Supplementary Methods

NCRI Myeloma XI trial patients

Patients with newly diagnosed symptomatic MM and were enrolled onto the National Cancer Research Institute (NCRI) Myeloma XI trial (NCT01554852, CRUK/09/014), a phase III, open-label randomised controlled trial. Patients were randomly assigned between triplet immunomodulatory drug (IMiD) induction of either cyclophosphamide, thalidomide, and dexamethasone or cyclophosphamide, lenalidomide, and dexamethasone. Patients exhibiting a suboptimal response (i.e. less than a very good partial response) were randomly assigned to pre-transplant treatment with a proteasome inhibitor triplet (cyclophosphamide, bortezomib, and dexamethasone) or no further therapy. All primary refractory patients received the proteasome inhibitor triplet. Older or less fit patients did not receive an autologous stem-cell transplant (non-intensive pathway), whereas young and fit patients received single high dose melphalan consolidation followed by stem cell support (intensive pathway). Patients were subsequently randomly assigned to either no maintenance, maintenance with lenalidomide or lenalidomide and vorinostat (**Supplementary Fig. 1**).

MMRF CoMMpass study patients

For 624 MM patients with WES data from the CoMMpass study IA9 data tranche, mutation VAFs were adjusted when present on recurrent amplified hyperdiploid chromosomes and R^2 could be called for 587 baseline samples. Following the censoring of cases where death had occurred ≤ 2 months, there were 221 CoMMpass patients that received an autologous transplant and received an IMiD containing therapy, and 213 CoMMpass patients that did not receive an autologous transplant and received an IMiD containing therapy available for analysis. Translocations status and copy number abnormalities from CoMMpass data were called from WGS long-insert, exome and RNA sequencing (FISH-seq). Additionally hyperdiploid cases with no detected translocation by FISHseq, but with a positive original FISH result for an IgH translocation were set as missing.

Myeloma XI Whole Exome Sequencing data

Whole exome sequencing of matched tumor-normal samples from the Myeloma XI trial has been previously described.[1,2] Briefly, plasma cells were isolated from bone marrow samples using CD138+ MACSorting (Miltenyi Biotech, Bisley, United Kingdom) and WES of germline and tumor samples performed using an Agilent SureSelect Human All Exon (38 Mb) capture probeset (Agilent, Santa Clara, CA, USA) in conjunction with Illumina HiSeq2000 technology (Illumina, San Diego, CA, USA). Variants were called using MuTect (version 1.1.4) according to best practice. The overall median depth was 60.1x, with the tumors samples at 61.0x median depth and the matched normal controls at 59.7x.

Exome sequencing data was available for 463 patients from the NCRI Myeloma XI trial. Of these, 107 patients could not be called for tumor neutrality, one patient was lost to follow-up and 22 patients died early in the study (< 2 months), leaving 333 for the current analysis for whom demographics are detailed in **Supplementary Table 1**. Progression-free survival (PFS) and overall survival (OS) were measured from trial entry; median follow-up time as of February 2016 was 47 months (range: 2 to 53 months). The median PFS was 26.9 months (95% confidence interval [CI]: 24.6-29.6 months), and the 3-year OS rate was 73.6% (95% CI: 69.4-77.9%). Response status was assessed at the end of induction treatment.

Myeloma XI MLPA and PCR-based translocation detection

Copy number at each locus for the Myeloma XI study was assessed by MLPA using the SALSA MLPA P425-B1 multiple myeloma probemix (MRC Holland, Amsterdam, The Netherlands), as previously described.[3] Multiplexed qRT-PCR was used to assess expression of IGH translocation partner genes and a FISH-validated translocation and cyclin-D (TC) classification-based hierarchical algorithm was applied to determine *IGH* translocation status, as previously described.[4]

Statistical analysis

All statistical tests were performed using R version 3.2.1. The relationship between neutral tumors (R^2>0.98) and categorical variables was examined by the Fisher exact test. The relationship between R^2 score and categorical variables was assessed using the Wilcoxon

rank sum test. A two-sided *P*-value <0.05 was considered significant. Differences between survival functions were tested using the log-rank test. Multivariable stepwise variable selection was performed using a standard backward-elimination approach, variables were retained at a level of significance *P*<0.05.

