

Supplementary Materials:

B-Cell Regeneration Profile and Minimal Residual Disease Status in Bone Marrow of Treated Multiple Myeloma Patients

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Table S1. Clinical and laboratory features observed at diagnosis for the MM patients included in this study (n = 162).

Disease Features		% Patient Distribution
Sex	Male	57%
	Female	43%
Age at diagnosis	< 65y	62%
	≥ 65y	38%
ISS stage	I	39%
	II	30%
	III	31%
Cytogenetic risk*	High	29%
	Low	71%
Serum β 2-microglobulin (mg/L)	<5.5	69%
	≥ 5.5	31%
Albumin (mg/L)	< 3.5	34%
	≥ 3.5	66%
LDH (U/L)	Normal	65%
	Increased	35%
Type of M-component	IgG	61%
	IgA	19%
	IgD	1%
	BJ	13%
	Oligosecretory	3%
	Non-secretory	3%

Abbreviations: ISS, international staging system; LDH, lactate dehydrogenase; BJ, Bence-Jones; NA, not available. High-risk cytogenetic include del(17/17p), t(4;14), t(14;16) and/or, t(14;20). *not available in 59/162 cases.

Table S2. Distribution of maturation-associated normal/residual B-cell and nPC cell population in BM of HD *vs.* MM patients studied at diagnosis and at different time points after therapy.

Cell population (%)	Multiple myeloma			
	HD (n = 14)	At diagnosis (n = 25)	End of Induction (n = 27)	Post-ASCT (day + 100) (n = 94)
Total B-cells	2.6 (1–4.6)	1.2^a (0.4–3.3)	0.8^a (0.3–5)	3.3^b (0.05–11.4)
Pre-germinal center B-cells	1.7 (0.4–3.3)	0.5^a (0.07–2.2)	0.5^a (0.1–4.5)	3.1^{a, b} (0.003–11.2)
BCP	0.3 (0.09–1.7)	0.01^a (<0.0002–0.8)	0.3^b (<0.0002–2.3)	1.5^{a, b} (0.002–10.3)
Stage I BCP	0.02 (0.006–0.2)	0.0005^a (<0.0002–0.1)	0.02^b (<0.0002–0.7)	0.2^{a, b} (<0.0002–0.9)
Stage II BCP	0.3 (0.09–1.5)	0.01^a (<0.0002–0.7)	0.2^b (<0.0002–2.1)	1.3^{a, b} (0.002–9.4)
Stage I / stage II BCP ratio	0.1 (0.04–0.2)	0.02^a (0–0.2)	0.1^b (0–1.6)	0.09^b (0–1.2)
Transitional/naïve B-cells	1.1 (0.3–2)	0.4^a (0.05–1.9)	0.2^{a, b} (0.004–2.5)	1.5^b (0.008–5.7)
Post-germinal center B-cells	0.8 (0.4–1.4)	0.4^a (0.1–1.6)	0.2^{a, b} (0.03–0.6)	0.1^{a, b} (0.009–0.9)
Memory B-cells	0.4 (0.06–1.3)	0.3 (0.05–1.5)	0.07^{a, b} (0.005–0.5)	0.03^{a, b} (0.0005–0.5)
nPC	0.3 (0.08–0.9)	0.04^a (0.005–0.5)	0.06^a (0.008–0.4)	0.08^{a, b} (0.002–0.8)
CD19 ⁺ nPC	0.2 (0.03–0.6)	0.03^a (0.002–0.4)	0.05^a (0.006–0.4)	0.07^{a, b} (<0.0002–0.8)
CD19 ⁻ nPC	0.1 (0.02–0.3)	0.006^a (<0.0002–0.08)	0.009^a (<0.0002–0.07)	0.007^a (<0.0002–0.2)
CD19 ⁺ /CD19 ⁻ nPC ratio	2 (0.7–8.6)	2.2 (0–18.6)	5.8^{a, b} (0–147)	8.6^{a, b} (0–42.4)
Mature B-cells ‡	1.5 (0.4–3)	0.8^a (0.1–2.8)	0.4^{a, b} (0.009–2.6)	1.5^b (0.004–5.9)
BCP/Mature B-cell ratio	0.2 (0.08–1.6)	0.005^a (0–2.5)	0.6^b (0–30.2)	1.1^{a, b} (0–13.2)

