In vivo and *in silico* studies on single versus multiple transplants for multiple myeloma

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High-dose therapy and autologous stem cell transplantation (HDT-ASCT) have significantly improved survival in multiple myeloma (MM). However, patients are not cured, responses are variable and only about 40% of patients achieve a complete response (CR). Optimal timing of the procedure and knowledge of the relapse kinetics may assist physicians when they consider this therapeutic modality for their patients. We analyzed myeloma tumor burden and kinetics before and after HDT-ASCT in a cohort of 265 patients. Disease burden was estimated from serial M-spike measurements and the data fitted to the Gompertz function to determine the general parameters for all patients. Functions that couple disease burden and kinetics with time to progression (TTP) were derived and used to determine the optimal timing of transplantation. Patients who achieve CR with the first episode of HDT-ASCT should not be routinely offered tandem transplantation but carefully monitored and transplanted at an optimal disease burden. If CR is not achieved with a first trial of HDT-ASCT, the probability of CR after a tandem second trial is ~10%. TTP after tandem transplants (with its higher associated mortality) cannot be superior to TTP achieved with optimally timed serial transplants. Individualized HDT-ASCT for patients with MM is possible and may optimize results. (*Cancer Sci* 2007; 98: 734–739)

Survival of patients with multiple myeloma (MM) has improved with the advent of high-dose therapy (HDT) with autologous stem cell transplantation (ASCT).[1–5] When compared to conventional therapy, HDT is associated with a significantly higher incidence of complete response (CR) that translates into a longer time to progression (TTP),[1,2] although the procedure is not curative. Several large randomized studies have addressed important issues in the field including the therapeutic equivalence of melphalan 200 mg/m^2 (MEL200) versus melphalan 140 mg/m^2 combined with total body irradiation (MEL-TBI),[6] as well as the impact of single versus tandem transplantation.[2] The role of maintenance therapy after HDT remains to be clarified.[7,8] Whereas some of the results were expected, others perhaps came as a surprise. For example, IFM94 showed that patients who achieve a very good partial response after the first transplant benefit from a second transplant even though the fraction of patients who reach a CR after the second transplant is less than 10%.[2] Because almost all patients will ultimately relapse, purging of the harvested autograft from contaminating myeloma cells was attempted but this had no impact on TTP.[9] Recently we reported that therapy with high-dose cyclophosphamide before stem cell collection does not improve CR rates or lengthen TTP.[10]

The tumor burden in any patient with cancer is not static as tumor cells are continually replicating or undergoing apoptosis. The difference between these two rates gives the net rate of tumor growth. It is usually difficult to estimate the rate of tumor growth *in vivo*, but in this respect MM is unique. The secretion of a monoclonal protein (M-protein) by the tumor cells provides a simple way to monitor disease burden, if one accepts some reasonable assumptions as discussed below. Sullivan and Salmon used such data to measure the growth kinetics and tumor burden in patients with MM before and after therapy.[11] They showed that growth of an untreated tumor can be described by the Gompertz function. This function has considerable appeal for tumor modeling[12,13] as two parameters are enough to define its life history. Initially the Gompertz function was thought to be simply a phenomenological descriptor of tumor growth dynamics, but recent models combined with an appreciation of intratumor cell kinetics gave new meaning to the function and put it on a more solid biological basis.[14,15] In order to understand recent observations concerning HDT-ASCT for MM, we developed a simple model based on tumor burden estimation at various time points in patients who underwent HDT-ASCT at our institution. We use this data to deduce realistic Gompertz functions for MM and subsequently use these functions to address the phenomenology of transplantation for this disorder. Our results provide compelling evidence that the underlying tumor kinetics explain many of the observations seen in large trials in MM. Based on these results, we propose new ways to optimize therapy.

Methods

Patients. Patients with MM who undergo HDT-ASCT at Mayo Clinic Rochester are maintained in a database that contains all of the relevant demographic, clinical and laboratory characteristics of these patients, which is continuously updated by the senior author. All patients give informed consent to be included in the database. Any patient with MM who is considered for HDT-ASCT undergoes evaluation of disease status before transplantation, which includes measurement of serum M-protein. Patients who undergo HDT-ASCT are typically reevaluated for the response approximately 100 days after the procedure. For the purpose of this study, patients with a serum M-protein <0.1 g/dL were excluded from analysis. This study was approved by the Mayo Foundation Institutional Review Board in compliance with both federal regulations and the Declaration of Helsinki.

