Myeloma Cell Dynamics in Response to Treatment Supports a Model of Hierarchical Differentiation and Clonal Evolution

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Abstract

Purpose: Since the pioneering work of Salmon and Durie, quantitative measures of tumor burden in multiple myeloma have been used to make clinical predictions and model tumor growth. However, such quantitative analyses have not yet been performed on large datasets from trials using modern chemotherapy regimens.

Experimental Design: We analyzed a large set of tumor response data from three randomized controlled trials of bortezomib-based chemotherapy regimens (total sample size \( n = 1,469 \) patients) to establish and validate a novel mathematical model of multiple myeloma cell dynamics.

Results: Treatment dynamics in newly diagnosed patients were most consistent with a model postulating two tumor cell subpopulations, "progenitor cells" and "differentiated cells." Differential treatment responses were observed with significant tumor-cidal effects on differentiated cells and less clear effects on progenitor cells. We validated this model using a second trial of newly diagnosed patients and a third trial of refractory patients. When applying our model to data of relapsed patients, we found that a hybrid model incorporating both a differentiation hierarchy and clonal evolution best explains the response patterns.

Conclusions: The clinical data, together with mathematical modeling, suggest that bortezomib-based therapy exerts a selection pressure on myeloma cells that can shape the disease phenotype, thereby generating further inter-patient variability. This model may be a useful tool for improving our understanding of disease biology and the response to chemotherapy regimens.

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Introduction

Multiple myeloma was the first metastatic malignancy for which quantitative measurements of tumor burden became available, allowing for mathematical and statistical approaches to studying this disease (1–4). Unlike other cancers for which serial measurements of tumor size are a challenging problem, it was shown in multiple myeloma that serum levels of myeloma protein (M-protein) are strongly correlated with tumor burden,
Translational Relevance
Relapse in multiple myeloma patients suggests the existence of progenitor cells responsible for tumor regrowth, but this population has never been confirmed. Here, we used mathematical modeling to show that the combined data from three clinical trials of bortezomib-based chemotherapy are consistent with a differentiation hierarchy where a myeloma progenitor cell population that is relatively resistant to therapy gives rise to a differentiated cell population that is sensitive to therapy. Thus, we conclude that bortezomib-based therapy exerts a selection pressure on myeloma cells, and the administration of rational combination treatments may reduce expansion of resistant clones, leading to more prolonged remissions. Our model could be used to improve future clinical trial design for multiple myeloma by using treatment effects of therapeutic agents on both differentiated and progenitor cells populations in order to predict how patients will respond to treatment.

Materials and Methods
To develop a model for multiple myeloma tumor cell dynamics, we utilized data from 682 newly diagnosed multiple myeloma patients who were treated with first-line melphalan and prednisone with bortezomib (the ‘VISTA VMP’ cohort) or without (the ‘VISTA MP’ cohort) within the randomized phase III VISTA trial (ref. 11; see SI, ‘Patient cohorts’). Serum M-protein measurements (g/dL) were analyzed uniformly by a central laboratory and were determined as surrogates for disease burden (15). By VISTA trial design, patients were to be treated in both cohorts for a maximum duration of 54 weeks, unless treatment was discontinued earlier due to toxicity or myeloma progression. Of the 682 randomized patients, 299 patients in the VISTA MP cohort and 300 patients in the VISTA VMP cohort were evaluated for their disease kinetics (Fig. 1A, SI, ‘Patient selection’ for exclusion criteria). To investigate the treatment effects of melphalan, prednisone, and bortezomib, we first utilized a statistical modeling approach to identify trends within the treatment response data (SI, ‘Statistical modeling’). We then aimed to design simple mathematical models, developed in a biologically intuitive way, to investigate the treatment dynamics of the disease; similar approaches have previously led to a mechanistic understanding of infectious diseases (16). Finally, we validated our model using M-protein data from two independent trials, the MMY-2001 and APEX trials, which include both newly diagnosed (11, 13) and relapsed multiple myeloma patients (8–10). The SI contains details on all patient cohorts, analyses, and models.