Supplementary References

1. Walker BA, Boyle EM, Wardell CP, et al. Mutational Spectrum, Copy Number Changes, and Outcome: Results of a Sequencing Study of Patients With Newly Diagnosed Myeloma. *J Clin Oncol*. 2015;33(33):3911-3920.
2. Walker BA, Wardell CP, Murison A, et al. APOBEC family mutational signatures are associated with poor prognosis translocations in multiple myeloma. *Nat Commun*. 2015;6:6997.
3. Boyle EM, Proszek PZ, Kaiser MF, et al. A molecular diagnostic approach able to detect the recurrent genetic prognostic factors typical of presenting myeloma. *Genes Chromosomes Cancer*. 2015;54(2):91-98.
4. Kaiser MF, Walker BA, Hockley SL, et al. A TC classification-based predictor for multiple myeloma using multiplexed real-time quantitative PCR. *Leukemia*. 2013;27(8):1754-1757.

Neutral tumor evolution in myeloma is associated with poor prognosis

SUPPLEMENTARY FIGURES AND TABLES

Supplementary Figure 1: Overview of the Myeloma XI trial design. ASCT, autologous stem-cell transplantation; CR, complete response; CRD, cyclophosphamide, lenalidomide, and dexamethasone; CRDa, attenuated CRD; CTD, cyclophosphamide, thalidomide, and dexamethasone; CTDa, attenuated CTD; CVD, cyclophosphamide, bortezomib, and dexamethasone; MR, minimal response; NC, no change; PD, progressive disease; PR, partial response; VGPR, very good partial response

Supplementary Figure 2: Distribution of VAF and R^2 across neutral and non-neutral samples. a) Top panel show distribution of the variant allele frequencies (VAFs) of sub-clonal mutations across all neutral samples in the Myeloma XI study, bottom panel show the cumulative mutations versus 1/allele frequency across all neutral samples in the Myeloma XI study. b) Top panel show distribution of the variant allele frequencies of the sub-clonal mutations across all non-neutral samples in the Myeloma XI study, bottom panel show the cumulative mutations versus 1/allele frequency across all non-neutral samples in the Myeloma XI study.

Supplementary Figure 3: Supplementary Figure 4: Median number of mutations versus VAF for neutral and non-neutral samples. a) Median count of mutations across neutral samples. b) Median count of mutations across non-neutral samples. Medians were assessed in VAF bin of 0.01.

Supplementary Figure 4: Violin plot of R^2 by subtype in Myeloma XI study patients at presentation. The distribution shows kernel density estimation where a broader shape represents a higher probability of a value**.** Thick black bar represents the interquartile range. Thin line represents the 95% confidence interval. The dotted line corresponds to the R^2=0.98 threshold for discriminating neutral from non-neutral tumors.

