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# The Arkansas approach to therapy of patients with multiple myeloma

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## Abstract

This chapter gives an account of the experience of the Arkansas myeloma program since 1989 with transplant-supported high-dose melphalan, novel agents, and prognostic factors as they relate to standard laboratory features, gene expression profiling, and magnetic resonance imaging (MRI). Incorporation of novel agents and new concepts, such as post-tandem transplant consolidation therapy, has improved the rate and duration of complete response and prolonged event-free and overall survival rates. With Total Therapy 2, median survival exceeds 8 years, while Total Therapy 3 with added bortezomib has sustained complete remissions in more than 90% of patients at 2 years which, when used as a survival surrogate in Total Therapy 2, assured a high 6-year survival rate of 75%. Gene expression profiling identified 15% of patients with very short survival. MRI-defined focal lesions are associated with poor outcome, while their resolution – although slower than the time course of attaining clinical complete remission – conferred superior survival. Representing a frequent source of recurrence, with genetic profiles (in both plasma and stromal cells) distinct from those in random bone-marrow samples, therapeutic efforts are directed at hastening onset and increasing frequency of focal lesion resolution.

### Keywords

multiple myeloma; autotransplant; therapy; prognostic factors; event-free survival; overall survival

The treatment of patients with multiple myeloma (MM) was largely palliative until, with the advent of high-dose melphalan (MEL), high rates of complete response (CR) could be obtained.[1] Our team introduced the use of autologous hematopoietic stem-cell support, initially with bone marrow[2,3] and later with chemotherapy plus growth-factor-mobilized peripheral-blood stem cells (PBSCs).[4,5] When the feasibility of repeated cycles of MEL administration was established,[6] this ‘tandem’ transplant approach was evaluated in the front-line setting of newly diagnosed symptomatic or progressive MM as part of Total Therapy 1 (TT1).[7,8] This concept was modeled after St Jude’s Children’s Hospital Total Therapy programs for acute leukemia which have achieved unprecedented progress in curing children afflicted with acute leukemia.[9] In this review article we describe our collective experience with autologous transplants and with non-transplant combination regimens as well as new agents in the

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treatment of newly diagnosed and previously treated patients in the context of prognostic factors to be used in future risk-based trials.

## TOTAL THERAPIES FOR PATIENTS WITH NEWLY DIAGNOSED MM

The underlying rationale for TT in MM was to apply, from the outset, all agents with proven single-agent activity toward sustained disease control and eventually cure. No more than 20% of patients had achieved CR status after a single application of MEL at a dose of 200 mg/m$^2$ (MEL200),[10–12] which we considered insufficient as a curative strategy since cures in acute leukemia seemed to require a CR threshold of at least 40%.[13] As mucositis prevented further MEL dose escalation, we developed the 'tandem' transplant approach toward maximizing the frequency of CR as a quintessential first step toward improving clinical outcomes beyond results obtained with standard melphalan-prednisone (MP) therapy that produced low CR rates (under 5%) and median survival on the order of 30–36 months.[**14]**

### Regimens and patient characteristics

Since 1989, 1202 newly diagnosed patients have received TT protocol therapies, including 231 enrolled in TT1, 668 in TT2 and 303 in TT3, with median follow-up times of 160, 60 and 24 months, respectively. Regimen details and clinical outcomes have been published previously. [7,15,16] TT1, a phase-II pilot study, evaluated MEL200-based tandem transplants after induction with three cycles of non-stem-cell-toxic VAD (vincristine, doxorubicin, dexamethasone [DEX]), followed by high-dose cyclophosphamide (HDCTX) for PBSC mobilization of at least 5 x 10$^6$ CD34 cells/kg, and a final cycle with EDAP (etoposide, DEX, arabinosyl-cytosine, cisplatin) that targeted a lymphoma-like, more immature MM compartment. Maintenance therapy consisted of interferon-α-2b (IFNα2b), based on promising results from the Royal Marsden group.[17] TT2**,** a phase-III trial, examined whether the up-front addition of thalidomide (THAL), with profound salvage potential in advanced and refractory MM,[18,19] could increase the frequency of CR and thereby extend event-free survival (EFS) and overall survival (OS). Compared to TT1, TT2 also applied more intensive growth-factor-supported multi-agent chemotherapy as induction prior to – and introduced consolidation chemotherapy after – MEL200-based tandem transplant; high-dose DEX pulsing was administered during the first year of IFN maintenance therapy. The recognition of bortezomib's activity in end-stage MM[**20]** and its synergy with THAL and DEX in VTD [21,22] led us to explore, in TT3, its addition to DT-PACE (DEX, THAL, cisplatin, doxorubicin, cyclophosphamide, etoposide), a highly effective combination in advanced MM. [23] Anticipating superior activity of VTD-PACE compared with induction and consolidation regimens employed in TT2 (see above), we applied only two instead of the previous four cycles in both phases of treatment. Previous 'drug-free' phases between induction and consolidation cycles in TT2 were 'bridged' with THAL + DEX to dampen post-chemotherapy cytokine elaboration with potentially deleterious anti-apoptotic effects on MM cells.[24] Maintenance consisted of monthly VTD during the first year and THAL + DEX during years 2 and 3.