Results
We analyzed the observed tumor burden trajectories from the three trials in the following order: (i) we applied statistical modeling to determine the trends in longitudinal tumor trajectories from newly diagnosed multiple myeloma patients in the VISTA trial, (ii) we built two biologically sound mathematical models in an attempt to recapitulate the observed trends, (iii) we compared our models’ predictions against observed results, and (iv) we validated our model with external data from the independent MMY-2001 and APEX trials and expanded our model to explain trends in refractory patients.

Statistical modeling of VISTA trial data
Before biology-based mathematical modeling, we first investigated different statistical approaches to identify the shape of the treatment response data, that is, the statistical model with the best fit to the M-protein data (Fig. 1D and E, SI, ‘Statistical modeling,’ Supplementary Fig. S1). We began with the data from the VISTA trial, which demonstrated a clear survival advantage for bortezomib, melphalan, and prednisone (VMP) versus melphalan and prednisone alone (MP) in newly diagnosed elderly patients (11). When studying the cohort-level patient dynamic of treatment response in both the VISTA MP and VMP cohorts, we identified a 2-phasic exponential model (i.e., a curve with a bend, or turning point; Supplementary Fig. S1) to be the best-fitting statistical model among 1-phasic exponential and 2-phasic exponential models for both cohorts (SI, ‘Treatment phase model fitting.’ Table 1). The summary statistics for the first slopes ($\beta_1$), second slopes ($\beta_2$), and turning points ($\tau$) when fitting 2-phasic exponential models to all patients in each cohort are shown in Table 2. First, we found that patients in the VISTA VMP cohort had a significantly steeper first slope on average than patients in the VISTA MP cohort ($P = 3 \times 10^{-13}$ Wilcoxon rank-sum test), corresponding to a faster initial response rate in the VMP cohort. Second, the difference between the cohorts’ second slopes was not statistically significant ($P = 0.2439$, Wilcoxon rank-sum test), and both cohorts were not significantly different from zero ($MP P = 0.2645$ and VMP $P = 0.08$, t-statistics). Third, the difference between the cohorts’ turning points was also not statistically significant ($P = 0.3852$, Wilcoxon rank-sum test).

We then performed individual patient analyses to ensure that the observed patterns at the cohort level are representative of sufficiently many individual patients such that we are not focusing only on a small subset of patients. Comparing between patients who displayed 1 versus 2-phasic exponential declines, we found that there was a significantly larger proportion of 2-phasic patients in the VISTA VMP cohort as compared to the VISTA MP cohort ($P < 10^{-16}$, Fisher exact test). Furthermore, 2-phasic patients in the VISTA VMP cohort had a significantly steeper first slope than such patients in the VISTA MP cohort ($P = 3 \times 10^{-15}$ for all patients with $\beta_1 < 0$, Wilcoxon rank-sum test), again indicating a faster initial response rate in the VMP cohort. The difference between cohorts in the second slope was not statistically significant. Finally, 2-phasic patients in the VISTA VMP cohort had a
smaller turning point than patients in the VISTA MP cohort, indicating that the time at which the response rate changes was shorter in the VMP cohort than the MP cohort ($P = 0.02$ for all 2-phasic patients with $\beta_1 < 0$ and $P = 0.01$ for all 2-phasic patients with $\beta_1 < 0$ and $\beta_2 < 0$, Wilcoxon rank-sum test).

When investigating the relationship between the best-fitting model and multiple myeloma stage (ISS stage) (17), we found that patients with advanced-stage disease in the VISTA MP cohort were significantly more likely to display a 2-phasic rather than a 1-phasic exponential trend after the initiation of therapy (Supplementary Table S4). For the VISTA VMP cohort, however, there was no significant association between multiple myeloma stage and the shape of the treatment response curve (Supplementary Table S4). In both cohorts, there were more deaths of patients with positive second slopes compared with patients with negative second slopes (Supplementary Table S5). Furthermore, for VISTA VMP patients displaying a 2-phasic M-protein depletion in the treatment phase, the first slope was significantly associated with the time to progression ($P = 0.005$, Cox model) when controlling for multiple myeloma stage.

