High-dose carfilzomib achieves superior anti-tumor activity over low-dose and recaptures response in relapsed/refractory multiple myeloma resistant to low-dose carfilzomib by co-inhibiting the β2 and β1 subunits of the proteasome complex

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Supplementary information:

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Supplementary Methods

Sampling and sample preparation

Patients' peripheral blood sample was collected into EDTA-coated tubes. Subsequently, peripheral blood mononuclear cells (PBMC) were purified from whole blood using Ficoll density gradient separation solution (Sigma-Aldrich) and washed twice with 0.04% phosphate buffered saline/bovine serum albumin (PBS/BSA). PBMCs were stored as dry cell pellets at -80°C until further processing.

Kd, KRD, and D-Kd regimens

In patients treated with Kd, KRD, and D-Kd combinations, we administered CFZ intravenously (IV) twice weekly. The dosing of CFZ was determined at the treating physicians' discretion, with approved CFZ doses of 20/56 mg/m^2, 20/27 mg/m^2, and 20/56 mg/m^2 in the Kd, KRD, and D-Kd combinations, respectively. In the KRD regimen, lenalidomide (LEN) was given QD orally, and LEN dose was determined by the treating physician, with a standard LEN dose of 25 mg QD (1). In D-Kd, daratumumab 8 mg/kg was administered intravenously on days 1 and 2 of the first cycle and at 16 mg/kg weekly for the remaining doses of the first two cycles, then Q2W in cycles 3-6, and Q4W thereafter (2). Dexamethasone, paracetamol, famotidine, and clemastine were administred before daratumumab treatment. The patients received anti-infective prophylaxis for *Pneumocystis jirovecii* (e.g., co-trimoxazole 960 mg QOD orally) and herpes virus (e.g., acyclovir 400 mg BID orally). All the patients were treated at the University Hospital of Würzburg.

Statistical evaluation

Descriptive statistics were analyzed using Microsoft Excel 2016 (Microsoft Corp., Redmond, WA, USA). Data are represented as absolute numbers and percentages or medians and ranges, unless otherwise stated. Survival analysis was performed using the Kaplan-Meier method. We calculated the percentage of proteasome subunit activity pre- versus 3 hours post-CFZ in each patient. Two-tailed unpaired Student's t-test was used to compare proteasome subunit inhibition in different subgroups. Fisher's exact test was used to compare the overall response rate (ORR) in RRMM patients treated with different doses of CFZ. One-way ANOVA was used to evaluate the differences in the characteristics of RRMM patients who received different CFZ-containing regimens (Kd, KRD, and D-Kd). The analyses were performed using GraphPad Prism 9.0 (GraphPad Software Inc., San Diego, CA, USA). Statistical significance was set at 0.05 (*P*-value <0.05). The data generated in this study are available upon reasonable request from the corresponding author.

Supplementary Table

Table S1: CFZ containing treatment regimens in 16 patients receiving CFZ dose escalation