Abbreviations: BCP, B-cell precursors; nPC, normal plasma cell; ‡ Mature B-cells (transitional/naïve B-cells plus memory B-cells); limit of detection of MRD of 0.0002% (2×10^{-6}); a *p*-value < 0.05 *vs.* HD, b *p* < 0.05 *vs.* MM at diagnosis (Mann Whitney-U test).

Table S3. Distribution of maturation-associated normal/residual B-cell and nPC cell populations in BM of MM patients studied at diagnosis and at different time points after therapy according to the patients' cytogenetic risk (standard *vs.* high risk).

Cell population (%)	At diagnosis (n = 21)		End of Induction (n = 22)		Post ASCT (day + 100) (n = 45)	
	Standard risk (n = 18)	High risk (n = 3)	Standard risk (n = 12)	High risk (n = 10)	Standard risk (n = 35)	High risk (n = 10)
Total B-cells	1 (0.4–2.1)	1.4 (0.5–3.3)	0.7 (0.25–2.7)	0.7 (0.3–5)	2.5 (0.6–11.4)	2.7 (1.1–4.4)
Pre-germinal center B-cells	0.4 (0.07–1.7)	1.1 (0.1–2.2)	0.4 (0.1–2.7)	0.3 (0.1–4.5)	2.4 (0.5–11.2)	2.6 (1.07–4.2)
BCP	0.009 (<0.0002–0.4)	0.4 (<0.0002–0.8)	0.2 (0.01–2.3)	0.07 (<0.0002–2.2)	1.2 (0.2–10.3)	1.4 (0.7–2.6)
Stage I BCP	0.0003 (<0.0002–0.06)	0.02 (<0.0002–0.1)	0.02 (0.001–0.3)	0.02 (<0.0002–0.7)	0.2 (0.009–0.9)	0.1 (0.04–0.4)
Stage II BCP	0.008 (<0.0002–0.4)	0.3 (<0.0002–0.7)	0.2 (0.01–2.1)	0.05 (<0.0002–2)	1 (0.1–9.4)	1.2 (0.5–2.3)
Stage I/stage II BCP ratio	0.01 (0–0.2)	0.07 (0–0.2)	0.1 (0.02–0.2)	0.3 (0–0.8)	0.1 (0.02–1.2)	0.1 (0.02–0.6)
Transitional/naive B-cells	0.4 (0.05–1.4)	0.3 (0.1–1.9)	0.2 (0.02–0.6)	0.2 (0.004–2.5)	1.3 (0.2–4.9)	1.1 (0.3–2.1)
Post-germinal center B-cells	0.4 (0.09–1.2)	0.4 (0.2–1.1)	0.2 (0.08–0.6)	0.2 (0.03–0.6)	0.08 (0.02–0.5)	0.08 (0.03–0.2)
Memory B-cells	0.07 (0.007–0.3)	0.09 (0.005–0.5)	0.07 (0.007–0.3)	0.09 (0.005–0.5)	0.03 (0.006–0.5)	0.03 (0.008–0.1)
nPC	0.03 (0.005–0.5)	0.1 (0.01–0.1)	0.06 (0.02–0.4)	0.1 (0.008–0.4)	0.05 (0.002–0.2)	0.04 (0.01–0.2)
CD19 ⁺ nPC	0.03 (0.002–0.4)	0.07 (0.005–0.1)	0.05 (0.02–0.4)	0.07 (0.007–0.4)	0.04 (0.0008–0.2)	0.03 (0.01–0.2)
CD19 ⁻ nPC	0.005 (<0.0002–0.008)	0.05 (0.007–0.05)	0.009 (0.002–0.06)	0.003 (0.0006–0.04)	0.005 (0.003–0.06)	0.007 (0.02–0.05)
CD19 ⁺ /CD19 ⁻ nPC ratio	4.5 (0–18.6)	1.4 (0.7–2.1)	6.9 (2.9–19.3)	7.2 (2.7–147)	6.9 (0–29.2)	8.3 (3.7–19.4)
Mature B-cells †	0.8 (0.1–1.8)	0.6 (0.3–2.8)	0.3 (0.03–0.7)	0.5 (0.009–2.6)	1.4 (0.3–5)	1.2 (0.3–2.2)
BCP/Mature B-cell ratio	0.004 (0–2.5)	0.1 (0–1.4)	0.9 (0.03–5.9)	0.2 (0–30.2)	0.9 (0.1–10.4)	1.5 (0.4–3.2)