Response definitions. The response criteria used were as defined by Blade *et al*.[16] A complete response (CR) required the absence of M-protein in the blood and urine as well as negative immunofixation ~100 days after transplant. In patients who achieved CR, the return of immunofixation-positive serum defined relapse. However, for tumor burden estimation, the date of the first measurable serum M-protein after relapse was considered. In the case of patients without CR (LCR), progression was defined as doubling of the serum M-protein level. For patients with LCR, the lowest serum M-spike after transplant was

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considered the new baseline for estimating the rate of tumor regrowth, and the time interval between the lowest M-spike and M-spike compatible with progression taken as the denominator.

Disease burden estimation. The methodology for disease burden estimation has been described previously (D. Dingli *et al.*, unpubl. data, 2006). Briefly, a 'typical' myeloma cell produces IgG at a rate $P \approx 12.1$ pg/day and the M-protein is cleared at a rate $k = 0.117$/day (i.e. 11.7%/day).[11,17] If $M(t)$ is the size of the serum M-protein and PV is the plasma volume, then disease burden at a given time, $N(t)$ is given by:

$$N(t) = \log\left[\frac{(1-k)}{p} M(t) PV\right] \tag{1}$$

Plasma volume was estimated from the patients' height, weight and hematocrit using established nomograms.[18] Patients who achieve CR have a disease burden $<10^9$ cells.[19] The difference between the pretransplant disease burden, N_{PT}, and burden at day 100, N_{100}, was taken as the maximum decrease in tumor burden. For patients who achieved CR, tumor reduction was estimated as:

$$\Delta N(t) = N_{PT} - 10^9 \tag{2}$$

Gompertzian tumor growth. Assuming that MM growth can be described by the Gompertz function, the number of tumor cells at any time, $N(t)$, is given by:

$$N(t) = N(0) e^{\frac{\alpha}{\beta}(1-e^{-\beta t})} \tag{3}$$

Growth is assumed to start from a single cell, $N(0) = 1$ and as $N(t \to \infty) = e^{\alpha/\beta}$ tumor growth requires that $\alpha > \beta$. The Gompertz function satisfies the relationship:

$$N(t_2) = N(t_1) e^{\frac{\alpha(t_1)}{\beta}[1-e^{-\beta(t_2-t_1)}]} \tag{4}$$

where $\alpha(t_1) = \alpha e^{-\beta t}$.

Defining as N_1 the disease burden after transplant (occurring at time t_1) and N_2 the disease burden at relapse (occurring at time $t_2 = t_1 + \gamma$) then:

$$\ln(N_2) - \ln(N_1) = \left[\frac{\alpha}{\beta} - \ln(N_1)\right](1 - e^{-\beta\gamma}) \tag{5}$$

Defining the functions $e(\alpha, \beta; i) = \ln[N_2(i)] - \ln[N_1(i)]$ and $f(\alpha, \beta; i) = \left[\frac{\alpha}{\beta} - \ln(N_1(i))\right](1 - e^{-\beta\gamma(i)})$, the parameters (α, β) can be determined by minimizing the positive definite function:

$$S(\alpha, \beta) = \sum_{i=1}^{M} [e(\alpha, \beta; i) - f(\alpha, \beta; i)]^2 \tag{6}$$

where $\{N_1(i), N_2(i), \gamma(i)\}$ result from the data associated with the cohort of patients who achieved a partial response.

We did not do a straightforward minimization of $S(\alpha, \beta)$ as the minimum may violate basic requirements pertaining to the choice of the specific cohort under analysis. Instead, our minimization of $S(\alpha, \beta)$ required additional constraints. Specifically, our fit required that for all LCR patients the Gompertz curve is compatible with a partial response, that is, a burden reduction of less than five orders of magnitude upon transplant (D. Dingli *et al.*, unpubl. data, 2006) as well as a rate of growth such that all patients have a tumor burden above 10^9 cells (a hallmark of partial response) 100 days after transplant. For patients who achieved CR, a similar procedure was carried out (further details provided in the 'Results' section).