No	Age	Gender	Regimen[§]	Dosing of agents other than CFZ/DEX/mAb/ADC[†]	Dosing of low-dose CFZ	Number of low-dose CFZ containing therapy cycles	CFZ dose escalation	Number of high-dose CFZ containing therapy cycles	Best response
1	57	Female	KD	/	20 mg/m^2 biw	1	56 mg/m^2 biw[#]	3	PR[‡]
2	45	Male	KCyD-Pom	Cy 300 mg/m^2 qw, POM 2 mg	27 mg/m^2 biw	2	56 mg/m^2 biw	6	VGPR
3	78	Male	KRD	REV 25 mg	27 mg/m^2 biw	1	36 mg/m^2 biw	7	VGPR
4	74	Male	D-KCyD	Cy 150 mg/m^2 qw	27 mg/m^2 biw	1	56 mg/m^2 biw	10	VGPR
5	69	Female	KCyD-BLENREP	Cy 250 mg on d1	27 mg/m^2 biw	1	56 mg/m^2 biw	1	SD
6	76	Male	D-KD	/	27 mg/m^2 biw	1	56 mg/m^2 biw	2	PR
7	61	Male	KRD	REV 25 mg	27 mg/m^2 biw	2	36 mg/m^2 biw	7	VGPR
8	60	Male	D-KCyD	Cy 300 mg/m^2 qw	27 mg/m^2 biw	1	56 mg/m^2 biw	5	VGPR
9	71	Male	DARA-KPD-PCE	POM 2 mg, CDDP 10 mg/m^2, Cy 400 mg/m^2, VP16 40 mg/m^2	27 mg/m^2 biw	3	56 mg/m^2 biw	3	PR
10	78	Female	D-KPD	POM 2 mg	27 mg/m^2 biw	2	56 mg/m^2 biw[$]	3	PR[‡]
11	64	Male	KRD	REV 25 mg	27 mg/m^2 biw	7	36 mg/m^2 biw	2	SD
12	77	Female	D-KCyD	Cy 300 mg on d1	27 mg/m^2 biw	3	56 mg/m^2 biw	1	SD
13	83	Male	DARA-KTD-PCE	CDDP 5 mg/m^2, Cy 200 mg/m^2, VP16 20 mg/m^2	27 mg/m^2 biw	3	36 mg/m^2 biw	6	SD
14	78	Female	ELO-KD	/	27 mg/m^2 biw	2	36 mg/m^2 biw	4	SD
15	61	Female	ELO-KD	/	15 mg/m^2 biw	8	36 mg/m^2 biw	5	SD
16	70	Male	KRD	REV 25 mg	27 mg/m^2 biw	4	36 mg/m^2 biw	1	PD

ADC - antibody drug conjugate; CDDP - cisplatin; CFZ - carfilzomib; Cy - cyclophosphamide; DEX - dexamethasone; D-KCyD - daratumumab, carfilzomib, cyclophosphamide, dexamethasone; D-KD - daratumumab, carfilzomib, dexamethosone; D-KPD - daratumumab, carfilzomib, pomalidomide, dexamethasone; DARA-KPD-PCE - daratumumab, carfilzomib, pomalidomide, dexamethasone, cisplatin, cyclophosphamide, etoposide; DARA-KTD-PCE - daratumumab, carfilzomib, thalidomide, dexamethasone, cisplatin, cyclophosphamide, etoposide; ELO-KD - elotuzumab, carfilzomib, dexamethasone; KCyD-BLENREP - carfilzomib, cyclophosphamide, dexamethasone, belantamab-mafodotin; KCyD-Pom - carfilzomib, cyclophosphamide, dexamethasone, pomalidomide; KD - carfilzomib, dexamethasone; KRD - carfilzomib, lenalidomide, dexamethasone; mAb - monoclonal antibody; POM - pomalidomide; REV - lenalidomide; VP16 - etoposide; [§] daratumumab, elotuzumab, dexamethasone and belantamab-mafodotin were given in standard dosing as approved in Germany; [†] the dosing of agents other than CFZ remained the same; [#] reduced to 45, 36, 27 and then 20 mg/m^2 biw due to fatigue grade 3; [$] reduced to 36 and then 27 mg/m^2 biw due to heart failure grade 3; [‡] patients No.1 and No.10 progressed after CFZ dose reduction due to fatigue and heart failure, respectively

Supplementary Figures

Figure S1: Multiplex activity-based protein profiling for assessment of proteasome active sites: (A) Structures of activity-based proteasome probes (ABPP) used for labelling the proteasome subunits in cell lysates. BODIPY(FL)-LU-112 labels the constitutive (c) and immune (i) -proteasome β2 subunits (β2c+i) and appears as a green color (Ex: 505 nm, Em: 511 nm), BODIPY(TMR)-NC-005-VS labels the β5c+i subunits and appears as a red color (Ex: 530 nm, Em: 560 nm) and Cy5-NC-001 labels the β1c+i subunits and appears as a blue color (Ex: 600 nm, Em: 690 nm). **(B)** Expected SDS-PAGE gel of all constitutive (c) and immuno-proteasome (i) subunits, labelled by the ABPP-cocktail.