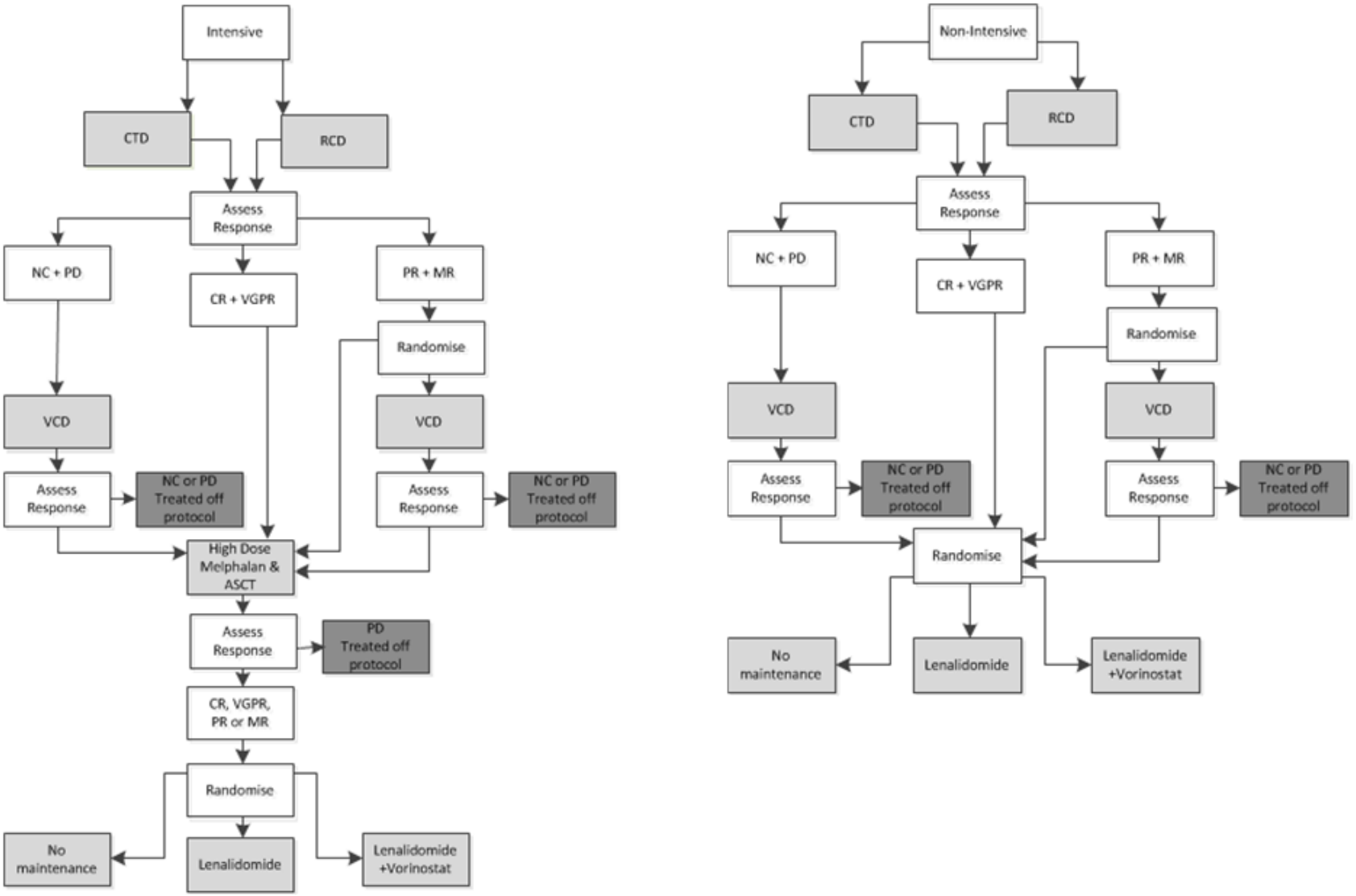
Supplementary Figure 5: **Violin plot of R^2 by subtype in CoMMpass study patients at presentation.** The distribution shows kernel density estimation where a broader shape represents a higher probability of a value. Thick black bar represents the interquartile range. Thin line represents the 95% confidence interval. The dotted line corresponds to the R^2=0.98 threshold for discriminating neutral from non-neutral tumors.

Supplementary Figure 6: Interaction of neutral and non-neutral tumours with microenvironment modulating agents. Far left diagrams depict clonal growth of sub-clones in neutral and non-neutral examples. Middle plots show typical distribution of the variant allele frequencies (VAFs) and a typical plot of cumulative mutations versus 1/allele frequency for neutral and non-neutral samples. Far right diagrams show the myeloma microenvironment. A myeloma cell is shown blue, an osteoclast in orange and bone marrow stromal cell in green. Purple arrows represent the relative response to IMiDs. Red stars represent mutations that allow adaption to microenvironment. Yellow stars represent proliferative mutations.

