Neutral tumor evolution in myeloma is associated with poor prognosis

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ABSTRACT

Recent studies suggest that the evolutionary history of a cancer is important in forecasting clinical outlook. To gain insight into the clonal dynamics of multiple myeloma (MM) and its possible influence on patient outcome we analysed whole exome sequencing tumor data for 333 patients from Myeloma XI, a UK phase III trial and 434 patients from the CoMMpass study, all of which had received immunomodulatory therapy (IMiD). By analysing mutant allele frequency distributions in tumors we found that 17-20% of MM is under neutral evolutionary dynamics. These tumors are associated with poorer patient survival in non-intensively treated patients, consistent with reduced therapeutic efficacy of micro-environment modulating IMiD drugs. Our findings provide evidence that knowledge of the evolutionary history of MM has relevance for predicting patient outcome and personalising therapy.

Key words: Tumor evolution, sequencing, multiple myeloma, prognosis

Main text: 1,066 words, 17 references

INTRODUCTION

Advances in the treatment of multiple myeloma (MM) in form of proteasome inhibitors and immunomodulatory drugs (IMiDs) have significantly improved patients' outcome, however MM remains a remitting-relapsing disease in most patients.[1]

Although rearrangements at the immunoglobulin (IGH) loci and hyperdiploidy (HRD) are key initiating events in MM oncogenesis, it is likely by inference from the study of other cancers, that the evolutionary history of MM is important in determining patient outcome.[2,3] This is because prognosis in cancer is strongly associated with the development of resistant sub-clones.[4] Recent studies of solid cancers have challenged the classical Darwinian model of cancer evolution based on a changing sub-clonal dominance.[5-7] Observations have suggested that after malignant transformation, sub-clones that have distinct mutational profiles that can coexist for long periods of time.[8,9] Such a model of neutral tumor evolution is consistent with only a handful of recurrent driver alterations identified to date, indicating that they all occurred in the primordial cancer cell and that subsequent clonal outgrowths are relatively rare.

The mutant allele frequency distribution has been shown to predict the expected pattern of sub-clonal mutations within a tumor under neutral evolutionary dynamics from a single baseline sample.[10] To gain insight into the clonal evolution of MM and its impact on phenotype we analysed whole exome sequencing (WES) tumor data from two independent series of MM patients.[11,12] We report that a high proportion of MM tumors are under neutral evolutionary dynamics and that these tumors are associated with a worse survival in patients receiving iMiDtherapy.

METHODS

We analysed WES tumor data from: (i) 333 patients from Myeloma XI (NCT01554852, CRUK/09/014) an open-label randomised controlled randomised phase III trial comparing thalidomide against lenalidomide at induction and lenalidomide maintenance against no maintenance in both transplant eligible and non-eligible patients (**Supplementary Methods, Supplementary Fig. 1**) [11,12]. Copy number changes in tumors were based on MLPA data and qRT-PCR used to assign translocation status.[13,14] (ii) 434 patients from the Multiple Myeloma Research Foundation's CoMMpass study, which had received IMiD therapy (NCT01454297; dbGaP accession phs000748.v5.p4; IA9 data tranche; **Supplementary Methods**). Translocations status and copy number abnormalities from CoMMpass data were called from whole genome sequencing, exome and RNA sequencing (FISH-seq). Hyperdiploid cases with no detected translocation by FISH-seq, but classified by conventional FISH were considered as missing.

Modelling tumor evolution

The distribution of mutant allele frequencies in each MM tumor was used to detect neutral evolution as previously described[10] (**Supplementary Methods**). Briefly, mutations were only included if the read depth was ≥ 10 and the number of mutant alleles was ≥3 and at least 12 mutations matching these criteria had to be present in a sample to be included.[10] Preliminary analysis showed that mosaic copy number changes, *e.g*. Hyperdiploidy could give rise to a false sub-clone status and all cases were corrected for copy number.[13-15] By excluding public mutations present at mutational frequencies ≥0.3, the influence of undetermined normal CD138 cell contamination was controlled. Mutations at a frequencies ≤ 0.12 were also excluded, since they reach the limit of reliable detectability in bulk sequencing data.[10] For each tumor sample the cumulative number of mutations, M(f), was tested for linearity with the inverse of the frequency ($1/f$) as predicted by $M(f)=\mu/\beta(1/f - 1/f_{max})$ for neutral tumor evolution. A tumor sample was considered to have evolved neutrally if $R^2 \geq 0.98$, as previously advocated.[10]

RESULTS AND DISCUSSION

Evidence of neutral evolution was shown in 20% of tumors (65/333) from the Myeloma XI trial (**Fig.1; Supplementary Fig.2 and 3)**. Evidence for neutral evolution was not influenced by sequencing depth, exome coverage or number of mutations (**Supplementary Table 1**). There was no significant association between neutral clonal evolution in tumors by age at diagnosis, sex or International Staging System (ISS) stage. In the CoMMpass study 17% of tumors (74/434) from patients treated with IMiDs showed evidence of neutral evolution.