Mathematical modeling of VISTA trial data

The fact that many patients displayed more complex response kinetics than a simple exponential decay of M-protein abundance over time suggests complex intra-patient disease dynamics. We therefore designed biologically sound mathematical models to explain the response dynamics. These models were created to relate the abundance of measurable M-protein to the numbers of different types of multiple myeloma cells, which are not directly detectable in the clinical trial data, offering an explanation for the observed trial results from a theoretical biology perspective. To validate the model-predicted and observed outcomes, we compared their turning points and the proportion of 1- and 2-phasic patients.

Figure 1.
Patient selection criteria and M-protein treatment response in the VISTA and APEX trials. A–C, flowcharts outlining the patient inclusion and exclusion criteria for quantitative analysis of M-protein treatment response. D–I, median longitudinal M-protein trajectories for each cohort in the three trials, and the associated numbers of patients at each time point: D, VISTA MP cohort; E, VISTA VMP cohort; F, MMY2001 VMP cohort; G, MMY2001 SVMP cohort; H, APEX DEX cohort; and I, APEX VEL cohort. The median M-protein values are indicated by the orange circles, and quartiles are indicated by the whiskers. The numbers of patients at each time point are shown by the histograms. Vertical yellow and green dashed lines indicate treatment initiation and termination times, respectively. For the VISTA and MMY-2001 trials (D–G), only patients who completed the entire treatment regimen are included. For the APEX trial (H and I), due to the small number of patients who completed the entire treatment regimen, only the M-protein response during treatment is shown.
We first postulated a model based on the existence of two genetically independent multiple myeloma clones. In this 'clonal evolution' model, tumor cells evolve independently, and there is no differentiation hierarchy within the myeloma cell population (SI, 'The clonal evolution model'). We found that there was a significant difference in the numbers of 1- and 2-phasic patients between the observed clinical data in the VISTA trial and the simulated data based on the clonal evolution model (observed 1-phasic patients/total number of patients: 55/234; in one simulation, 1-phasic patients/total number of patients: 113/234; \( P = 5 \times 10^{-6} \), Fisher exact test). Furthermore, the estimated turning points for 2-phasic patients were significantly different between the observed clinical data in the VISTA trial and the simulated data based on the clonal evolution model, 90 ± 69, median 72 days in VISTA data; 186 ± 81, median 169 days in simulated data; \( P = 2 \times 10^{-12} \), two-sample t test (SI, 'The clonal evolution model'). Thus, a clonal evolution model, which considers two genetically different multiple myeloma clones, was unable to recapitulate the tumor dynamics observed from the VISTA trial data.

We then designed a second model that describes the differentiation hierarchy of the hematopoietic system; in this hierarchical model, we postulated that myeloma cells in part recapitulate normal differentiation and are composed of two populations: myeloma 'progenitor' cells and 'differentiated' cells (Fig. 2A and SI, 'The differentiation hierarchy model'). In the context of this model, normal stem cells reside on top of the hierarchy and give
rise to progenitor cells, which in turn produce differentiated cells. In addition to normal cells, the bone marrow of multiple myeloma patients also includes multiple myeloma cells. Multiple myeloma progenitors reside on top of the multiple myeloma hierarchy and give rise to multiple myeloma differentiated cells, which in turn produce M-protein. Multiple myeloma progenitors produce none or only low amounts of M-protein, which we neglected in the mathematical model. We found good agreement between the VISTA trial data and predictions of the mathematical model (Fig. 2D and E and SI, ‘The differentiation hierarchy model’). The pink shaded region denotes the time during which patients receive treatment. D–G, concordance between observed population-level multiple myeloma protein trajectories (blue intervals, error bars indicating the observed quartiles) and the multiple myeloma protein levels predicted by the mathematical model (solid blue lines). The parameter values for the first and second slopes used to generate panels D, E, and F are listed in Table 2. For G, the model predicted trajectory is generated using the same first and second slopes as E. All ancillary parameters for D–G are identical and are listed in SI, ‘Parameter values for the hierarchical and hybrid models’.