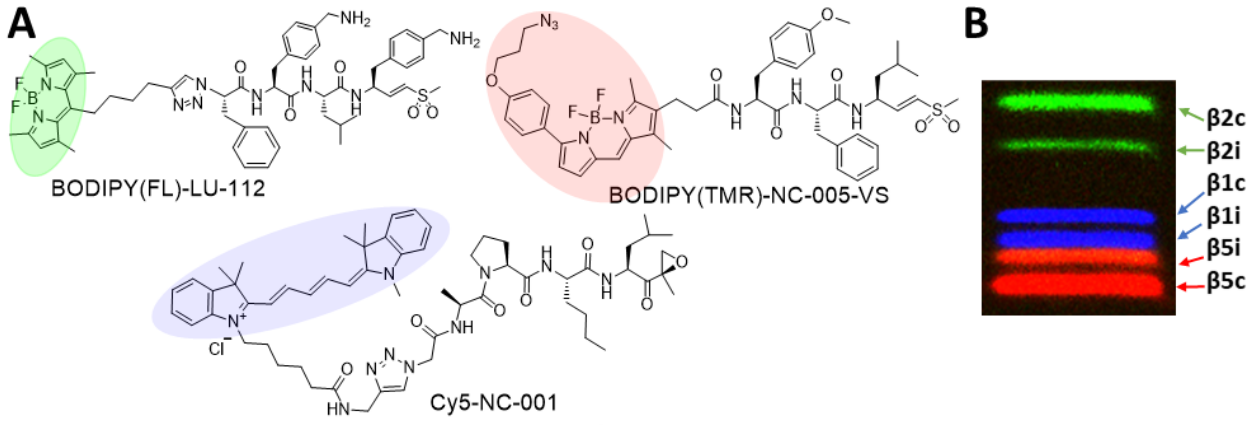


Figure S2: Overall response rate (ORR) in MM patients treated with Kd, KRD, and D-Kd. **(A)** shows that patients who received high-dose CFZ showed higher ORR than patients with low-dose CFZ in the entire group (77.8% vs 57.9%, *P*=0.05) of patients. **(B-D)** demonstrates ORR (high-dose vs low-dose CFZ) in subgroups the Kd, KRD and D-Kd regimens. Kd: 73.8% vs 15.4%, *P*=0.003; KRD: 90.9% vs 67.8%, *P*=0.16; D-Kd: 66.7% vs 50.0%, *P*>0.9999. CFZ - carfilzomib; D-Kd - carfilzomib, daratumumab, dexamethasone; Kd - carfilzomib, dexamethasone, KRD - carfilzomib, lenalidomie, dexamethasone.