Patient characteristics were similar in the three regimens. Importantly, one third had cytogenetic abnormalities (CAs) recognized as the single most adverse feature among standard prognostic factors (SPFs).[25] However, the proportion of patients over the age of 65 years had increased from 9% in TT1 to 20% in TT2 to 28% in TT3. Significantly higher proportions of patients completed the intended therapies in TT3, especially first and second transplants (94% and 83% versus 85% and 67%, respectively, in TT2).

### Clinical outcomes

The frequency of CR increased from 40% with TT1 to >60% with TT2 plus THAL (versus 40% without THAL) and remained at approximately 60% with TT3 (although the timing of

its onset was accelerated) (Figure 1a). Importantly, CR duration, measured from its onset (medians of 18 months with TT1, 12 months with TT2, 8 months with TT3), was significantly extended from medians of 2.5 years with TT1 to 5.5 years with TT2 (no difference between arms) and to a 3-years estimate with TT3 of >90% (Figure 1b). EFS was similarly prolonged from medians of 3 years with TT1 to 5 years with TT2 (71 months with thalidomide versus 51 months on the control arm, $P = 0.002$) to a 3-years estimate of >80% with TT3 (Figure 1c); the corresponding values for OS are 6 years with TT1and 8.5 years with TT2 (THAL versus no THAL, $P = 0.169$) while the 3-years estimate with TT3 is 85% (Figure 1d). As consequence of greater dose density and THAL+DEX bridging therapy in TT3, the cumulative relapse rate following each protocol step over the ensuing 12 months was significantly decreased in comparison with TT2 (Figure 1e). Despite greater regimen intensity and advanced age in TT3, these improvements were accomplished without increasing the 1-year cumulative treatment-related mortality beyond 5%.

On multivariate analysis of baseline SPF, OS was adversely affected by CA (HR = 1.53, $P = 0.002$), creatinine (≥2 mg/dL) (HR = 1.47, $P = 0.016$), age (≥65 years) (HR = 1.35, $P = 0.013$), $\beta_2$-microglobulin (B2M) (≥4 mg/dL) (HR = 1.29, $P = 0.027$) and C-reactive protein (CRP) (>4 mg/dL) (HR = 1.28, $P = 0.011$); in addition, compared to TT3, TT1 and TT2 (both arms) also imparted shorter OS (TT1: HR = 2.00, TT2 without THAL: HR = 1.44, TT2 with THAL: HR = 1.21; $P = 0.0002$). The superiority of TT3 was also confirmed by pair-mate analyses matching patients by SPF or GEP features (not shown).

## Gene expression profiling (GEP)

GEP studies on CD138-purified plasma cells were available in 351 of the 668 patients entered on TT2. Results revealed significant prognostic implications of three models: an unsupervised seven-group model,[26] a model derived from comparisons with subjects having monoclonal gammopathy of undetermined significance (MGUS),[27] and a supervised model based on TT2 survival outcomes.[28] In the context of TT3, the GEP-defined high-risk group (70-gene model)[28] was a powerful discriminator of OS, EFS and CR duration (Figure 2). In comparison to TT2, TT3 prolonged CR duration in low-risk MM, EFS in both risk groups, and OS in the *FGFR3* subgroup.

