Allogeneic anti-BCMA CAR T Cells Show Tumour Specific Killing Against Primary Multiple Myeloma Cells from Different Genomic Sub-Groups

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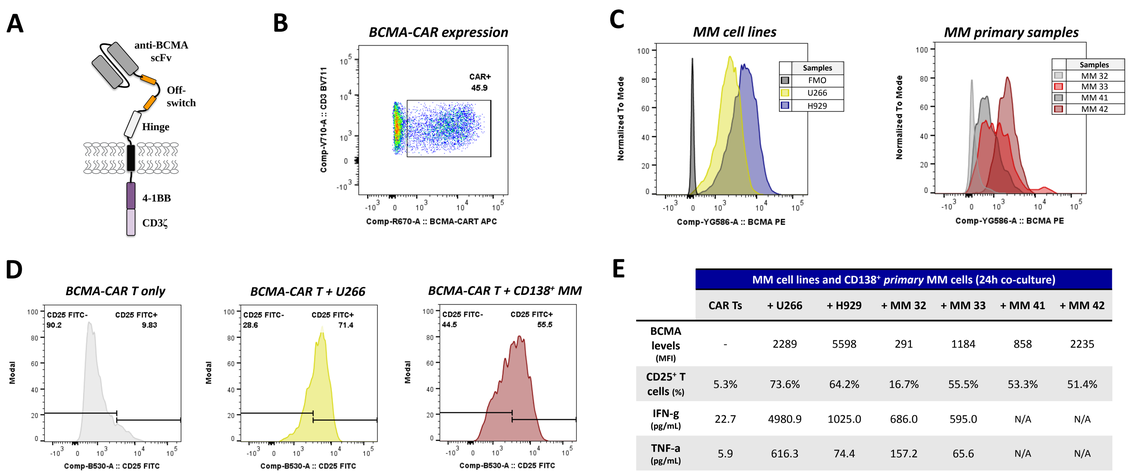
INTRODUCTION

Autologous anti-BCMA CAR T cells have been successfully used in clinical trials for the treatment of relapsed refractory Multiple Myeloma (rrMM), achieving high initial response rates (>80%). However, these therapeutic responses are not durable with patients relapsing on average after 12-18 months[1,2]. Poor T cell fitness, CAR T cell exhaustion, the immunosuppressive effect of the tumour microenvironment and BCMA negative tumour escape are some of the factors contributing to treatment failure. In this study we describe for the first time the activity of an allogeneic anti-BCMA CAR T cell product derived from young healthy donors (HD) against *primary* MM cells using patient bone marrow (BM) biopsies. In addition, we compare the performance of HD and MM patient-derived anti-BCMA CAR T cells.

[1] Raje N et al. NEJM 2019; 380(18):1726-1737 ; [2] Zhao WH et al. J Hematol Oncol. 2018; 11(1):141.

RESULTS

1. Allogeneic anti-BCMA CAR T cells become strongly activated upon exposure to MM *primary* cells.



	MM cell lines and CD138+ *primary* MM cells (24h co-culture)						
	CAR Ts	+ U266	+ H929	+ MM 32	+ MM 33	+ MM 41	+ MM 42
BCMA levels (MFI)	-	2289	5598	291	1184	858	2235
CD25+ T cells (%)	5.3%	73.6%	64.2%	16.7%	55.5%	53.3%	51.4%
IFN-g (pg/mL)	22.7	4980.9	1025.0	686.0	595.0	N/A	N/A
TNF-a (pg/mL)	5.9	616.3	74.4	157.2	65.6	N/A	N/A

Figure 1 – **(A)** Schematic representation of the second generation anti-BCMA CAR construct with 4-1BB and CD3z intracellular domains. **(B)** Representative dot plot showing the percentage of anti-BCMA CAR+ T cells present in the allogeneic product, measured at Day 14 by FACS using an anti-idiotype Ab. **(C)** BCMA levels of MM cell lines (U266 and H929) and MM *primary* cells from different MM patients (MM32, MM33, MM41 and MM42) measured by FACS. FMO was used as control. **(D)** Representative images of CD25 expression on anti-BCMA CAR T cells after 24h of co-culture with the MM cell line U266 and isolated CD138+ MM *primary* cells (measured by FACS). CD25 is a known T cell activation marker. **(E)** Table summarizing the different BCMA levels of the MM cell lines and MM *primary* cells, the activation achieved by the anti-BCMA CAR T cells (CD25+) after exposure to the MM cells for 24h at 1:1 E/T ratio, IFN-g and TNF-a secretion and MM cell killing. BCMA levels, percentage of CD25+ CAR T cells and MM killing were measured by FACS. IFN-g and TNF-a secretion (pg/mL) was detected in the supernatant by Luminex FLEXMAP 3D. MM *primary* cells were isolated from the BM using a CD138+ selection kit.