In both the Myeloma XI and CoMMpass series tumors with IGH translocations were more likely to show evidence of neutral evolution than hyperdiploidic tumors; Respective median R^2 values for Myeloma XI and CoMMpass tumors being 0.963 vs. 0.956 (P=0.002), and 0.957 vs. 0.947 (P=0.034) (**Fig. 2** , **Supplementary Fig. 4 and 5**).

In both series of patients that received non-intensive therapy, (*i.e.* no high-dose alkylating consolidation), neutral tumour evolution was associated with worse progression free survival (PFS) and overall survival (OS); In the Myeloma XI trial, median PFS was 15.6 as compared with 20.5 months (Logrank P=0.019) and median OS was 27.3 compared with 49.6 months (P<0.001) for neutral and non-neutral tumors, respectively. In the CoMMpass study, median PFS was 18.7 as compared with 28.1 months (P=0.036) and median OS was 21.3 and not reached (P=0.029), respectively. In contrast no difference was shown for patients in receipt of intensive alkylating therapy based on high-dose melphalan and autologous transplantation.

To address the possibility of potential co-linearity between tumor evolution status and established genetic risk factors in non-intensively treated patients that may have confounded outcome we performed a multi-variable survival analysis (**Supplementary Table 2**). Neutral evolution was shown to be prognostically independent of ISS, adverse IGH translocations, gain(1q) and TP53 deletion.

The observation that tumors with IGH translocations have a higher degree of evolutionary neutrality than hyperdiploid tumors may reflect the fact that early mutational events brought about by IGH translocations provide increased tumor fitness as compared to hyperdiploidy.

Importantly, IGH translocations are present in all sub-clones, thus potentially mediating relative tumor independence from external factors such as microenvironment growth factors that might in a weaker oncogenic context contribute to sub-clonal selection.[16]

Tumor microenvironment factors are well established to influence MM cell survival and proliferation.[17] Therapy with IMiDs modulates the tumor microenvironment, but in the context of neutral evolution and presence of early clonal strong oncogenic driver events this mechanism of therapy may be less efficacious. This contrasts with intensive alkylator therapy, which targets the tumor cell directly and non-specifically through DNA adduct formation. This 'de-bulking' effect may reset the sub-clonal structure, potentially reducing the impact of a neutral or non-neutral evolutional tumor history (**Supplementary Fig. 6**), which may explain the similar survival in both groups of intensively treated patients.

In summary, we demonstrate that a significant proportion of MM is under neutral evolutionary selection. Importantly, such tumors tend to confer a poorer patient survival in the context of microenvironment modulating therapies. Our findings therefore provide further evidence that knowledge about the evolutionary dynamics of MM has potential to inform treatment decisions.

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AUTHOR CONTRIBUTIONS

Conception and design of the study: D.C.J, R.S.H and M.F.K

Acquisition of data: D.C.J, B.A.W, J. J, C.P, C.W, G.J, D.C, W.G, R.O, M.T.D, G.C, F.D, G.M and M.F.K

Analysis of data: D.C.J, O.L, J.S.M, D.C, R.S.H and M.F.K.

Writing, review or revision of manuscript: D.C.J, R.S.H and M.F.K

Disclosure of Conflicts of Interest

G.J. has received consultancy fees, honoraria, travel support, research funding and speakers bureau from Celgene and Takeda and consultancy fees, honoraria and speakers bureau from Janssen, MSD, Roche and Amgen. M.T.D. owns equity and is a board member of Abingdon Health. G.C. has received consultancy fees, honoraria, research funding and speakers bureau from Celgene, Janssen, Takeda and Amgen; consultancy fees, honoraria and speakers bureau from Sanofi; consultancy fees, honoraria from BristolMyers Squibb and Glycomimetics. F.D. received consultancy fees and honoraria from Celgene, Takeda and Janssen. C.P. received consultancy fees and travel support from Celgene and consultancy fees from Takeda Oncology. J.J received honoraria and research funding from Celgene. G.M. received consultancy fees and honoraria from Takeda and Janssen; consultancy fees, research funding and honoraria from Celgene; research funding from Janssen. M.F.K. has received consultancy fees, research funding and honoraria from Celgene; consultancy fees and honoraria from Amgen and Janssen; consultancy fees and travel support from Takeda and BMS; consultancy fees from Chugai. All other authors have no relevant financial relationship(s) to disclose.

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FIGURE LEGENDS

Figure 1: Influence of neutral evolutionary dynamics on overall survival and progression-free survival in Myeloma XI and CoMMpass studies. Kaplan-Meier curves comparing neutral cases (R2 ≥ 0.98) versus non-neutral cases (A) progression-free survival (PFS) of Myeloma XI cases in the non-intensive treatment arm; (B) overall survival (OS) of Myeloma XI cases in the non-intensive treatment arm; (C) PFS of Myeloma XI cases in the intensive treatment arm; (D) OS of Myeloma XI cases in the intensive treatment arm; (E) PFS of non-autologous transplant CoMMpass cases receiving an IMID; (F) OS of non-autologous transplant CoMMpass cases receiving an IMID; (G) PFS of autologous transplant CoMMpass cases receiving an IMID; (H) OS of autologous transplant CoMMpass cases receiving an IMID. The red line depicts the survival curve for tumors with neutral evolutionary dynamics and the black line depicts the survival curve for tumors with non-neutral evolutionary dynamics. Horizontal ticks on the survival curves show censored cases.