Transplant modeling. Defining $n = \ln(N)$, Eq. (3) can be written in the form $n(t) = K(1 - e^{-\beta t})$ (where $K \equiv \alpha/\beta$). The instantaneous growth rate is given by the time derivative of $n(t)$, $\dot{n}(t)$, which can be written in terms of $\dot{n}(t)$ as $n(t) = \alpha(1 - n(t)/K)$. Clearly, the higher the tumor burden, the lower its growth rate. This can have a dramatic impact on the tumor relapse time, as illustrated in Figs 1 and 2.

An important parameter in transplant is the TTP. Given the general (Gompertz) parameters (α, β), the TTP associated with a single transplant, T_1, is given by:

$$T_1 = \frac{1}{\beta} \ln\left(1 + \frac{L}{K - n_0}\right) \tag{7}$$

where L stands for the $\log_e$-reduction with a CR and n_0 is the log-burden before transplant.

The probability $p(n)$ for achieving CR after transplant is given by:

$$p(n) = \frac{1}{1 + e^{r(n-s)}} \tag{8}$$

with $r = 0.928$ and $s = 26.086$ (D. Dingli *et al.*, unpubl. data, 2006). This function is effectively 1 for $n < 21$ and 0 when $n > 32$, strongly limiting the window of disease burden for which HDT-ASCT will lead to a CR. The optimum disease burden n_0 is found when the quantity $g(n) = T_1(n) \times p(n)$ is maximal (Fig. 3).

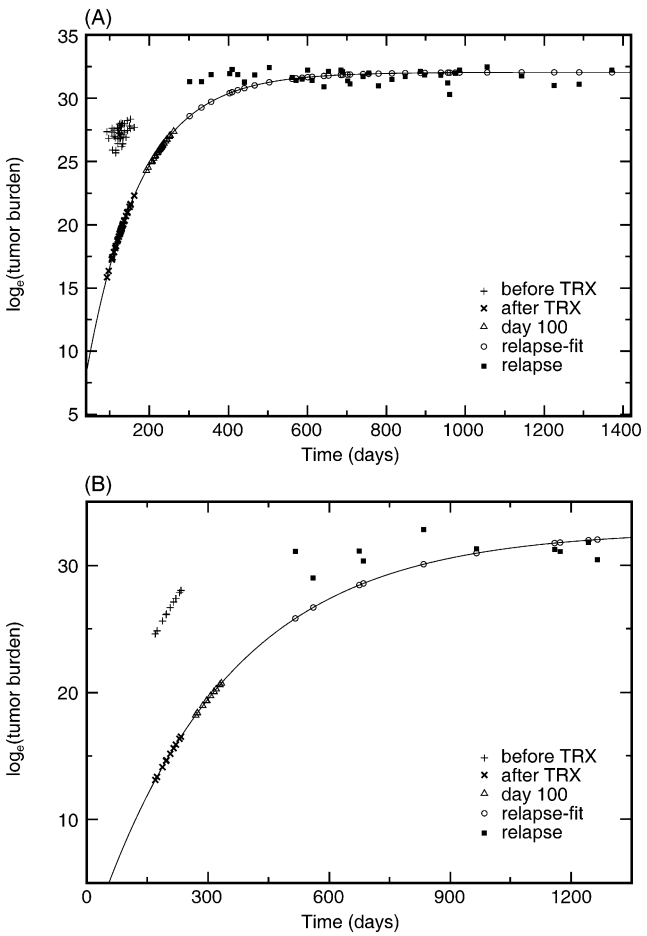

Fig. 1. Optimized Gompertz curves for myeloma growth kinetics after high-dose therapy and autologous stem cell transplantation (HDT-ASCT). Patients who (A) did not achieve a complete response (LCR) had a faster-growing tumor (β) compared to (B) patients who achieved a complete response (CR). The nadir tumor burden immediately after transplant for patients who achieved CR was estimated based on the constraints imposed on the fitting (see 'Methods'). Tumor burden was plotted as the natural logarithm for mathematical convenience.