GEP studies were also performed 48 hours after a test dose of bortezomib in order to determine whether there were any MM- or microenvironment-associated changes with prognostic implications. Suppression within 48 hours of bortezomib administration of a microenvironment-associated gene (*MAG1*) identified a subset of patients with a superior prognosis.[29] Additional studies are performed serially during remission and at relapse in order to determine: (1) whether a normalization of the microenvironment (in comparison with normal subjects) can serve as a surrogate marker for extended CR duration,[29] and (2) whether MM relapse represents an outgrowth of a small subset of high-grade MM cells present at diagnosis, or results from a transformation event.

A total of 220 patients enrolled on TT2 had information on all standard prognostic factors (SPF) as well as novel prognostic factors, including MRI-defined focal lesion number (MRI-FL), CA, fluorescence in-situ hybridization (FISH-)derived amplification of chromosome 1q21 (amp1q21) and deletion of 13q14, and GEP high-risk signature.[30] Five multivariate analysis-based survival models were derived, incorporating these factors in a stepwise fashion: SPF only (model 1), with progressive addition of CA (model 2), MRI-FL (model 3), (FISH) (model 4), and GEP (model 5). The $R^2$-value, a statistical measure of the clinical outcome variability, increased progressively from 18% in model 1 to 38% in model 5, implying that with information on GEP, nearly 40% of survival variation could be captured. The hazard ratio for OS was highest for GEP high-risk (3.01, $P < 0.001$), followed by amp1q21 (1.71, $P = 0.05$) in model 5. According to the presence of none (49%), one (35%), or both of these risk features

(16%), 3-year survival decreased progressively from 90% to 78% to 40% ($P < 0.0001$). Thus, the dominance of molecular genetics over SPF justifies the generation of quantitative reverse transcriptase polymerase chain reaction (RT-PCR) methodology ('MM genetic kit') for optimal risk stratification of patients participating in therapeutic trials. As a means of examining the risk over time of recurrence from CR and death, hazard rates were compared in TT3 and TT2 protocols among patients with GEP baseline data. In TT2, the risk of recurrence (Figure 3a) and death (Figure 3b) among patients with high-risk MM decreased to levels observed for those with low-risk disease at approximately 4 years from treatment start; in the case of TT3, the hazard rates in high-risk patients were lower than those in TT2 and decreased to levels observed in low-risk MM 2 years from initiation of protocol therapy.

## MRI examinations

As part of TT2, MRI examinations were performed at baseline and serially thereafter in order to determine their relevance to patient outcome.[31] A higher proportion of patients without metastatic bone-survey-defined focal lesions (MBS-FL) had MRI-defined FL than vice versa. MRI-FL number was associated with lower albumin levels but higher CRP, lactate dehydrogenase (LDH), and creatinine levels. When examined by multivariate analysis, MRI-FL but not MBS-FL conferred poor shorter EFS and OS. Present in 74% at baseline, MRI-FL resolved with a time lag of ~18 months in relation to the onset of clinical CR; resolution of MRI-FL imparted superior OS.

GEP comparisons of random iliac crest biopsies without focal lesions, CT-guided fine-needle aspirates, and biopsies from sites of MRI-FL revealed significantly higher *DKK1* expression levels in plasma cells[32] and higher levels of several ME-associated genes (MAG) in the focal lesions (Shaughnessy, unpublished). Considering that MRI-FL is a key discriminator of MM and MGUS, that MM relapse frequently occurs at sites of original involvement, and that MRI-FL can be the sole manifestation of relapse (non-secretory relapse), we postulate that such focal lesions with different plasma-cell and MAG features may harbor MM stem cells. Thus, future therapeutic trials should incorporate MRI examinations at baseline and periodically after therapy with the goal of accelerating MRI-CR.

