

Supplementary methods section – MRD by NGS after FCR

Blood was collected into acid-citrate-dextrose or heparin tubes. Samples were centrifuged at 1500rpm for 10 minutes, then plasma aliquoted into 2 mL cryogen vials, which are stored at -80C. Mononuclear cells were separated using Ficoll Hypaque®. Subsequently, the mononuclear cells were re-suspended in PBS and centrifuged again at 1500rpm for 10 minutes. The supernatant was then discarded and the cell pellets re-suspended in FBS with 10% DMSO and aliquoted into cryogen vials with 1mL FBS/vial. Vials were stored long-term in liquid nitrogen.

Bone marrow was collected in heparin tubes. It was centrifuged and plasma aliquoted off as outlined above. Subsequently, the buffy coat and red cells were placed in 10mL of lysis buffer for 10 minutes, re-centrifuged and supernatant discarded. The cell pellet was re-suspended in PBS and centrifuged a third time to wash cells, re-centrifuged and the supernatant discarded. The pellet was then re-suspended in FBS with 10% DMSO and aliquoted into cryogen vials with 1mL FBS/vial. Vials were stored as above.

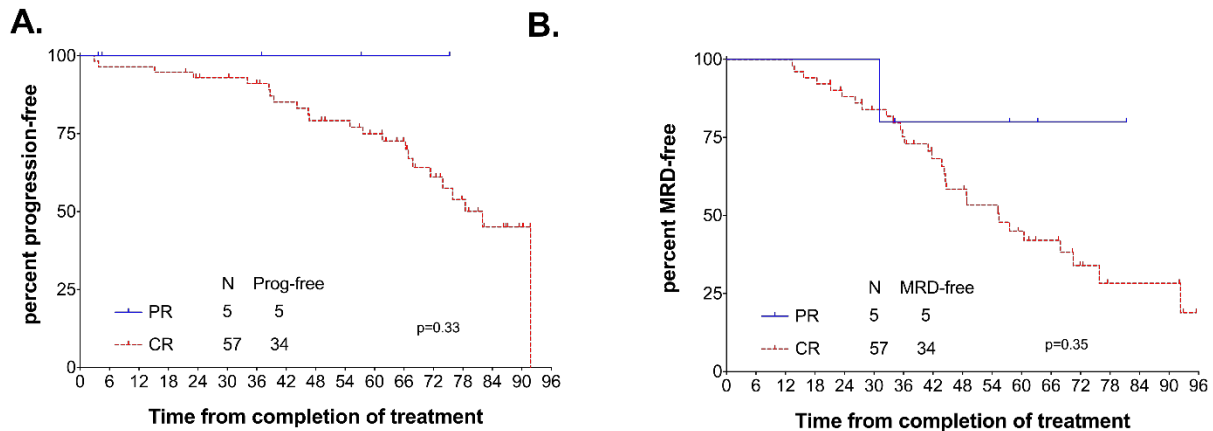
Supplementary Table 1 – Univariable analysis for likelihood of U-MRD by NGS according to pre-treatment characteristics in the total treated cohort.

Characteristic	Ref level	N	OR (95% CI)	p-value
Age <65	≥65	176	0.75 (0.23-2.46)	0.63
Rai stage 0-II	III-IV	176	2.47 (0.76-7.97)	0.13
β ₂ -microglobulin <4.0g/L	≥4.0g/L	172	1.37 (0.48-3.92)	0.56
ZAP70 Neg	ZAP70 Pos	157	1.83 (0.60-5.58)	0.29
FISH trisomy 12	Not Trisomy 12	172	4.22 (1.36-13.07)	0.01
IGHV mutated	Unmutated	175	5.99 (1.84-19.51)	0.003

Supplementary Table 2 – Univariable analysis for PFS in patients MRD+ in bone marrow.

Prognostic factor	Hazard ratio for progression or death	P value
Age ≥ 65 vs < 65	2.6 (0.7-9.1)	0.15
Rai stage III-IV vs 0-II	0.9 (0.3-2.4)	0.89
B2M ≥ 4.0 g/l vs < 4.0 g/l	2.4 (0.97-6.1)	0.06
ZAP70+ vs ZAP70-	1.2 (0.4-3.1)	0.77
UM-IGHV vs M-IGHV	3.2 (1.1-9.5)	0.03
Del(11q) or del(17p) vs other	1.9 (0.7-5.0)	0.19

Supplementary Figure 1.



Supplementary Figure 1. A. PFS according to clinical response. B. Time to MRD re-emergence (B) according to clinical response.