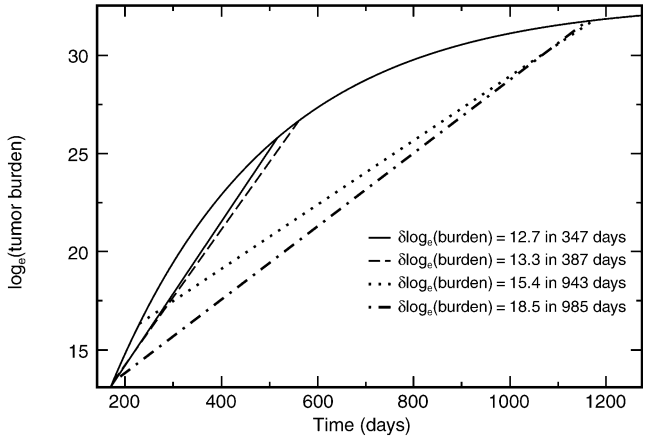

Fig. 2. The nadir of the tumor burden with high-dose therapy and autologous stem cell transplantation (HDT-ASCT) dictates the rate of tumor regrowth. The lower the nadir, the higher the net rate of tumor growth, as can be seen from these pairwise comparisons.

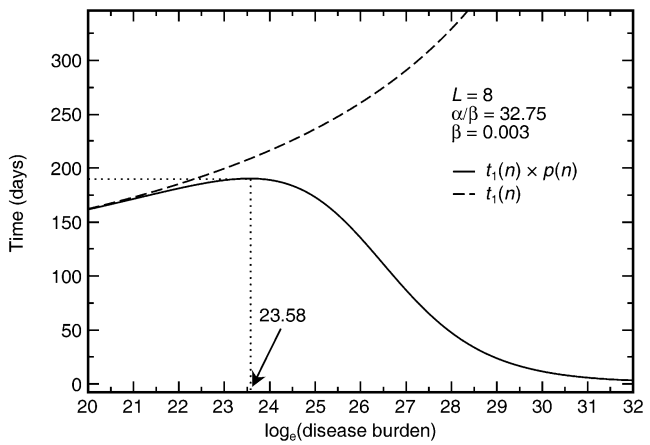

Fig. 3. There is an optimal time and disease burden that maximizes the benefit (in terms of prolonging time to progression [TTP]) from transplant. The profile of the function $g(n)$ (defined in the main text) is plotted as a function of the log-disease-burden. It shows the one-humped nature of this function, with a maximum value that takes place when the probability $p(n)$ for complete response (CR) is ~90%. Parameters in the figure are: $\alpha = 0.067$, $\beta = 0.002$, $s = 26.086$ and $L = 8$.

To calculate the TTP, T_2, for a patient undergoing two transplants, we denote by Δ the time interval between the first and the second transplant (Fig. 4) and by ε the time it takes for the tumor burden to relapse to n_0. We obtain:

$$T_2(\Delta) = \Delta + \varepsilon = \Delta + \frac{1}{\beta}\ln\left[\frac{L + e^{-\beta\Delta}(K - n_0 + L)}{K - n_0}\right] \qquad (9)$$

In general, the time interval Δ is constrained to the interval $[\Delta_{min}, \Delta_{max}]$. This fixes the actual critical value of the Gompertz parameter β, β_{crit}, above which a second transplant is not feasible:

$$\beta_{crit} = \frac{1}{\Delta_{min}}\ln\left[1 + \frac{L}{K - n_0}\right] \qquad (10)$$

Statistical analysis. All comparisons between groups were carried out with non-parametric tests with the Mann–Whitney test for continuous variables and the χ^2-test for nominal variables. Differences between groups were considered significant if $P < 0.05$.

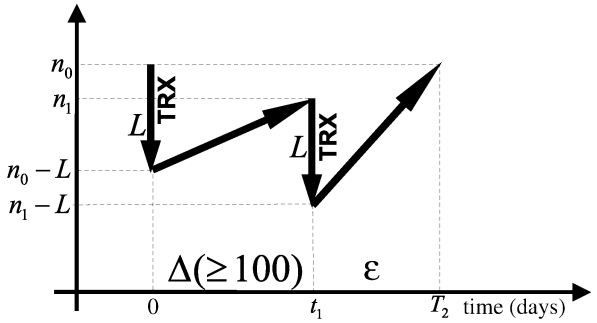

Fig. 4. The relationship between multiple transplants and time to relapse. Time to progression (TTP) from two optimally timed transplants can be twice what can be achieved with a single transplant. This performance cannot be achieved with tandem transplants.