## The role of clinical CR as a surrogate marker for long-term clinical outcomes with TT trials

The prognostic implications of time-dependent onset of CR on OS and EFS were examined in the context of SPF and GEP-derived data, available in a subset of 326 patients enrolled in TT2.[33] With knowledge of gene array data (70-gene model), CR conferred superior survival to the 13% of patients deemed at high risk (hazard ratio, 0.22; $P < 0.001$) but not to the remainder (hazard ratio, 0.69; $P = 0.17$); consequently, those with low-risk MM fared well regardless of CR achievement whereas CR was critical to extended EFS and OS in the high-risk subgroup, with obvious therapeutic ramifications. The unexpected lack of prognostic implications of CR for OS fits in with our recent observations that lower CR rates after TT2 in patients with a documented prior history of MGUS or smoldering MM[34] and in those with GEP-defined MGUS-like MM had no adverse consequences for EFS and OS.[27] These findings are consistent with the re-establishment of an MGUS-like condition in an 'MGUS-evolved' type MM following elimination of the transformed MM tumor-cell population. In examining the prognostic implications of baseline serum free-light chain (SFLC) levels, we also recently reported that high levels (>75 mg/dL) were associated with a higher CR rate but inferior EFS and OS.[35] Thus, high CR rates in high-grade MM may not translate into superior OS in case of rapid tumor regrowth or insufficiently profound tumor cytoreduction beyond the clinical CR threshold. Therefore, while recognizing that the pursuit especially of MEL dose intensity toward increasing CR frequency overall has paid off in terms of prolonging OS in general, the above results call into question an indiscriminate reliance on CR as an early clinical surrogate for OS. As an alternative, we examined whether a finite CR duration of at least 2 years, observed

in the majority of patients achieving CR on TT3, might be a more robust surrogate for long-term OS. Indeed, in a landmark analysis of patients treated with TT2, those remaining in CR at 2 years enjoyed a far superior OS (75% at 6 years) than those not achieving CR (median, 4.4 years) or, worse, those relapsing within 2 years after CR onset (median, 2.7 years) (Figure 4).

### Lessons from Total Therapies

We are drawing the following conclusions from our TT trial experience: (1) increasing CR frequency can – but does not necessarily – translate into improved EFS and OS;[36–38] (2) improved CR duration, EFS and OS with TT2 without THAL versus TT1 – despite similar CR rates – attests to the role of post-transplant consolidation introduced in TT2;[39] (3) improved CR duration and EFS (and borderline of OS) with TT3 versus TT2 plus THAL – despite similar CR rates – can be attributed to the addition of bortezomib and the greater compliance with intended therapy steps in TT3 versus TT2; (4) extrapolating from TT1 and TT2 results, the markedly higher proportion of patients with sustained CR in TT3 should result in a significant increase in 10-year event-free survival to at least 50%, from 15% with TT1 and an estimated 30% with TT2; (5) according to hazard rate estimates over time, risks of recurrence and death among patients with high-risk MM decreased to levels associated with low-risk disease much earlier, i.e. 2 years instead of 4 years after initiation of TT2; and (6) 2-year CR should be evaluated formally as a short-term clinical endpoint, especially to advance more rapidly the prognosis of patients with high-risk MM best captured by GEP.

## FUTURE DIRECTIONS OF CLINICAL TRIAL RESEARCH AT ARKANSAS

### Old versus new[40]

The availability of the immunomodulatory agents, thalidomide and lenalidomide, and of the proteasome inhibitor bortezomib, proven effective as salvage therapy for advanced and refractory MM, has spawned trials with these new agents alone and in combination with DEX, each other, and, most recently, with standard-dose MEL in newly diagnosed patients. Remarkably high PR and near-CR/CR rates have been reported, especially in trials employing new agent combinations with MEL and doxorubicin. However, follow-up in these studies is short, so that information on the durability of remissions induced by these new agents and subsequent salvage potential after relapse is not available. We are envisioning trials that are designed to ensure sustained progress, achieved thus far with the concepts of dose intensification and incorporation of new agents proven effective in advanced disease into the up-front management of MM. A sensible place for exploration of new agent combinations is in the setting of the elderly and those with significant comorbidities.