Abbreviations: BCP, B-cell precursors; nPC, normal plasma cell; † Mature B-cells (transitional/naïve B-cells plus memory B-cells); limit of detection of MRD of 0.0002% (2×10^{-6}); *, $p < 0.05$ for comparison between MM samples standard risk *vs.* high-risk (Mann Whitney-U test). High-risk cytogenetic include del(17/17p), t(4;14), t(14;16) and/or, t(14;20).

Table S4. Distribution of maturation-associated normal/residual B-cell and nPC cell populations in BM of MM patients studied at day+100 post-autologous stem cell transplantation, according to the type of induction therapy received.

Cell Population (%)	Post ASCT (day+100) (n=93)	
	PI plus IMiDs plus steroids (n = 43) ^a	PI plus steroids or IMiDs plus steroids (n = 50) ^b
Total B-cells	2.5 (1.1–11.4)	4.3 * (0.05–11)
Pre-germinal center B-cells	2.4 (0.5–11.2)	4 * (0.003–11)
BCP	1.2 (0.06–12.3)	1.9 * (0.002 -6.2)
Stage I BCP	0.2 (0.002–0.9)	0.2 (<0.0002 -0.6)
Stage II BCP	1 (0.06–9.4)	1.8 * (0.002–6)
Stage I/stage II BCP ratio	0.1 (0.02 -1.2)	0.8 * (<0.0002–0.5)
Transitional/naive B-cells	1.3 (0.09–5)	1.7 * (0.0008–6)
Post-germinal center B-cells	0.1 (0.02 -0.6)	0.2 * (0.009–0.9)
Memory B-cells	0.03 (0.005–0.5)	0.03 (0.005–0.2)
nPC	0.05 (0.02–0.6)	0.1 * (0.008–0.8)
CD19 ⁺ nPC	0.04 (0.008–0.4)	0.1 * (<0.0002–0.8)
CD19 ⁻ nPC	0.007 (0.003–0.2)	0.01 (<0.0002 -0.1)
CD19 ⁺ /CD19 ⁻ nPC ratio	6.3 (<0.0002–24.1)	11 (<0.0002–42.4)
Mature B-cells ¥	1.3 (0.1–5)	1.8 * (0.004–6)
BCP/Mature B-cell ratio	0.9 (0.09–13.2)	1.2 (<0.0002–4)
% (n/n) MRD+ status	60% (26/43)	58% (29/50)
PFS in months	32	NR

Abbreviations: BCP, B-cell precursors; nPC, normal plasma cell, MRD minimal residual disease, PFS progression free-survival; ¥ Mature B-cells (transitional/naïve B-cells plus memory B-cells); limit of detection of MRD of 0.0002% (2x10⁻⁶); * *p* < 0.05 for comparison between different of types of protocols induction to prior ASCT (Mann Whitney-U test). ^a in this group was included: PI+IMiDs+steroids (n = 40), PI+IMiDs+steroid+MoAb (n = 1), PI+IMiDs+steroids+Chemotherapy (VDL-Page, n = 2); ^b in this group was included: PI+ cyclophosphamide+steroid (n = 22), cyclophosphamide+IMiDs+steroids (n = 19), PI+IMiDs+steroids+ cyclophosphamide (n = 3) PI+steroid (n = 3), IMiD+steroid (n = 2).