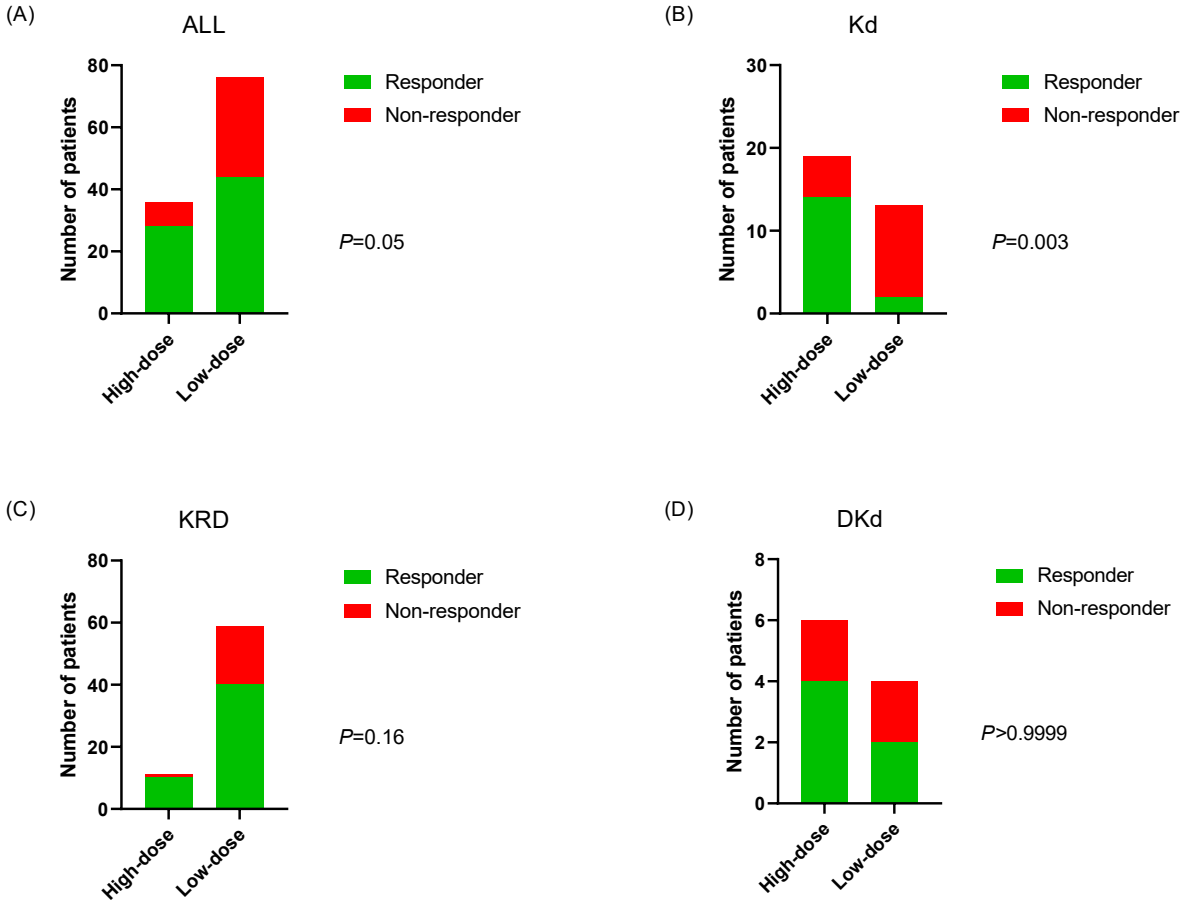
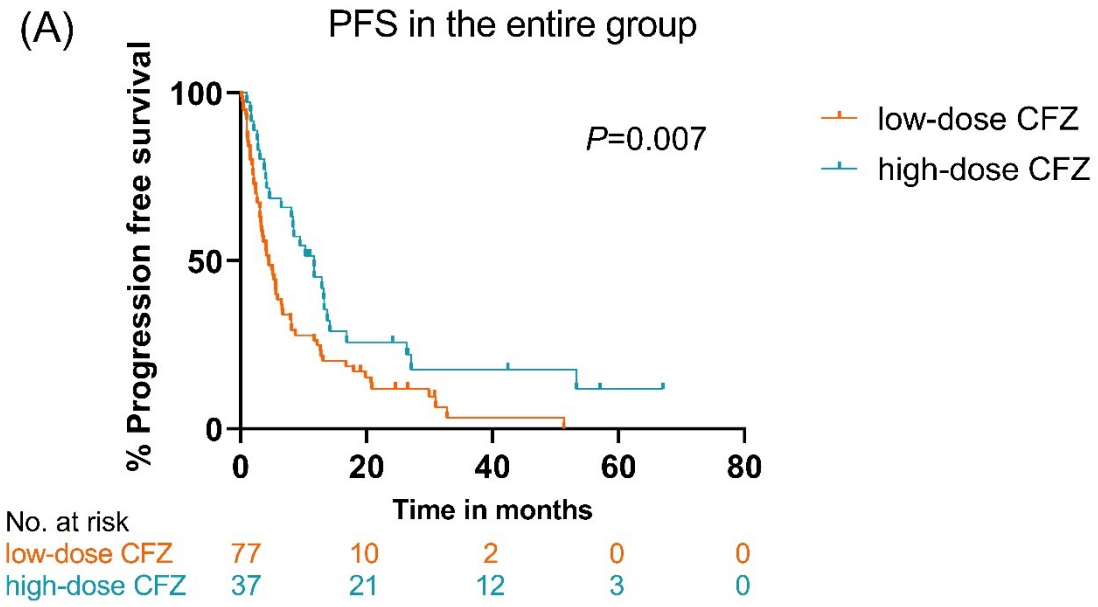


Figure S3: Survival outcome in the entire group of MM patients that received CFZ-containing regimens: Kd, D-Kd and KRD: The figures demonstrate **(A)** PFS and **(B)** OS of patients treated with high-dose CFZ versus low-dose CFZ in the entire group. CFZ - carfilzomib; D-Kd - carfilzomib, daratumumab, dexamethasone; Kd - carfilzomib, dexamethasone, KRD - carfilzomib, lenalidomide, dexamethasone; OS - overall survival; PFS – progression-free survival.