Validation of the hierarchical model using additional trial data

We then utilized data from two independent clinical trials to test the hierarchical model’s ability to explain patient responses: the MMY-2001 trial (13) comparing bortezomib-melphalan-prednisone (VMP) versus siltuximab plus VMP (SVMP) in newly
diagnosed multiple myeloma patients \( (n = 106, \text{Fig. 1B}) \) and the APEX trial comparing high-dose dexamethasone (DEX) versus single-agent bortezomib (VEL) in refractory patients \( (n = 669, \text{Fig. 1C}) \).

For these two validation data sets, we first performed the same statistical analysis as before to estimate the shapes of the treatment response curves (Fig. 1F–I). For both the MMY-2001 and APEX trials, we found that the cohort-level M-protein dynamics during treatment were best explained by the 2-phasic exponential model (Table 1); summary statistics are shown in Table 2. Gratifyingly, we found the first slopes of patients in the VISTA VMP and MMY-2001 VMP cohorts were not significantly different \( (P = 0.2082, t \text{ test}) \). For the MMY-2001 trial, we found that there was a statistically significant difference between VMP and SVMH cohorts in terms of the first slopes \( (P = 0.0005, t \text{ test}) \). Interestingly, although the addition of siltuximab significantly increased the initial reduction of M-protein levels, it failed to improve progression-free or overall survival \( (13) \). In terms of the second slopes, there was no significant difference between MMY-2001 VMP and SVMH cohorts \( (P = 0.7586, t \text{ test}) \), and both cohorts were not significantly different from zero \( (\text{VMP} P = 0.8953 \text{ and } \text{SVMH} P = 0.2123, t \text{ test}) \). For the APEX trial, we found that patients in the VEL cohort had a significantly steeper first slope than patients in the DEX cohort \( (P = 0.0035, t \text{ test}) \). Also, the difference between cohorts in terms of the second slope was not statistically significant \( (P = 0.8635, t \text{ test}) \). However, unlike the VISTA and MMY-2001 trials, in the APEX trial the second slopes were significantly positive \( (\text{DEX} P = 0.01231 \text{ and } \text{VMP} P = 0.001, t \text{ statistics}) \). These increases are consistent with disease progression and likely signify the development of drug-resistant multiple myeloma cells. In all three trials, patients displayed significant reductions in M-protein levels immediately upon receiving bortezomib-based treatment; however, the long-term effects of these medications varied.

We then aimed to validate the hierarchical model using data from the MMY-2001 and the APEX trials. We found that, for the MMY-2001 trial consisting of newly diagnosed patients, the hierarchical model was able to recapitulate the population-level M-protein trajectories; the MMY-2001 VMP cohort could be recapitulated using the same parameters as for the VISTA VMP cohort (Fig. 2C). The correlations between observed mean, median, and model predicted values were 0.82 and 0.78, respectively. The SVMH cohort could be recapitulated using slope estimates obtained from the SVMH patient data and keeping all other parameters unchanged (Fig. 2F and Table 2).

For the APEX trial, we observed increasing trends in M-protein values already during the treatment phase in many patients; this may contribute to the increases of the M-protein levels in the APEX trial (SI, ‘Rebound dynamics’). Unlike the APEX trial, we observed rebounding patients in the APEX trial within 100 days after the start of treatment, while still on treatment \( (\text{median: } 74.21 \text{ days; mean: } 83.20; \text{SD: } 41.88 \text{ for the rebounding patients in the APEX DEX cohort and median: } 68.02 \text{ days; mean: } 69.14; \text{SD: } 27.80 \text{ for the rebounding patients in the APEX VEL cohort}) \).