### Additional accrual into TT3

The encouraging results that have emerged from TT3 in the first 303 patients, together with important translational aspects of molecular genetics and imaging, have prompted us to accrue another 100 patients into a follow-up trial of TT3 in order to clarify whether: (1) baseline GEP data and bortezomib-induced alterations in gene expression can be validated as a potential prelude to risk-adapted therapy in Total Therapy 4; (2) gene expression data of remission bone-marrow biopsies can serve, in comparison with normal donor samples, as an indication of the depth of remission; (3) early suppression of 18F-2-fluoro-2-deoxy-D-glucose (FDG) uptake on follow-up PET scanning, or lack thereof, can identify likely success or failure of TT3 in the context of GEP data; and (4) resolution of MRI-FL is a critical positive feature for long-term disease control.

### Total Therapy 4 (TT4)

A risk-adapted approach is envisioned in TT4, such that patients with *low-risk MM* shall be randomized between the current TT3 versus TT3-LITE, applying only one cycle of VTD-PACE each for induction and consolidation and VRD (bortezomib, lenalidomide, DEX) instead of VTD for maintenance. For *high-risk MM*, one cycle of dose-escalated VTD-PACE will be employed with PBSC collection. Patients achieving near-CR will continue with dose-dense (every 21–28 days) and dose-intense VTD-PACE for an additional four cycles, each supported by a stem-cell boost, followed by four cycles of M-VRD (melphalan 10 mg/m$^2$/day for 4 days plus VRD) with PBSC boosts as needed and 3 years of monthly VRD maintenance. In patients failing to achieve near-CR, fractionated high-dose MEL at 300 mg/m$^2$ will be applied in three equal fractions of 100 mg/m$^2$ on days 1, 4 and 7, along with VRD followed by PBSC support on day 8. After VRD bridging therapy, a further MEL300-VRD cycle will be applied, to be followed by M-VRD and VRD as for patients achieving near-CR after VTD-PACE. The primary clinical endpoint will be 2-year CR.

## PREVIOUSLY TREATED MM

### THAL in refractory MM[18,19]

A phase-I/II study of dose-escalated THAL (maximum daily dose of 800 mg/d) was conducted in 1998. Of 169 patients with advanced and refractory MM enrolled, 67% had CA; 76% had received one and 53% two prior transplants. PR status was achieved by 30%, including 10% who entered CR. Median durations of OS and EFS were 20 months and 6 months, respectively; at 72 months, nearly 20% of the patients are currently alive, and 10% remain event-free 7 years after treatment initiation.

### Bortezomib–thalidomide–dexamethasone (VTD) salvage therapy[21,22]

A phase-I/II trial of this combination was performed between March 2002 and December 2004. A starting dose of 1.0 mg/m$^2$ of bortezomib was administered on days 1, 4, 8, and 11, with a cycle length of 21 days. THAL was added with the second cycle at a starting dose of 50 mg/day, with dose escalation to 100 mg, 150 mg, and 200 mg in cohorts of ten patients, with the possibility of added DEX pulsing after cycle 2 in case PR status was not achieved. Bortezomib was then increased to 1.3 mg/m$^2$ in subsequent patient cohorts, using the same THAL dose escalation scheme and allowing for addition of DEX pulsing. The trial accrued 85 patients, 76% of whom had CA, and of whom 92% had had one and 65% two prior autotransplants. Eventually, 61% achieved PR status, including 7% who achieved CR. The dose-limiting toxicity of the combination was peripheral neuropathy grade 3, which was observed when THAL was increased to 150 mg with bortezomib 1.3 mg/m$^2$. The median durations of EFS and OS were 9 months and 22 months, respectively, but were shorter in the presence of CA (data not shown); at 3 years, 30% of patients are alive and 10% are event-free.