Results

Common Gompertz function for myeloma. In their study, Sullivan and Salmon found that MM growth followed approximately a Gompertz function, although different individuals have different combinations of the Gompertz function parameters (α, β).[11] Here, we shall infer common Gompertz growth functions for MM from a large cohort of patients. This allows us to demonstrate the importance of tumor kinetics in evaluating the response to HDT-ASCT, without compromising the essence of the conclusions drawn.

Our clinical data come from analysis of a cohort of 265 patients with MM treated at the Mayo Clinic Rochester (D. Dingli *et al*., unpubl. data, 2006) and are divided into two groups: (i) patients who achieved CR, for whom we can estimate the disease burden immediately before transplant, at relapse and the time interval between transplant and relapse; and (ii) patients who achieved less than CR (LCR) for whom we can estimate the disease burden immediately before transplant, 100 days after transplant, as well as the burden at relapse together with the time between transplant and relapse.

There were no differences between the CR and LCR groups with respect to known prognostic parameters, including β_2 microglobulin ($P = 0.22$), plasma cell labeling index ($P = 0.117$), aneuploidy ($P = 0.55$), deletion 13 ($P = 0.358$), $t(4;14)$ ($P = 0.092$) and C-reactive protein ($P = 0.58$).

Assuming that all LCR individuals fell on the same growth curve, we determined the best Gompertz function that accommodated the data corresponding to these patients. Using a constrained least squares fitting as described in the 'Methods', we estimated (α, β) for the cohort. Our results show that $K \equiv \alpha/\beta = 32.04$ and $\beta = 0.0074$/day. Note that whereas the ratio α/β is similar to the value estimated by Sullivan and Salmon,[11] the specific value for β is considerably smaller. As elaborated in the 'Methods', a simple unconstrained fitting would lead to values remarkably similar to those obtained by Sullivan and Salmon, that are twice as large as the ones reported here. However, the constraints imposed ensure that the Gompertz function is compatible with a cohort of patients who achieve only a partial response. The optimal values obtained together with the associated curve and data are depicted in Fig. 1A.

Given these parameter estimates, we tested whether the data for the CR cohort would fit into the Gompertz function obtained for the LCR patients, but this was not possible. In order to achieve a reasonable fit, we assumed that all CR patients underwent the same reduction in their disease burden with HDT-ASCT. Subsequently, we deduced a common Gompertz curve constrained by the known tumor burden before transplant and at relapse together with the fact that the maximum log reduction

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with transplant cannot be higher than five orders of magnitude (D. Dingli *et al*., unpubl. data, 2006) and with a tumor burden less than 10^9 cells at day 100 after transplant (compatible with CR). The values obtained ($K \equiv \alpha/\beta = 32.75$, $\beta = 0.0030$) show that for patients who achieved CR, the best common Gompertz curve reflects a tumor that is growing at a slower rate, as shown in Fig. 1B. As for α it mostly rescales to meet a similar asymptotic value when $t \to \infty$.

Myeloma growth kinetics. Using the common Gompertz growth function defined for the cohort of patients who achieved CR, we compared the post-transplant tumor growth rates for these patients (Fig. 2). We compared two matched pairs of patients who had similar TTP (±40 days) after achieving CR. For the first pair, with average TTP of 360 days, the Gompertz curve predicts that both individuals dropped to the same log-burden upon transplant, into a region of rapid tumor growth. The tumor burden increases by $\log_e \approx 13$ in those 360 days but the 40 days difference for this pair implies an additional tumor increase of $\log_e \approx 0.6$ for the patient with longer TTP. The second pair of patients reveals the striking effect of log tumor reduction with transplant. Similar to the previous pair, these two patients have comparable TTP (40 days apart) and reach a similar burden at relapse. However, they start from different burdens after HDT-ASCT, and this has dramatic effects on the overall rate of tumor regrowth until relapse is diagnosed. The patient with a higher disease burden after transplant experienced an increase in tumor burden of $\log_e \approx 15.4$, whereas for the patient with a lower disease burden post-transplant, the disease burden increased by as much as $\log_e \approx 18.5$ in an interval of time only 40 days longer. The message from Fig. 2 is clear: optimal timing of HDT-ASCT is critical to achieve the best possible TTP.