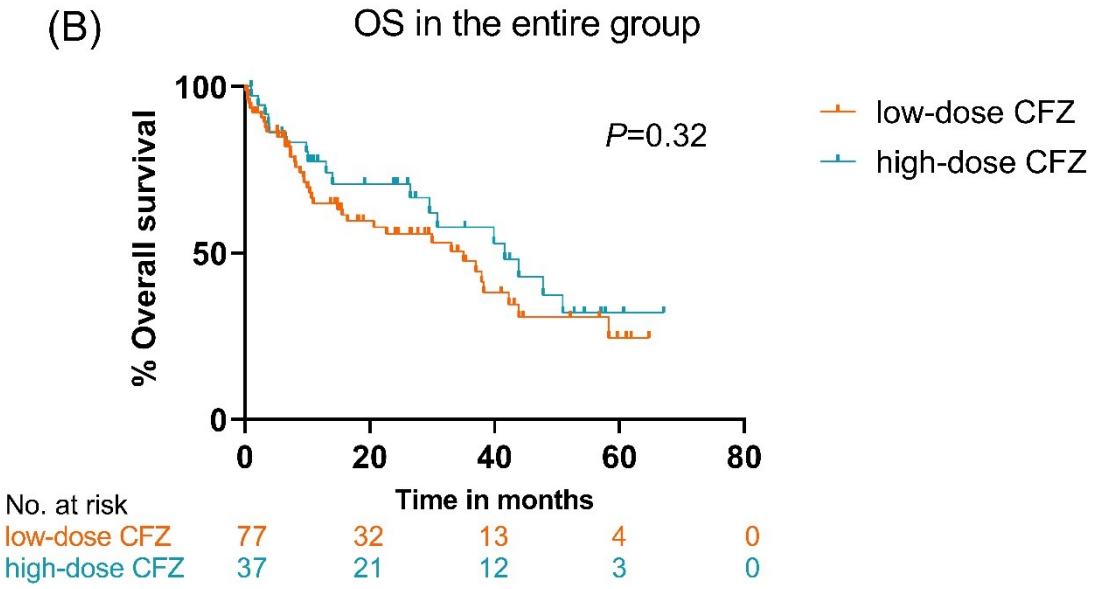


Figure S4: Hematologic adverse events in MM patients treated with Kd, KRD, and D-Kd: In the entire group, there was no significant difference in **(A)** anemia, **(B)** leukopenia, **(C)** thrombopenia, and **(D)** neutropenia between the high-dose versus low-dose carfilzomib groups of patients.