### Phase-II evaluation of rapidly recycled high-dose DT-PACE (HD-DTPACE) with PBSC boost [41]

Due to dose- and time-interval–limiting mucosal toxicity, MEL200 cannot be rapidly recycled, so that relapse may occur prior to the ‘timely’ administration of second transplant within 2–3 months after the first. Achieving similar CR rates in high- and low-risk MM is deceiving as far as long-term success of therapy is concerned because, in high-risk MM, the depth of tumor mass reduction is likely to be less profound, and tumor re-growth may proceed sooner. Thus, it appears worthwhile to test the hypothesis that rapidly recycled non-normal stem-cell-toxic HD-DTPACE (with scheduled PBSC boosts after each cycle to avoid cumulative myelotoxicity) can produce more profound net tumor cytoreduction without significant re-growth between cycles. Off-protocol pilot data in 30 patients, mostly with CA, are summarized

in Table 1. Of 16 patients receiving one cycle, five achieved CR, two near-CR, and eight PR; of eight receiving two cycles, one achieved CR, three near-CR and three PR; of three patients receiving three cycles, two achieved near-CR and two PR; all three receiving four cycles achieved near-CR. Thus, among all 30 patients treated, the rates of CR, near-CR and PR were 20%, 27% and 43%, respectively. There were no deaths in this series of patients.

### Fractionated MEL-VTD as salvage transplant regimen[42]

There is synergistic interaction between MEL and immunomodulatory agents, THAL and lenalidomide, as well as the proteasome inhibitor bortezomib.[43] To date, we have applied, in an off-protocol setting, MEL-VTD as follows: bortezomib administered at 1.0 or 1.3 mg/$m^2$ on days 1, 4, and 7 along with THAL at 100 or 200 mg/day for 7 days, DEX at 20 or 40 mg on the days of and after bortezomib administration, and MEL at 50, 60, 70, 80, 90, or 100 mg/$m^2$ after each bortezomib dose, for total MEL doses of 150, 180, 210, 240, 270 or 300mg/$m^2$ (Table 2). Despite highly unfavorable patient and disease features (CA present in 69% of all 94 patients), MEL-VTD promoted high frequencies of near-CR and CR in almost 70%, with frequent resolution of MRI-FL. Toxicities – especially grade >2 stomatitis – were noted in fewer than 10% of subjects. Tandem MEL300-VTD transplants were given to 11 patients, including two who had previously been exposed to a total dose of 400 mg/$m^2$ as part of an original MEL200-based tandem transplant regimen (total, 1000 mg/$m^2$).

An important component of a currently accruing formal trial of MEL300-VTD is a pharmacogenomic approach evaluating the 24-hour versus baseline GEP changes after a MEL test dose of 10 mg/$m^2$. Repeat analysis 48 hours after the first of three therapeutic MEL100 doses will allow us to link test-dose- and therapeutic-dose-induced GEP changes with clinical outcome. By examining both plasma-cell and MAG expression changes, we hope to identify MEL-unique and prognostically relevant tumor and stromal cell gene alterations as a prelude to future routine in vivo drug sensitivity testing.

### The Arkansas experience with more than 3000 transplants for MM (‘ARK 3000’)[44]

We performed MEL200-based transplants in more than 3000 patients with MM (‘ARK3000’). Three treatment groups were distinguished: (1) TT-P, comprising patients on TT protocols for newly diagnosed disease that included protocol-based induction therapy; (2) non-TT-P, comprising patients receiving other protocol-based transplants in case of prior treatment; and (3) non-P, comprising patients given off-protocol transplants due to protocol ineligibility or patient/physician preference. As expected, the TT-P group had more favorable baseline features in terms of CA, B2M, CRP, and albumin, compared with previously treated patients; pre-transplant CR was higher, and a second transplant was completed more frequently. Post-transplant outcomes (OS, EFS and CR duration not shown) with TT-P were superior to outcomes after non-TT-P, which in turn were better than those in the non-P group (Figure 5a). Multivariate analyses of pre-transplant parameters and type of treatment intervention revealed TT protocol therapies to be independent favorable features for OS, EFS and CR duration (Table 3). As shown in Figure 5b, five subgroups could be discerned that exhibited distinctly different OS according to the number of favorable parameters: absence of CA of chromosome 13 or hypodiploidy (CA13/hypo), low B2M, low CRP, high albumin, and high platelet count.