Supplementary Table 1: Clinical demographics of Neutral and Non-Neutral groups in Myeloma XI

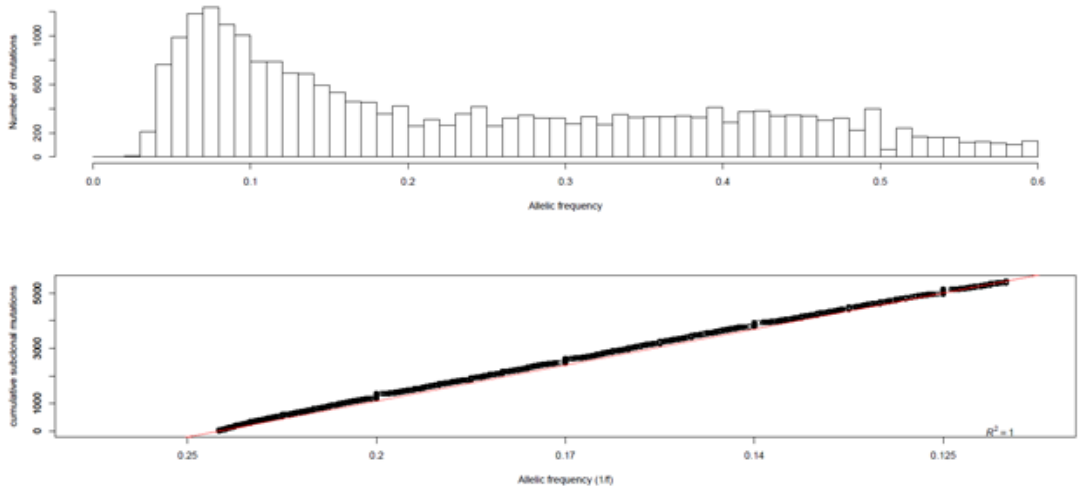
Supplementary Table 2: Multi-variant analysis of prognostic clinical markers with neutral tumor status. Multivariable stepwise variable selection was performed using a standard backward-elimination approach, variable were retained at a level of significance $P<0.05$.

Supplementary Figure 1: Overview of the Myeloma XI trial design

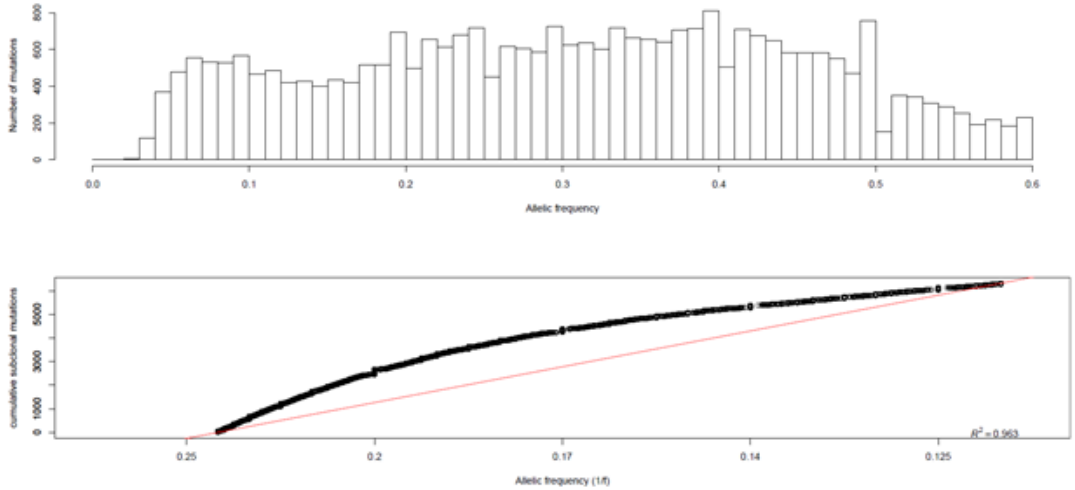


Supplementary Figure 2: Distribution of VAF and R^2 across neutral and non-neutral samples