Figure 2: Association of neutral evolutionary dynamic with IgH translocations in Myeloma XI and CoMMpass studies. Violin-plot of the neutral evolutionary dynamics measured by R2 by (a) Myeloma XI (b) CoMMpass. 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 R2=0.98 threshold for discriminating neutral from non-neutral tumors. Statistical differences between experimental groups were evaluated by Wilcoxon rank sum test. $P < 0.05$ was considered statistically significant.

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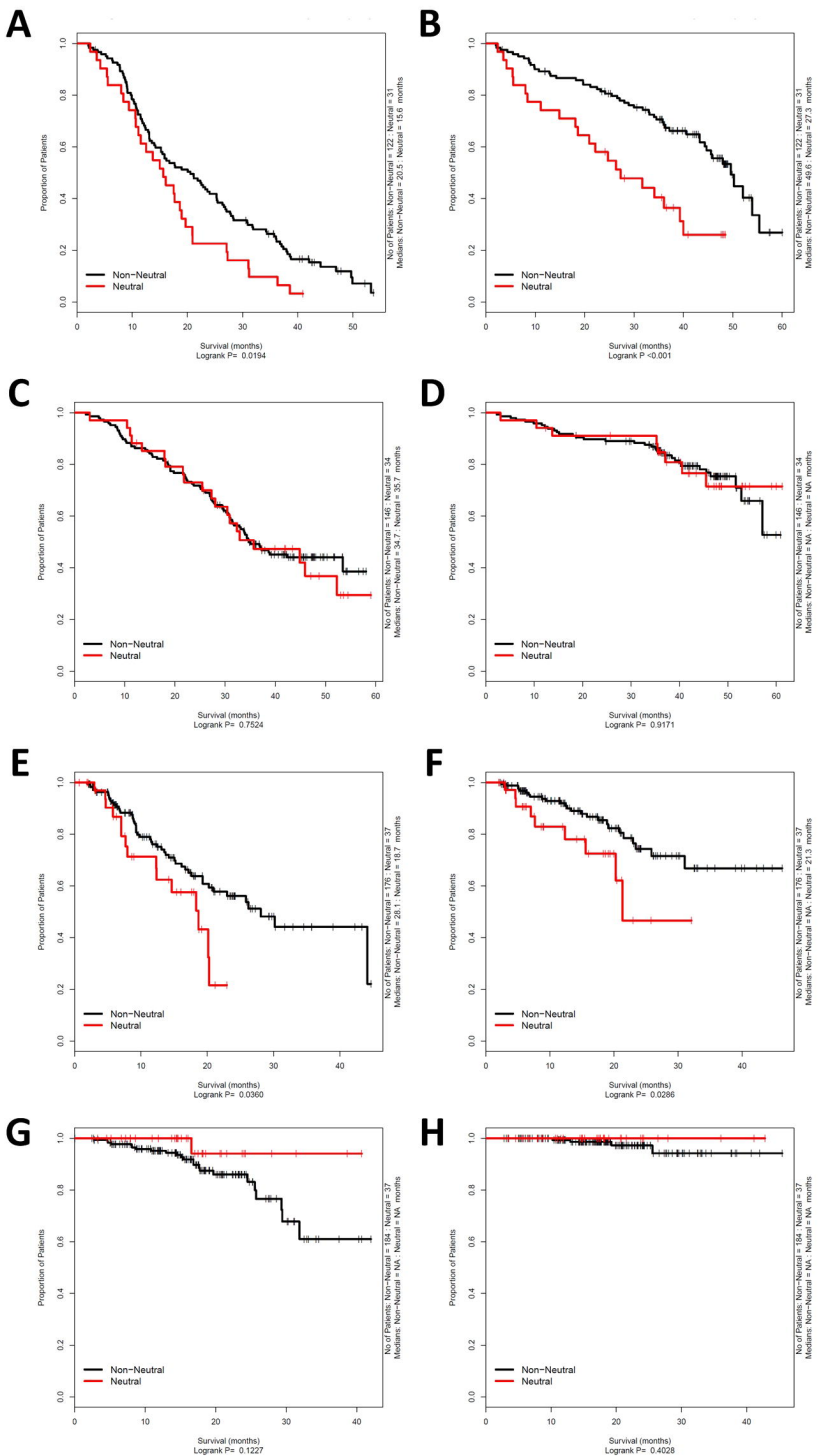

Figure 2: Association of neutral evolutionary dynamics with IgH translocations in Myeloma XI and CoMMpass studies