumar SK, Dimopoulos MA, Kastritis E, et al. Natural history of relapsed myeloma, refractory to immunomodulatory drugs and proteasome inhibitors: a multicenter IMWG study. Leukemia. 2017;31(11):2443-2448.
5. Pick M, Vainstein V, Goldschmidt N, et al. Daratumumab resistance is frequent in advanced-stage multiple myeloma patients irrespective of CD38 expression and is related to dismal prognosis. Eur J Haematol. 2018;100(5):494-501.
6. Gill SK, Unawane R, Wang S, et al. Inferior outcomes of patients with quad and penta-refractory multiple myeloma (MM) compared to those of patients who have been quad and penta exposed. Blood. 2021;138 (Supplement 1):4742.
7. Vogl DT, Dingli D, Cornell RF, et al. Selective inhibition of nuclear export with oral selinexor for treatment of relapsed or refractory multiple myeloma. J Clin Oncol. 2018;36(9):859-866.
8. Guo R, Lu W, Zhang Y, Cao X, Jin X, Zhao M. Targeting BCMA to treat multiple myeloma: updates from the 2021 ASH Annual Meeting. Front Immunol. 2022;13:839097.
9. Munshi NC, Anderson LD Jr, Shah N, et al. Idecabtagene vicleucel in relapsed and refractory multiple myeloma. N Engl J Med. 2021;384(8):705-716.
10. Berdeja JG, Madduri D, Usmani SZ, et al. Ciltacabtagene autoleucel, a B-cell maturation antigen-directed chimeric antigen receptor T-cell therapy in patients with relapsed or refractory multiple myeloma (CARTITUDE-1): a phase 1b/2 open-label study. Lancet. 2021;398(10297):314-324.
11. Du J, Wei R, Jiang S, et al. CAR-T cell therapy targeting B cell maturation antigen is effective for relapsed/refractory multiple myeloma, including cases with poor performance status. Am J Hematol. 2022;97(7):933-941.
12. Frigault MJ, Bishop MR, Rosenblatt J, et al. Phase 1 study of CART-ddBCMA for the treatment of subjects with relapsed and refractory multiple myeloma. Blood Adv. 2023;7(5):768-777.
13. Harush O, Asherie N, Kfir-Erenfeld S, et al. Preclinical evaluation and structural optimization of anti-BCMA CAR to target multiple myeloma. Haematologica. 2022;107(10):2395-2407.
14. Kumar S, Paiva B, Anderson KC, et al. International Myeloma Working Group consensus criteria for response and minimal

residual disease assessment in multiple myeloma. Lancet Oncol. 2016;17(8):e328-e346.
15. Theunissen P, Mejstrikova E, Sedek L, et al. Standardized flow cytometry for highly sensitive MRD measurements in B-cell acute lymphoblastic leukemia. Blood. 2017;129(3):347-357.
16. Lee DW, Santomasso BD, Locke FL, et al. ASTCT consensus grading for cytokine release syndrome and neurologic toxicity associated with immune effector cells. Biol Blood Marrow Transplant. 2019;25(4):625-638.
17. Laurent SA, Hoffmann FS, Kuhn PH, et al. Gamma-secretase directly sheds the survival receptor BCMA from plasma cells. Nat Commun. 2015;6:7333.
18. Ghermezi M, Li M, Vardanyan S, et al. Serum B-cell maturation antigen: a novel biomarker to predict outcomes for multiple myeloma patients. Haematologica. 2017;102(4):785-795.
19. Raje N, Berdeja J, Lin Y, et al. Anti-BCMA CAR T-cell therapy bb2121 in relapsed or refractory multiple myeloma. N Engl J Med. 2019;380(18):1726-1737.
20. Gazeau N, Beauvais D, Yakoub-Agha I, et al. Effective anti-BCMA retreatment in multiple myeloma. Blood Adv. 2021;5(15):3016-3020.
21. Mu W, Long X, Cai H, et al. A model perspective explanation of the long-term sustainability of a fully human BCMA-targeting CAR (CT103A) T-cell immunotherapy. Front Pharmacol. 2022;13:803693.
22. Cohen AD, Garfall AL, Dogan A, et al. Serial treatment of relapsed/refractory multiple myeloma with different BCMA-targeting therapies. Blood Adv. 2019;3(16):2487-2490.
23. Montes de Oca R, Alavi AS, Vitali N, et al. Belantamab mafodotin (GSK2857916) drives immunogenic cell death and immune-mediated antitumor responses in vivo. Mol Cancer Ther. 2021;20(10):1941-1955.
24. Carpenter RO, Evbuomwan MO, Pittaluga S, et al. B-cell maturation antigen is a promising target for adoptive T-cell therapy of multiple myeloma. Clin Cancer Res. 2013;19(8):2048-2060.
25. Skerget M, Skopec B, Zadnik V, et al. CD56 expression is an important prognostic factor in multiple myeloma even with bortezomib induction. Acta Haematol. 2018;139(4):228-234.
26. Lebel E, Nachmias B, Pick M, Gross Even-Zohar N, Gatt ME. Understanding the bioactivity and prognostic implication of commonly used surface antigens in multiple myeloma. J Clin Med. 2022;11(7):1809.
27. Pellat-Deceunynck C, Barille S, Jego G, et al. The absence of CD56 (NCAM) on malignant plasma cells is a hallmark of plasma cell leukemia and of a special subset of multiple myeloma. Leukemia. 1998;12(12):1977-1982.
28. Li G, Huang XJ, Niu T, et al. [Expression of CD56 in multiple myeloma cells and its relationship with extramedullary disease and extramedullary relapse]. Zhongguo Shi Yan Xue Ye Xue Za Zhi. 2021;29(2):553-556.
29. Chang H, Samiee S, Yi QL. Prognostic relevance of CD56 expression in multiple myeloma: a study including 107 cases treated with high-dose melphalan-based chemotherapy and autologous stem cell transplant. Leuk Lymphoma. 2006;47(1):43-47.
30. Hayden PJ, Sirait T, Koster L, Snowden JA, Yakoub-Agha I. An international survey on the management of patients receiving CAR T-cell therapy for haematological malignancies on behalf of the Chronic Malignancies Working Party of EBMT. Curr Res Transl Med. 2019;67(3):79-88.
31. Till BG, Jensen MC, Wang J, et al. CD20-specific adoptive immunotherapy for lymphoma using a chimeric antigen receptor with both CD28 and 4-1BB domains: pilot clinical trial results. Blood. 2012;119(17):3940-3950.
32. Prommersberger S, Reiser M, Beckmann J, et al. CARAMBA: a first-in-human clinical trial with SLAMF7 CAR-T cells prepared by virus-free Sleeping Beauty gene transfer to treat multiple myeloma. Gene Ther. 2021;28(9):560-571.
33. Kebriaei P, Singh H, Huls MH, et al. Phase I trials using Sleeping Beauty to generate CD19-specific CAR T cells. J Clin Invest. 2016;126(9):3363-3376.
34. Townsend MH, Bennion K, Robison RA, O'Neill KL. Paving the way towards universal treatment with allogenic T cells. Immunol Res. 2020;68(1):63-70.