a) Neutral - 65 samples

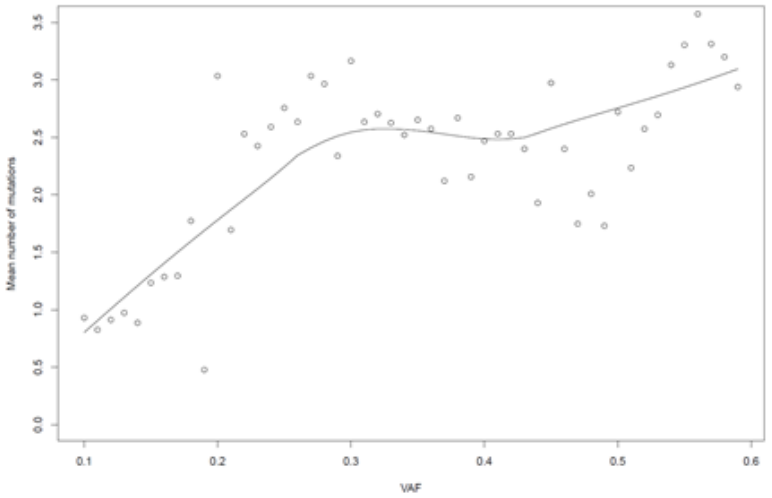


b) Non-neutral -268 samples

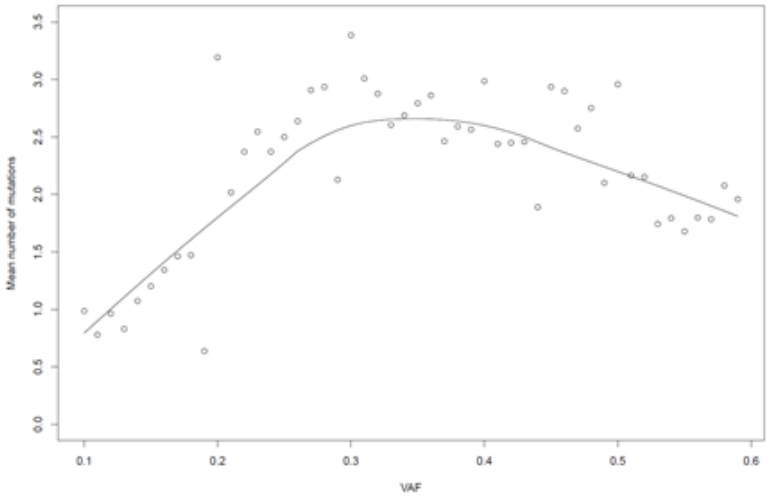


Supplementary Figure 3: Median number of mutations versus VAF for neutral and non-neutral samples