If we consider that with HDT-ASCT the log-reduction is constant, this clearly indicates that careful timing of transplant will optimize the overall success of therapy. We shall elaborate more on this issue below, but Fig. 2 shows that the location of tumor burden on the Gompertz function strongly determines the dynamics of tumor regrowth: patients with a lower tumor burden after transplant have a higher rate of regrowth (D. Dingli *et al*., unpubl. data, 2006).

Optimal disease burden and response to transplant. One of the benefits of transplant is that it significantly reduces the disease burden and allows patients to be free of therapy with its associated side-effects. Thus, we were interested to find how long it takes for a patient to relapse to a preset disease burden, assuming that after transplant the patient achieved a CR, with a tumor burden reduction of $\approx 10^{3.5}$, (D. Dingli *et al*., unpubl. data, 2006) that is a $\log_e$-reduction $L = 8$. The relapse time is given by Eq. (7), which suggests that TTP increases with decreasing β and with increasing n_0 (pretransplant disease burden). In other words, the slower the rate of growth of the tumor and the higher the log-burden before transplant, the longer it takes for the patient to relapse back to the pretransplant disease burden. However, the probability of achieving CR is strongly dependent on the disease burden before transplant (D. Dingli *et al*., unpubl. data, 2006). Thus, there is an optimum disease burden that maximizes the TTP. This optimum burden can be found by maximizing the function $g(n) = t_1(n) \times p(n)$ – cf. Eqs (7) and (8). This function is one-humped, as shown in Fig. 3, and mainly determined by the decaying function $p(n)$, being only mildly sensitive to (α, β, L). Note that whereas the underlying values of (α, β, L) may vary from patient to patient, our analysis does not depend on their specific values.

When is HDT-ASCT beneficial? The aim of HDT-ASCT is to obtain CR with its associated improvement in survival and freedom from therapy. However, whereas the probability of achieving CR is related to the pretransplant tumor burden, the duration of the response is dictated by the kinetics of the tumor (i.e. β). If the Gompertz function for a patient is determined, the probability of CR as well as the expected duration of the response can be estimated using Eq. (7). This will provide additional information that can guide decision making as it is doubtful whether a patient should undergo HDT-ASCT if the time to relapse is very short (cf. 'Methods'). Moreover, a value of β suggesting that the tumor will reach the pretransplant burden before 100 days, Eq. (10), will seriously question the wisdom of a transplant, let alone a tandem transplant.

How many transplants and timing between transplants. Clinical studies suggest that patients who do not achieve CR with the first transplant may benefit from a second transplant, possibly because they have a lower disease burden before the second transplant. To test this hypothesis, we utilized the Gompertz function for patients with LCR, to estimate their disease burden at day 100 after the first transplant. Subsequently, we used our probability function $p(n)$, Eq. (8) (D. Dingli *et al*., unpubl. data, 2006) to estimate the probability of achieving CR after a second cycle of HDT-ASCT in the cohort of 165 patients. Our function predicts that 8.9% of the patients with LCR will achieve CR if transplanted again approximately 100 days after the first transplant. This value is in excellent agreement with clinical evidence.[2,5]

Although in experienced centers the mortality associated with one cycle of HDT-ASCT is 1–2%, mortality for tandem transplants increases up to 6–8%.[2,5,20] Some centers now routinely offer a second, tandem transplant even to patients who achieve a CR with the first transplant. Therefore, we evaluated the incremental increase in TTP from a second transplant versus one transplant, given that the patient achieved CR with the first procedure. In other words, if TTP after one transplant is T_1 and TTP for tandem transplants is T_2, we tested whether T_2 can ever exceed twice the value of T_1. The process is illustrated in Fig. 4. Mathematically, $T_2 = \Delta + \varepsilon$, but Δ is constrained to the interval $[\Delta_{min}, \Delta_{max}]$ at all times. Indeed, the value of Δ_{min} is fixed by the specificities of the transplant itself because, in general, a patient undergoes a second transplant after a time interval of $\Delta_{min} \approx 100$ days. However, Δ_{max} depends on the growth rate of the tumor. Mathematically, the burden at the moment of the second transplant cannot exceed $n_0 + L$ otherwise relapse will never occur. In general, the effective Δ_{max} will be smaller than this extreme limit, as one must make sure that the probability of achieving CR with a second transplant is high, which is given by the probability function $p(n)$.