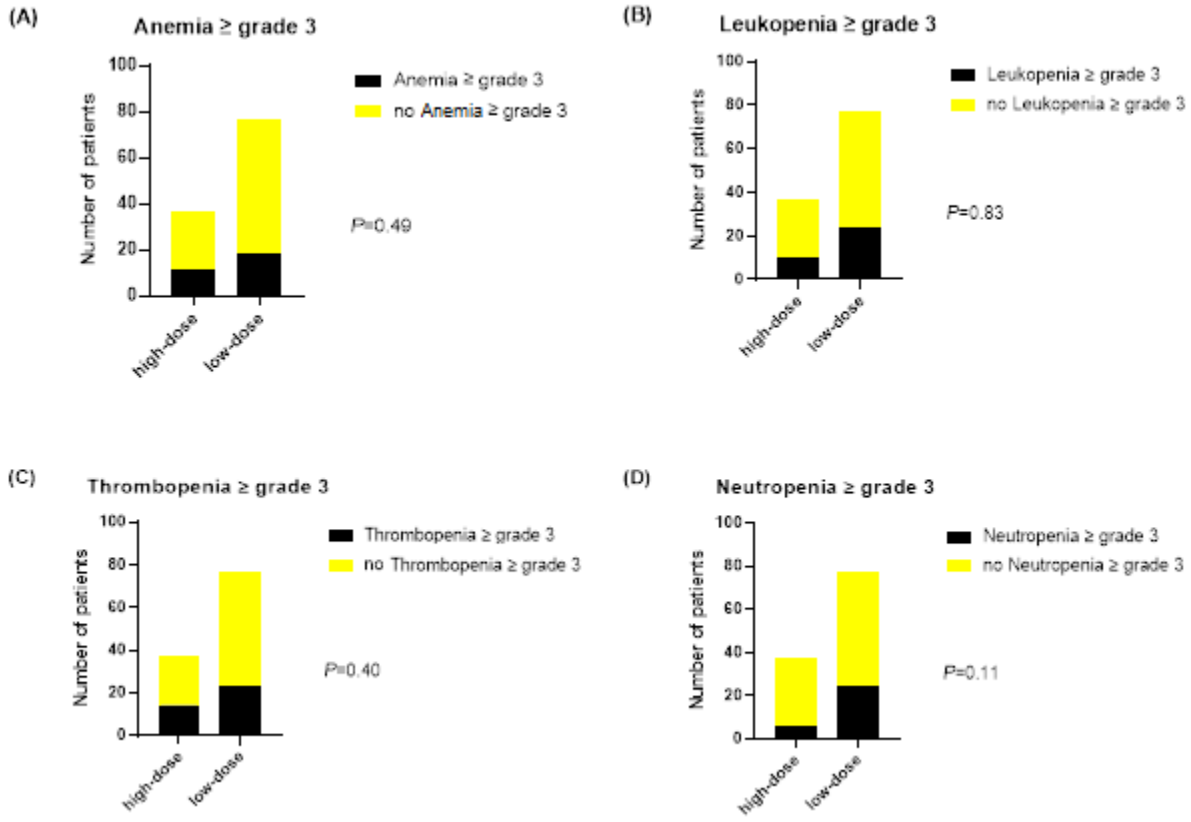


Figure S5: Proteasome subunit activity in a patient (patient #1 in Table S1) who had been treated with CFZ at 20 mg/m^2 and 36 mg/m^2: We observed a decrease of β2c+i and β5c+i proteasome subunit activity after CFZ dose escalation. CFZ - carfilzomib