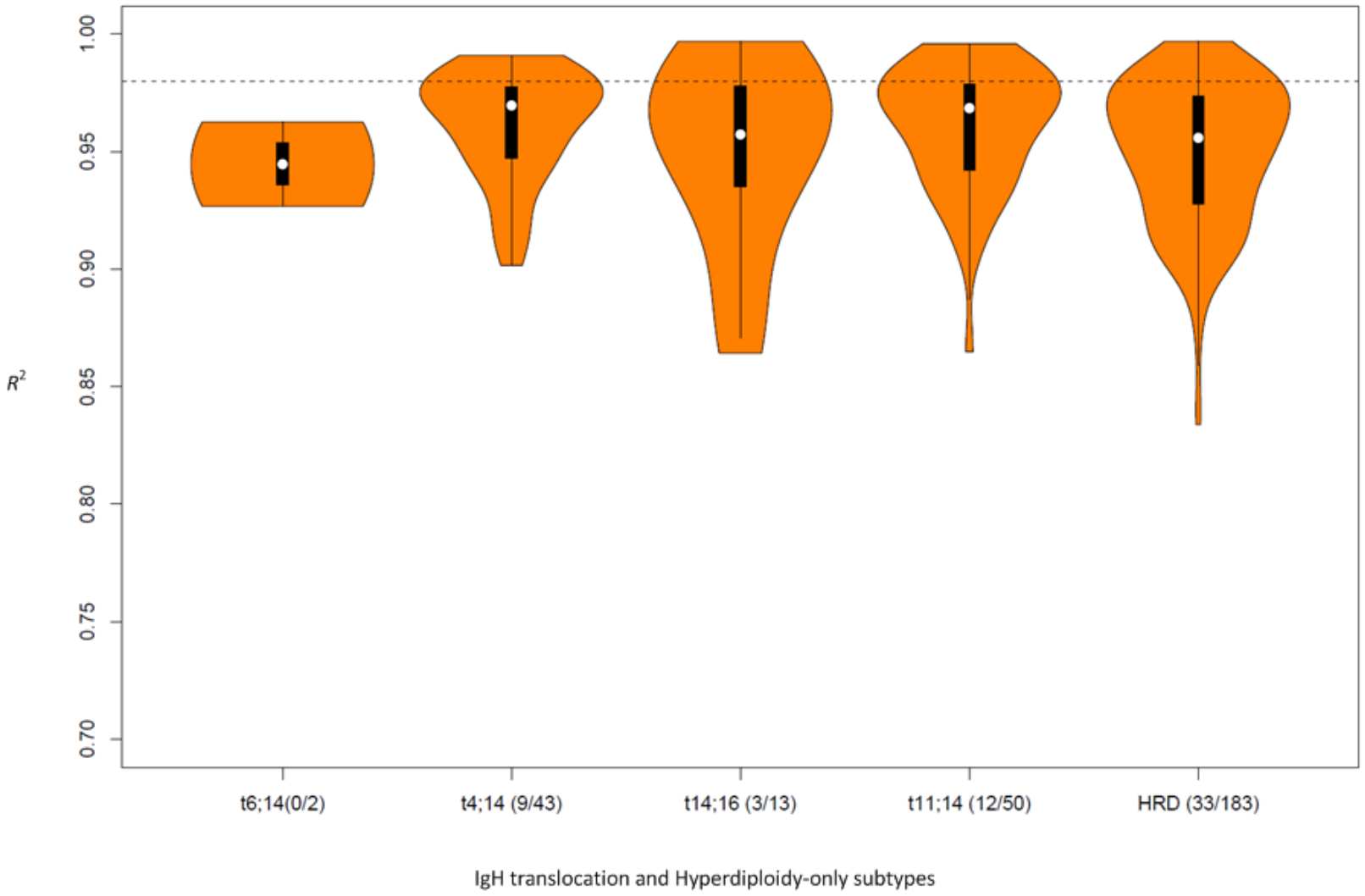
a) Neutral - 65 samples



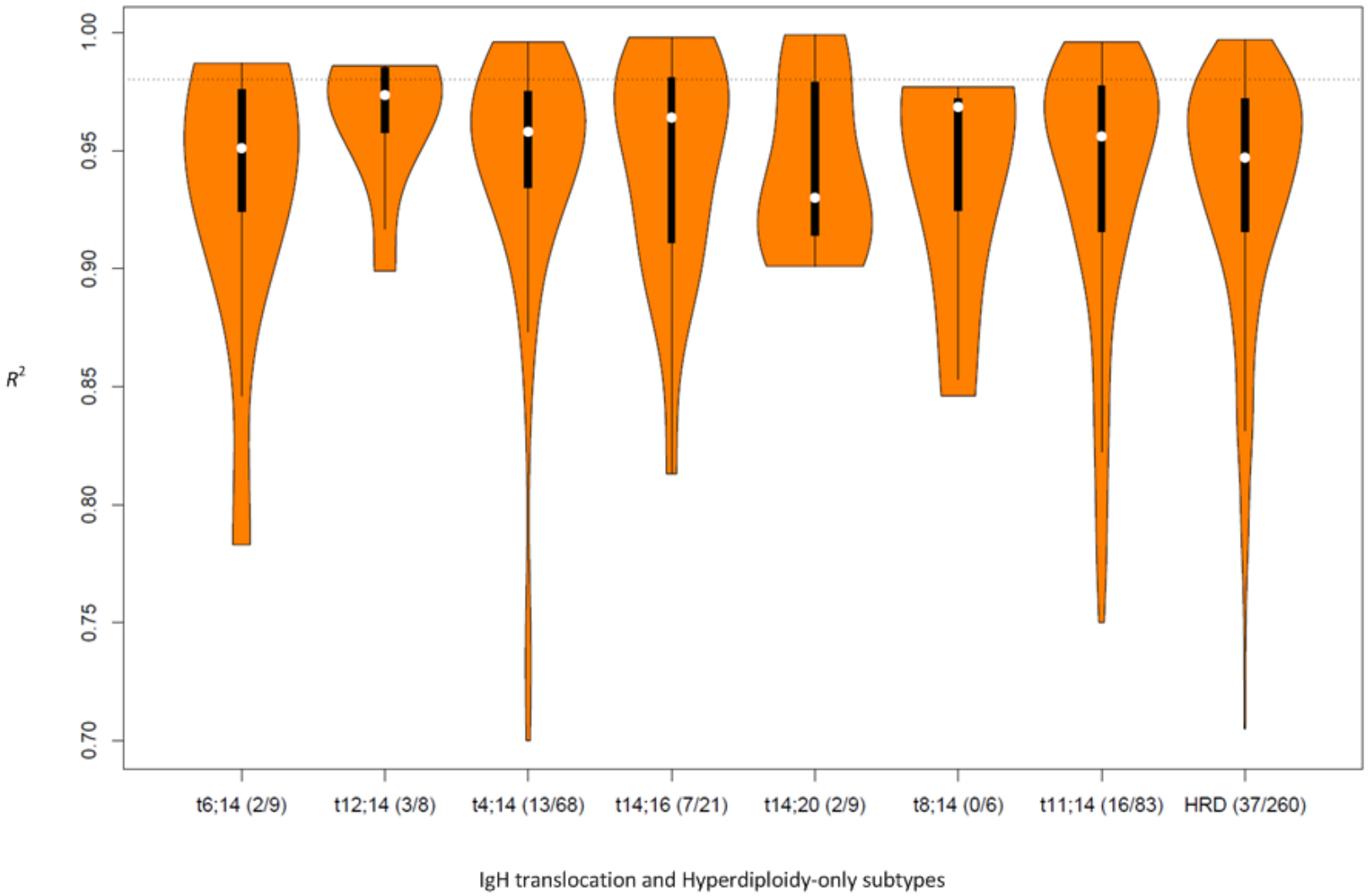
b) Non-neutral -268 samples



Supplementary Figure 4: Violin plot of R^2 by subtype in Myeloma XI study patients at presentation

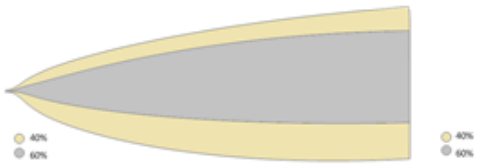


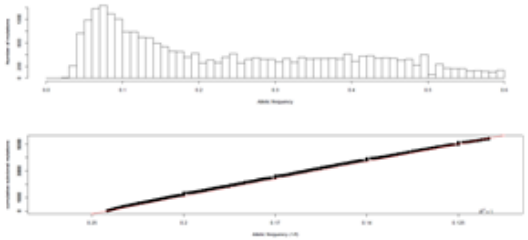
Supplementary Figure 5: Violin plot of R^2 by subtype in CoMMpass study patients at presentation

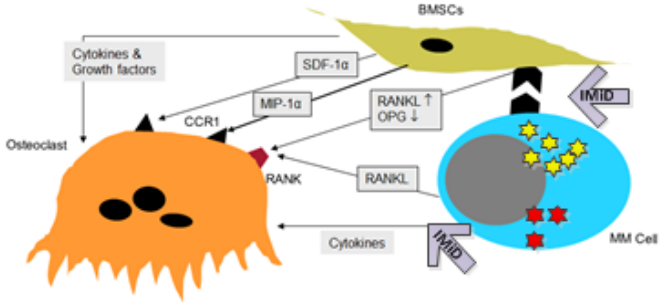


Supplementary Figure 6: Interaction of neutral and non-neutral tumours with microenvironment modulating agents

Neutral samples







Neutrally evolving tumors → Linear accumulation of mutations High R^2 (R^2 >0.98) → Fewer adaptive mutations due to lesser dependence on the microenvironment