According to Eq. (10) (see 'Methods'), for $\beta < \beta_{crit}$ the choice $\Delta = T_1$ will lead to $T_2 = 2T_1$. For values of $\Delta > T_1$, although $T_2 > 2T_1$ we cannot overlook that the probability for CR with the second transplant becomes suboptimal, and therefore there is a finite risk that the second transplant does not lead to CR. Beyond the intricacies of mathematical reasoning, however, clinical practice shows that other factors may lower the probability of achieving CR with a second transplant. Hence, $\Delta = T_1$ seems the adequate choice that, in view of Eq. (10), will only be advised to the extent that the patient-specific β satisfies $\beta < \beta_{crit}$. Based on the clinical data available from our cohort of patients, we estimate that the optimal time between two transplants is of the order of 7 months, similar to what has been observed in a large cohort of patients undergoing multiple transplants for the disease.[20]

Discussion

HDT and stem cell transplantation have improved survival in patients with MM.[1–5,21–23] However, it is clear that not all patients benefit from the procedure and tumor burden and kinetics as well as the biology of the disease determine the outcome of this therapeutic modality. HDT-ASCT is associated with substantial morbidity and tandem transplantation has a higher mortality, even in centers with a long experience in the field.[2,5,20] For any therapeutic intervention there is a cost in both morbidity and mortality and this cost must be compensated adequately by potential gains either in survival or quality of life, such as time

off therapy. It is becoming clear that although achieving CR with HDT-ASCT is important,[24] perhaps equally important is the duration of the response (TTP).[10,25] Hence, knowledge of the specific kinetic characteristics for a given patient may allow optimal use of this potentially toxic therapy.

In the present work, we have extended our observations about the impact of the pretransplant disease burden in MM (D. Dingli *et al*., unpubl. data, 2006) to include tumor growth kinetics in order to understand the results of HDT-ASCT for this disease. Perhaps the most striking observation is the fact that patients who achieve CR have a lower tumor burden before transplant and slower tumor growth compared to patients who do not achieve CR. This can be inferred from the significantly different Gompertz functions obtained for patients who achieve CR compared to the LCR cohort ($\beta = 0.003$ and $\beta = 0.0074$, respectively). The lower β (0.003) in the CR cohort translates into a slower rate of tumor growth and consequently a longer TTP. Moreover, our results also suggest that patients with kinetically very active disease may not benefit from a single, let alone tandem, transplant due to rapid relapse.[26] These results are compatible with observations showing the inferior outcome of patients with a high plasma cell labeling index.[25,27] The importance of pretransplant disease burden is also highlighted by the prediction of CR with tandem transplants. Our model, based on actual M-protein measurements, predicts that 8.9% of the patients who did not achieve CR with the first transplant will achieve this goal after a second transplant if carried out around 100 days after the first. This value is in excellent agreement with what has been observed in several studies[2,5] and further validates our model and its conclusions.

These results beg the question of what is different between patients who achieved CR and those that did not have such a response. In our previous studies of this cohort of patients, we demonstrated the impact of pretransplant disease burden on the probability of achieving CR (D. Dingli *et al*., unpubl. data, 2006). In the present report we demonstrate the critical importance of tumor dynamics on the duration of the response to therapy. These two parameters are naturally intertwined as clearly tumor burden at any time is due to prior tumor growth. Unfortunately, transplant seems to reduce only the tumor burden and serves to reset the clock, if we assume that the characteristic Gompertz function that describes each individual tumor is unaltered by HDT. Progress in therapy will require approaches that alter the growth kinetics of the tumor (decrease β) for more meaningful prolongation of responses to therapy or potential for a cure. We hope that current genome[28]- and proteome[29]-based approaches will identify valuable targets that make such therapies possible.