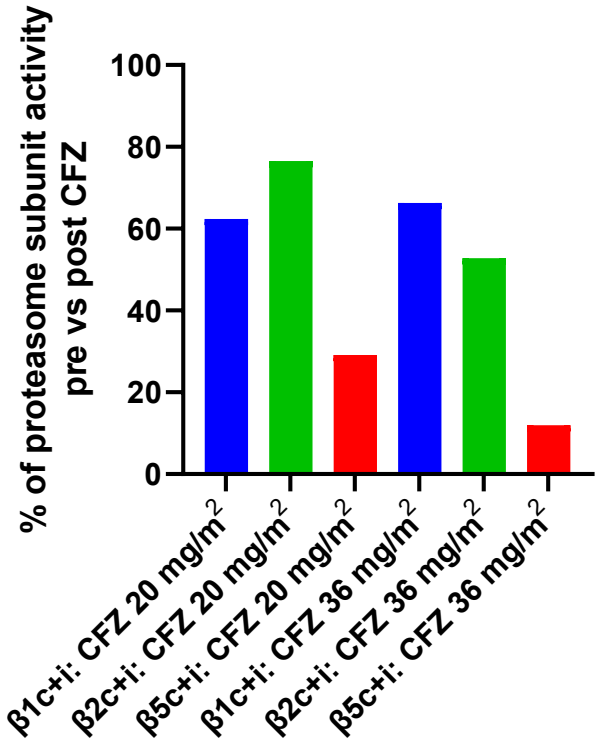
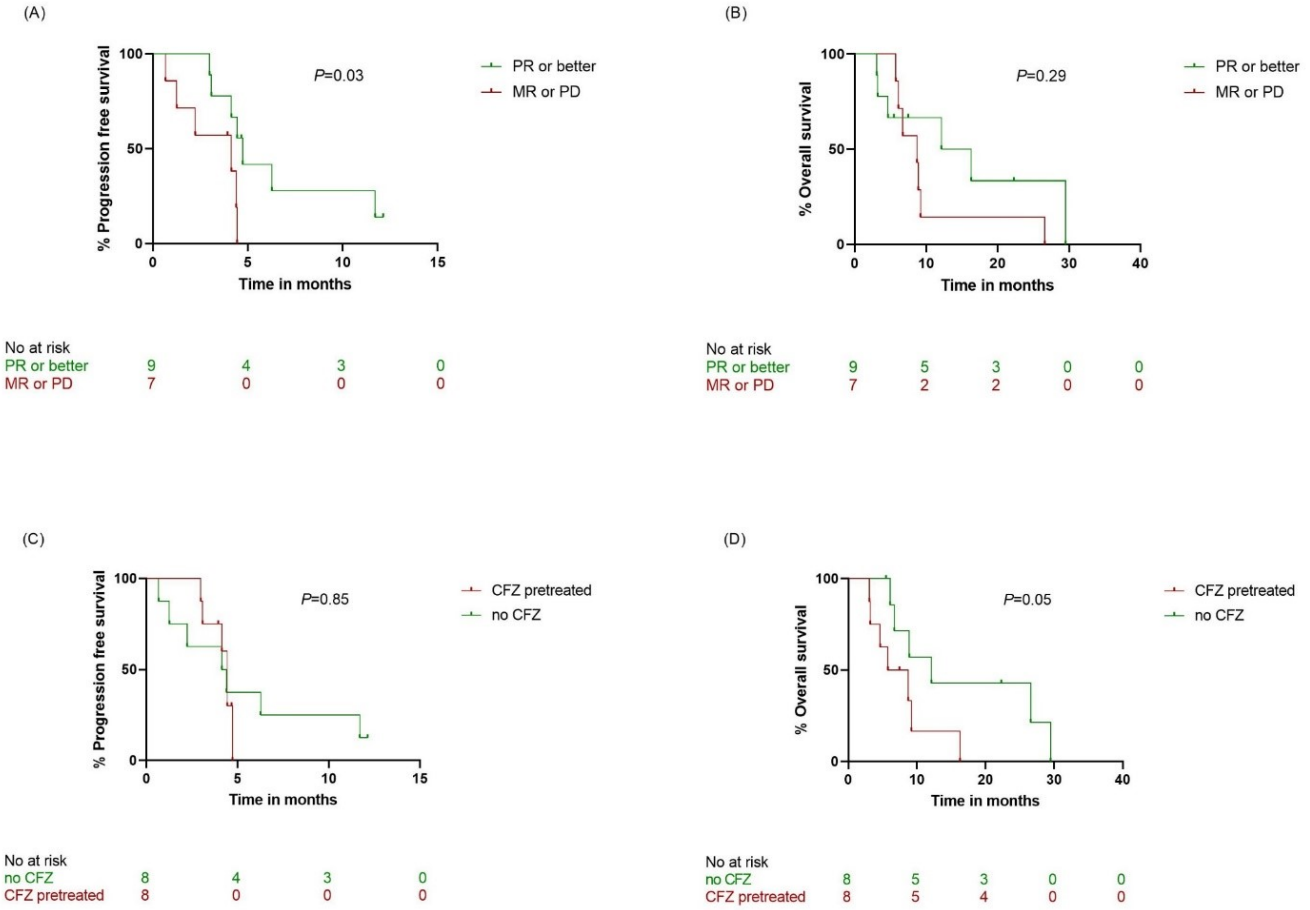


Figure S6: Survival analyses of MM patients who received CFZ dose escalation: (A-B) demonstrated that patients who achieved partial remission or better had superior progression free survival (PFS) compared to those progressing after CFZ dose escalation (median PFS: 4.72 vs 4.13 months). **(C-D)** displayed that patients who were not pretreated with CFZ in prior lines of therapies had superior overall survival (OS) in comparison to those having received CFZ previously (median OS: 12.13 vs 7.24 months). CFZ - carfilzomib; MR - minor response; PD - progressive disease; PR – partial remission



References

1. Groen K, van de Donk N, Stege C, Zweegman S, Nijhof IS. Carfilzomib for relapsed and refractory multiple myeloma. Cancer Manag Res. 2019;11:2663-75.
2. Dimopoulos M, Quach H, Mateos MV, Landgren O, Leleu X, Siegel D, et al. Carfilzomib, dexamethasone, and daratumumab versus carfilzomib and dexamethasone for patients with relapsed or refractory multiple myeloma (CANDOR): results from a randomised, multicentre, open-label, phase 3 study. Lancet. 2020;396(10245):186-97.