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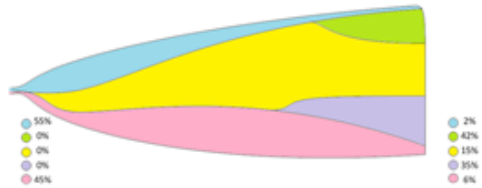
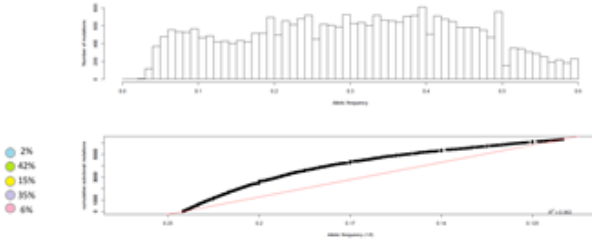
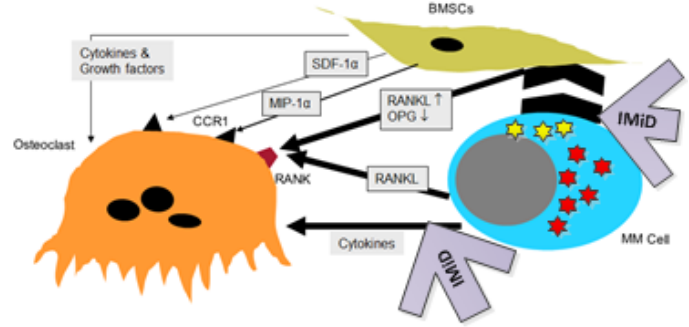
Worse outcome in response to microenvironment modulating agents

Proliferative Mutation
Adaptive Mutation

Non-neutral







Evolving under Darwinian selection → More clonal accumulation of mutations low R^2 (R^2 <0.98) → More adaptive mutation with greater dependence on the microenvironment

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Better outcome in response to microenvironment modulating agents

Supplementary Table 1: Clinical characteristics and demographics of patients

	Neutral	Non Neutral
Number of cases	65	268
Median age at MM diagnosis	68	67
Gender		
Male	36 (55.4%)	167 (62.3%)
Female	29 (44.6%)	101 (37.7%)
ISS		
I	12 (18.5%)	88 (23.5%)
II	26 (40.0%)	86 (39.0%)
III	24 (36.9%)	73 (33.0%)
NA	3 (4.6%)	21 (4.5%)
Serum albumin (g/l)	35	36
B2 microglobulin (g/l)	4.6	4
Lactate dehydrogenase IU/L	261.5	274
WHO performance stage		
0	17 (26.2%)	86 (32.0%)
1	28 (43.0%)	98 (36.6%)
2	9 (13.9%)	37 (13.8%)
≥3	2 (3.0%)	14 (5.2%)
NA	9 (13.9%)	33 (12.3%)
Bone disease		
Yes	47 (72.3%)	196 (73.1%)
No	18 (27.7%)	68 (25.4%)
NA	0 (0%)	4 (1.5%)
Heavy chain paraprotein		
IgG	38 (58.5%)	157 (58.6%)
IgA	16 (24.3%)	75 (28.0%)
IgD	0 (0%)	6 (2.2%)
LCO	10 (15.4%)	22 (8.2%)
NA	1 (1.5%)	8 (3.0%)
Light chain paraprotein		
Lambda	23 (34.7%)	89 (33.2%)
Kappa	42 (65.3%)	182 (67.8%)
Hyperdiploidy		
Yes	35 (46.3%)	157 (58.6%)
No	28 (52.4%)	110 (41.0%)
NA	2 (1.2%)	1 (0.4%)
Sequencing		
Mutations	131	134.5
Coding mutations	52	50
Non Synomous mutations	39	37
TiTv	2.8	2.73
Median depth	62	60
COVERAGE_20X	87.4	86.9

Supplementary Table 2: Multi-variant analysis of prognostic clinical markers with neutral tumor status

PFS multivariate non-intensive

Approximate Estimates after Deleting Factors

	Coef	S.E.	Wald Z	*P*
R^2	0.6769	0.2447	-2.766	0.00568
1q gain	0.5921	0.2012	2.943	0.003249
TP53 mut/bi-allelic	1.2648	0.4819	2.625	0.008677

Deleting Factors : HRD, ISS=3, Male, Adverse Translocations, MYC Translocation

OS multivariate non-intensive

Approximate Estimates after Deleting Factors

	Coef	S.E.	Wald Z	*P*
R^2	-1.2418	0.3306	-3.756	1.72×10^{-4}
ISS=3	0.7131	0.2765	2.579	9.91×10^{-3}
1q gain	0.9601	0.2907	3.302	9.59×10^{-4}
TP53 mut/bi-allelic	2.1613	0.5298	4.079	4.52×10^{-5}

Deleting Factors : HRD, Male, Adverse Translocations, MYC Translocation