2. Allogeneic anti-BCMA CAR T cells efficiently target *primary* MM cells within the BM niche.

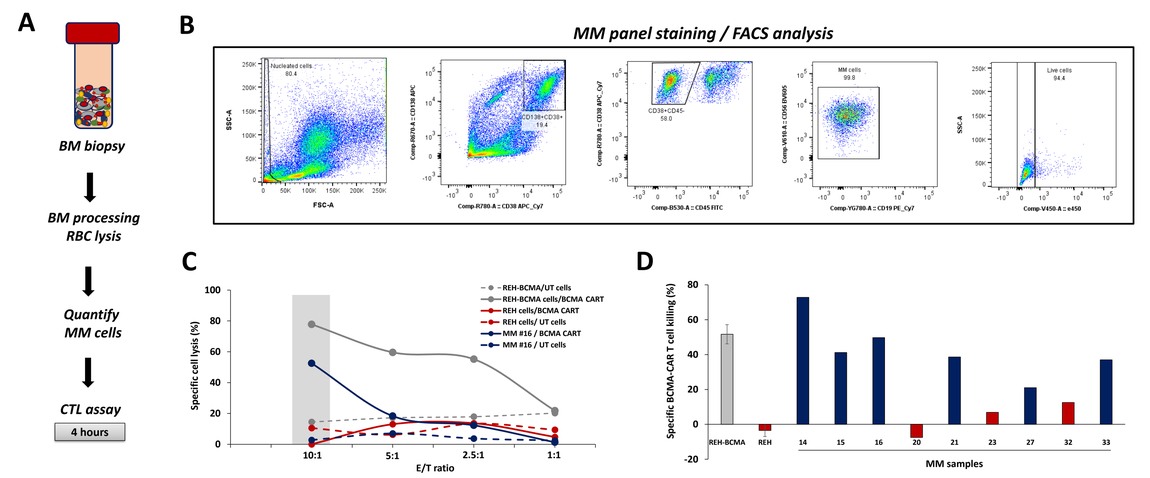


Figure 2 – **(A)** Schematic diagram illustrating whole BM biopsy processing before the CTL assay. **(B)** Gating strategy to identify viable MM cells within the bulk BM biopsy by FACS. MM cells can be distinguished from normal plasma cells and other BM microenvironmental cells based on CD138+CD38+CD45-CD19-CD56+ profile. **(C)** Specific MM killing (%) assessed at the end of the 4 hour CTL assay by FACS (using the MM gating strategy described above). anti-BCMA CAR T cells are co-cultured with REH-BCMA (a BCMA positive control cell line), REH (a BCMA negative control cell line) and the processed MM biopsy using different E/T ratios. Untransduced T cells (UT) were used to measure background T cell killing. **(D)** Specific anti-BCMA CAR T cell killing (%) against different MM BM samples (14, 15, 16, 20, 21, 23, 27, 32 and 33) at 10:1 E/T ratio. The percentage was quantified as: (% MM cell death with BCMA-CAR T cell - % MM cell death with UT cell) / % spontaneous MM cell death. REH and REH-BCMA were used as negative and positive controls.

3. Allogeneic anti-BCMA CAR T cells efficiently kill MM *primary* cells irrespective of genomic subgroup and independent of BCMA expression.

A

BM sample	Patient age	Genomic subgroup	Disease Stage	Treatment regimens
#14	64	-	2	VTD, Melphalan Auto, Len, Dara
#15	73	FGFR3 gain, 16q23 (4p) loss (**std risk**)	1	Newly diagnosed, Nil
#16	88	-	2	Newly diagnosed, Nil
#20	62	-	Normal PCs	-
#21	79	p53 del (**high risk**)	2	VMP, DVD
#23	81	Normal (**std risk**)	1	Newly Diagnosed, Nil
#27	64	p53 del, 1q+, 1p del (**high risk**)	2	Carfilzomib/Cyclo/Dex
#32	89	IgH rearrangement (but not 4,14 or 11,14) (**std risk**)	IgGk MGUS 8% PCs	-
#33	57	Normal (**std risk**)	1	VTD, Melphalan Auto

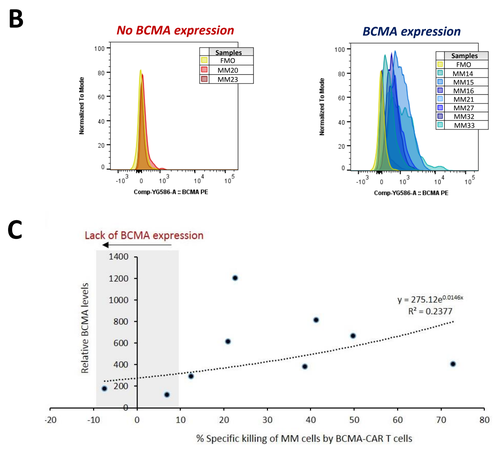


Figure 3 – **(A)** List of MM BM samples used to test the activity of the allogeneic anti-BCMA CAR T cell product, including different patient characteristics: patient age, genomic subgroup, disease stage and prior lines of treatment. **(B)** BCMA levels of all the MM *primary* samples tested in the CTL assay, measured by FACS. FMO was used as negative control (in yellow). Two MM primary samples were negative for BCMA (shown in red), while seven MM primary samples were positive for BCMA, with a range of expression as shown in the histograms. **(C)** Correlation between relative BCMA levels and specific MM killing by anti-BCMA CAR T cells. Relative BCMA levels were measured by FACS and normalized as described: *BCMA levels (MFI) in MM cells – BCMA levels (MFI) in endogenous BM T cells (*used as an internal negative control in the BM sample).