### Development of a hybrid mathematical model for multiple myeloma cell dynamics

On the basis of these observations, we attributed the differences in M-protein dynamics between newly diagnosed and relapsed patients to the existence and expansion of a resistant clone in relapsed patients \( (\text{Fig. 3A}) \). A dominant resistant clone existing before treatment explains the increasing M-protein values during treatment among 1-phasic patients in the APEX trial; an expanding resistant clone during treatment explains the initial declines followed by increases in M-protein values; and the presence of a small resistant clone explains continuing 2-phasic declines in the remaining patients \( (\text{Fig. 3B–D}) \). Therefore, we extended our mathematical model to take into account resistant clone(s) \( (\text{Fig. 3A, SI, ‘The hybrid model’}) \). In this extended model, in addition to normal cells and sensitive multiple myeloma cells, there is a resistant clone that originally arose from the sensitive progenitor multiple myeloma cells. This resistant clone gives rise to a similar differentiation hierarchy as the sensitive cells. The observed M-protein values are the sum of M-protein values generated from the sensitive and resistant clones; the amount of M-protein secreted by each clone is proportional to the size of the clone and the relative secretion rates, which are considered to be similar. The time at which resistance arises determines the relative proportion of sensitive and resistant cells. In newly diagnosed patients, the M-protein contribution from the resistant cells during treatment is negligible; this is supported by the observation that only a relatively small number of patients \( (7\% \text{ and } 21\% \text{ in VISTA VMP and MP cohorts, respectively}) \) experienced increases in M-protein values while on treatment in the VISTA trial. In contrast, in the relapsed patients from the APEX trial, because of prior treatment-induced clonal selection, the M-protein contribution from the resistant clone is sufficiently large and stable in size \( (\text{between } 1\% \text{ and } 10\% \text{ to alter the treatment response trajectory, leading to rebounds in a subset of patients while on treatment in the APEX trial (SI, ‘Rebound dynamics’}) \). Unlike the clonal expansion model, the resistant cells do not need to be substantial in size at the beginning of the first treatment to drive the rebound trajectory; rather, through multiple rounds of treatment-induced selection, the fraction of resistant cells may increase sufficiently much to alter the response trajectory. This hybrid model was able to explain the M-protein dynamics for both newly diagnosed and relapsed myeloma patients in response to chemotherapy \( (\text{Fig. 2D and E and Fig. 3E and F}) \). Our model suggests that treatment-induced clonal selection may contribute to the increases of the M-protein levels in the refractory patients while on treatment.
The hybrid mathematical model accurately predicts the dynamics of M-protein response in all three trials. A, illustration of the hybrid mathematical model. Normal, sensitive, and resistant multiple myeloma cells are shown in black, blue, and red, respectively. The dashed arrow indicates the mutation event that gives rise to the first resistant cell. Solid downward arrows indicate the direction in the differentiation hierarchy. Circular arrows indicate cell regeneration within each differentiation level. Double-lined arrow indicates the production of M-protein from differentiated multiple myeloma cells. B, illustration of effects of the time at which resistance arises on the observed M-protein trajectories. Left, resistance emerges very early; middle: resistance emerges early; right, resistance emerges late. The blue lines denote the contribution to the changes in the observed M-protein values of sensitive multiple myeloma cells; the red lines denote the contribution to the changes in the observed M-protein values of resistant multiple myeloma cells; and the black dots represent the observed total M-protein levels from both sensitive and resistant multiple myeloma cells. Although the sensitive cells’ response to treatment remains identical in all three subpanels, the timing at which resistance arises determines the observed M-protein response. Similar to the VISTA trial, the initial steep decline in M-protein (middle and right subpanels) is attributed to the reduction in the sensitive differentiated multiple myeloma cells; the shallow decline in M-protein is interpreted as the result of the reduction in the number of sensitive multiple myeloma progenitor cells. Vertical dashed lines in yellow and green indicate treatment start and end dates. C and D, the panels display the model-predicted abundances of normal (black) stem cells; normal (black), sensitive multiple myeloma (blue), and resistant multiple myeloma (red) progenitor cells; and normal (black), sensitive multiple myeloma (blue), and resistant multiple myeloma (red) differentiated cells over time (years) for early (C) vs. late (D) emergence of resistance for the APEX DEX cohort, as predicted by the mathematical framework (see SI, ‘The hybrid model’). C and D have identical parameter values except for the time at which resistance arises (for the full set of parameter values, see SI, ‘Parameter values for the hierarchical and hybrid models’). The time at which resistance arises dictates whether a rebound occurs during the treatment phase. E and F, M-protein treatment response dichotomized based on rebound status for APEX DEX and VEL cohorts. Rebound during treatment (purple): patients with at least one positive slope during the treatment phase; no rebound during treatment (orange): patients with all negative slope(s) during the treatment phase. Observed median M-protein values (>10 observations) from each subgroup are shown in dots. Lines show model-predicted M-protein trajectories. Within each cohort, all parameter values are identical, except the time at which resistance arises (for the full set of parameter values, see SI, ‘Parameter values for the hierarchical and hybrid models,’ and Supplementary Table S7).
Discussion