Our results also support the practice to withhold a second (tandem) stem cell transplant for patients who achieve CR with the first transplant. Even under the best of conditions, tandem transplant will not lead to a doubling of the TTP despite an increase in morbidity and mortality. Knowledge of the Gompertz function illustrates why the TTP for patients undergoing tandem transplantation is only increased by a few months compared to what is achieved with the first transplant (32 vs 23 months).[2] An early second transplant may simply lower the tumor burden to an even steeper part of the Gompertz curve with its associated higher growth rate that counters the potential gain in TTP for the same log reduction in tumor burden (Fig. 2). Similar reasoning explains the apparent lack of benefit from graft purging[9] and cyclophosphamide mobilization[10] as both procedures lead to a lower tumor burden with a higher rate of tumor growth after HDT-ASCT. Thus, timing of the first transplant based on an estimation of the patient-specific (α, β) will optimize the likelihood of CR. Hence, patients who achieve CR with the first transplant can be observed and retransplanted at the time of early relapse, preferably when their disease burden is again close to optimal (Fig. 3).

Our results suggest that the optimum time for a second transplant can be determined if the growth kinetics of the tumor in a patient are estimated by close serial observation of the serum M-spike. By timing the second transplant such that tumor burden is similar to the burden before the first HDT-ASCT, the probability of achieving a second (new) CR with its associated longer TTP will be high and the therapeutic benefit maximized. In principle, the duration of the response to the second transplant could be similar to that achieved with the first transplant assuming there is no change in tumor kinetics or the emergence of a clone resistant to the conditioning regimen. Spacing out the two transplants can possibly decrease mortality without compromising the benefit of the procedure, a feature which will be facilitated in tumors with an associated small β.

Any modeling rests on assumptions and our work has its own set. Our probability function $p(n)$, Eq. (8), implicitly assumes that on average myeloma cells produce M-protein at a constant rate and that clearance of the protein is also at a constant rate over a wide range of concentrations of the protein. These assumptions are, however, based on observation.[11,17,30] We also assume that the tumor growth kinetics before and after transplant are unaltered, compatible with the Norton–Simon hypothesis.[12] Finally, perhaps optimistically, we assumed that the log reduction in tumor burden associated with a second transplant is the same as that from the first transplant. If the burden reduction is less, the gain in TTP from the second transplant will naturally decrease.

Personalized HDT-ASCT for myeloma? Individualizing therapy for patients with any disease is a major goal of modern therapeutics. Our studies suggest that this may be possible for patients with myeloma when HDT-ASCT is being considered. If a patient can be safely observed without therapy for some time, serial measurements of the serum M-spike allow estimation of the kinetics of the tumor burden and its associated Gompertz parameters. Using our probability function $p(n)$, the probability of a CR with HDT-ASCT can be determined.[31] Moreover, knowledge of the pretransplant tumor burden and the patient-specific Gompertz parameters (together with the maximum potential tumor reduction with transplant) will allow an estimate of the duration of the response to HDT (Eq. 7). If the estimated time to relapse is short, one might consider alternative therapies with lower toxicity. However, whenever the rate of tumor growth is sufficiently slow, HDT-ASCT may be the preferred therapeutic option. Assuming that HDT does not alter the Gompertz parameters for the tumor, Eqs (7) and (9) will provide accurate estimates for the optimal time to proceed to a second transplant. Assuming that $\beta \approx 0.003$, as we observed in patients with CR, the time between transplants would amount to 7 months in most patients. Given that this estimate is based on the assumption of an optimal disease burden before the second transplant together with the inherent variability in the behavior of the disease and response to HDT in different patients, this result is very close to what has been reported by Vesole *et al*. for a large cohort of patients[20] and further supports our methodology and results.

In conclusion, optimizing therapy for patients with MM requires an understanding of the determining role of both tumor burden and kinetics before any therapeutic intervention. In principle, the optimal time for HDT-ASCT for a patient can be estimated if tumor growth can be followed by a time sequence of disease burden assessment before therapy in that patient. This will allow the determination of the characteristic Gompertz function that is specific for such a patient. From the function, both disease burden and growth kinetics can be predicted as well as the probability of a response to therapy. Clearly, identification of the kinetics of tumor growth may provide vital information regarding the feasibility of designing treatment that is best suited for each individual patient.

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