4. Allogeneic anti-BCMA CAR T cells from young healthy donors show higher CD4/CD8 ratio and reduced dysfunctionality compared to MM-derived CAR T cells.

A

	PB sample	Age	Disease progression	Treatment regimen
Early MM group	MM 24	70	newly diagnosed	Nil
	MM 37	79	newly diagnosed	Nil
	MM 18	68	2nd relapse	VCD, RVD
	MM 25	58	2nd relapse	CTD, VCD
	MM 30	69	1st relapse	VTD, Melphalan Autograft
	MM 38	39	1st relapse	Car/Cyclo/Dex

	PB sample	Age	Disease progression	Treatment regimen
rrMM group	MM 14	64	4th relapse	VTD, Melphalan Autograft, CD, RD, VD, Dara
	MM 26	71	3rd relapse	CTD, Melphalan Autograft, BTD, VCD
	MM 29	57	4th relapse	PAD, Melphalan Auto, KCD, CRD, Melphalan, Auto#2, Dara
	MM 36	61	3rd relapse	CTD, Melphalan, Auto#2

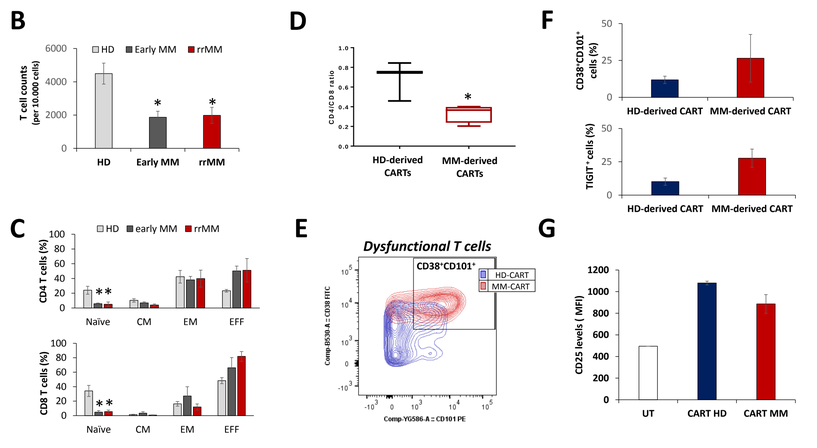


Figure 4 – **(A)** List of MM patients used to generate MM-derived anti-BCMA CAR T cells from peripheral blood samples: *early MM group* (n=6) refers to patients at diagnosis or up to two prior lines of treatment, *rrMM group* (n=4) refers to patients with 3 or more prior lines of treatment (eligible for anti-BCMA CAR T cell clinical trials). Healthy donor-derived anti-BCMA CAR T cells were also generated from healthy male volunteers (HD), age <30 (n=6). Patient ID, patient age, disease progression stage and treatment regimens are shown for each MM patient. **(B)** Comparison of T cell counts (per 10.000 PBMCs) from HD PBMCs *versus* early MM and rrMM PBMCs (at baseline Day 0). **(C)** Memory phenotype of CD4 and CD8 T cells from the different groups, measured by FACS, using CD45RO and CD62L to quantify: naïve, central memory (CM), effector memory (EF) and effector (EFF) T cells. **(D)** CD4/CD8 ratio of anti-BCMA CAR T cells derived from the different groups (at Day 14) measured by FACS. **(E)** Representative dot plot showing permanently dysfunctional T cells, characterized by CD38+CD101+, in HD-derived *versus* MM-derived CAR T cells. **(F)** Quantification of CD38+CD101+ and TIGIT+ T cells on HD-derived *versus* MM-derived CAR T cells (n=4). **(G)** Levels of the CD25 activation marker on UT, HD-derived CARTs and MM-derived CARTs (n=3), measured by FACS, after co-culture with U266 MM cells for 7 days. Data represent mean ± SEM. * $p < 0.05$, paired, 2-tailed t test.

CONCLUSIONS AND FUTURE DIRECTIONS

- To our knowledge, this is the first study showing that allogeneic anti-BCMA CAR T cells are therapeutically active against *primary* MM cells, in a clinically relevant model that includes the BM microenvironment;
- HD-derived anti-BCMA CAR T cells were shown to have distinct phenotypic and functional characteristics compared to rrMM-derived anti-BCMA CAR T cells;
- This work lends further support to the development of a first-in-human Phase 1 clinical trial for the treatment of rrMM patients using this allogeneic anti-BCMA CAR T cell therapy.

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