Here, we present a comprehensive quantitative analysis of myeloma cell growth and treatment response based on three large randomized trials (total sample size n = 1,469). Our analysis of responses to induction therapy in newly diagnosed patients in the VISTA trial revealed complex but structured kinetic patterns that support a hierarchical two-cell population mathematical model for multiple myeloma. This model was able to recapitulate the observed two-phasic decline patterns observed in the majority of newly diagnosed patients. Importantly, an alternative clonal selection model of myeloma cell growth did not fit the trial data. The hierarchical model suggests the existence of a multiple myeloma progenitor cell population that has self-renewal capacity and distinct growth kinetics and gives rise to the differentiated multiple myeloma cell population. The observation of a significantly larger proportion of 2-phasic patients in the VISTA VMP cohort as compared with the VISTA MP cohort when performing the individual level analysis signifies the potential treatment effect of bortezomib in combination with melphalan and prednisone on increasing the death rate of multiple myeloma progenitor cells during therapy. Our model of multiple myeloma progenitors, which are nonsecretory and relatively drug resistant compared with differentiated cells, is supported by experimental evidence (18–20).

We used the two additional independent sets of clinical trial data to validate and extend the hierarchical model. All trial data supported the model for disease response to treatment; however, inter-patient variability of disease kinetics was noted to be significant at the time of disease relapse, requiring the adjustment of growth parameters in the model and suggesting that distinct multiple myeloma progenitor subclones were selected by induction treatment. The existence of clonal selection between myeloma subclones was also supported by the analysis of relapsed or refractory patients in the APEX trial. The varied trajectories of disease relapse observed necessitated the development of a hybrid model with preexisting drug-resistant myeloma cell clones. Importantly, mathematical modeling in myeloma has not been attempted previously on this scale. In addition, statistical analyses reveal different M-protein trajectories even among patients randomly assigned to the same treatment cohorts. Significant association between slopes in the trial data. The trial data. The current goal of treatment in multiple myeloma is to achieve deep complete remissions. Mathematical modeling based on treatment responses can provide an additional method for testing biologic hypotheses relevant to disease outcomes in patients with multiple myeloma. Here, we presented a model based on a large amount of available clinical data, but models such as this one can also be used to improve the design of new clinical trials in myeloma by using treatment effects of specific agents on both differentiated and progenitor cells populations as endpoints (31). Mathematical modeling as suggested here might eventually be useful for investigating the efficacies of novel treatment modalities. For instance, if the effects of an agent on individual myeloma cell populations are known, our mathematical model can help predict how diverse patient populations will respond to treatment. As multiple myeloma clones are present at diagnosis, the administration of rational combination treatments at relapse may reduce their expansion and therefore lead to deeper and more prolonged remissions, again an area where the model may have utility (32, 33). Finally, mathematical models are limited by the availability of uniformly collected quantitative patient data. The utility of mathematical models such as this one would be improved by collecting additional quantitative data centrally from future clinical trials.

Disclosure of Potential Conflicts of Interest

Helgi van de Velde holds ownership interest (including patents) in Johnson & Johnson. Constantine S. Mitsiades reports receiving commercial research grants from Janssen Pharmaceuticals and Novartis. Jesus San Miguel is a consultant/advisory board member for Bristol-Myers Squibb, Celgene, Janssen, and...
References