### Third transplants

Among 251 patients receiving a third transplant, 120 with at least 3 years elapsing since their second transplant enjoyed a subsequent survival of almost 2 years as opposed to 8 months among the reminder. Their better outcome was accounted for by more favorable laboratory features, such as lower incidences of CA (especially the CA13/hypodiploidy variety) at any time or within 6 months from first transplant and LDH elevation prior to first transplant. Thus, further stem-cell-supported therapies should be considered for the further management of

patients with durable preceding remissions, especially when new agent-associated toxicities such as neuropathy become overwhelming or pancytopenia develops that precludes administration even of the modestly myelosuppressive lenalidomide. We have also applied MEL-based transplants in the setting of treatment-induced myelodysplasia (t-MDS) or acute leukemia, provided that PBSCs had been collected at a time when no MDS-associated CAs (MDS-CA) were present on bone-marrow examination. Maintaining further transplant options requires collection of adequate PBSC quantities, in our practice a minimum of 20 x $10^6$ CD34/kg.

## SPECIAL CONDITIONS

### Renal failure[45]

As part of both TT and non-TT regimens, we have applied MEL-based autotransplants to 239 patients with renal failure, as defined by creatinine levels >2 mg/dL. Their baseline laboratory features, as a group, showed higher frequencies of anemia, thrombocytopenia, and hypoalbuminemia, and elevations of CRP and LDH; as expected, due to the renal route of clearance, B2M levels were markedly higher in the renal failure group. Fewer of these patients were in CR at the time of first transplant, and fewer proceeded with a second transplant; those who did had a greater time lapse from first transplant. Figure 5c depicts the survival outcomes of the 'ARK3000' population according to the three serum creatinine levels. Survival was significantly superior in the creatinine ≤2 mg/dL group versus survival of those with renal failure, although no difference was apparent when the median cut-point of 3.6 mg/dL was considered in the renal failure group.

### Advanced age[46]

From the early days of our involvement with MM transplants, we have tried to make stem-cell-supported high-dose therapies available to the typical MM patient over the age of 65 years. As depicted in Figure 5d, survival, while similar among the four subgroups up to age 64 years, shortened as age increased beyond 64 years, and particularly beyond 74 years.

### Pancytopenia and metronomic therapy[47]

Patients with pancytopenia, especially transfusion-dependent thrombocytopenia, present a major challenge when requiring MM therapy, particularly when hematopoietic compromise cannot be explained by extensive MM induced bone-marrow involvement. Such patients suffer either from cytogenetically or morphologically recognizable myelodysplastic syndrome (MDS) or from profound hematopoietic stem-cell damage without MDS as a consequence of extensive standard-dose or transplant-supported high-dose therapies. Based on the concept that low-dose continuous drug application of certain cytotoxic drugs (cyclophosphamide, etoposide, doxorubicin) may effect tumor inactivation by non-traditional mechanisms, such as via anti-angiogenic effects, we explored mainly low-dose doxorubicin (ADR) along with VTD (sometimes with added rapamycin, an inhibitor of TOR, the target of rapamycin).[48] A dramatic response was observed in a 66-year-old patient with advanced and refractory Waldenström macroglobulinemia, with eventual recovery of hemoglobin and platelets. The collective experience with this 'metronomic therapy' is summarized in Table 4. Remarkably, the majority of the 21 patients had been previously exposed to – and had become resistant to – standard VTD; 71% had CA. First-cycle responses included CR and near-CR in four of 17 patients (24%), with an additional nine achieving PR status.

### Secondary myelodysplasia[49]

MDS is a well-recognized potential complication of stem-cell-toxic therapy for systemic malignancies, including MM. Among 3077 patients receiving MEL-based transplants between

October 1989 and December 2006, 2814 had cytogenetic studies performed post-transplant, a routine procedure in our program as part of each bone-marrow examination. A total of 110 subjects developed MDS-CA after a median of 32 months post-transplant, reaching 7% at 15 years, although only 21 patients developed clinical MDS and five additional patients developed acute myeloblastic leukemia (AML). According to multivariate analyses, MDS-CA development was associated with older age, longer time intervals from initial MM diagnosis to HDT, and hematopoietic stem-cell compromise, as evidenced by lower CD34 yield.

## Research agenda

The Arkansas experience with transplant-based and new agent therapies in the context of translational research has led to a research agenda for the coming years with emphasis in the following areas:

- further advancement of GEP studies of MM and stroma beyond baseline risk identification, including (a) in-vivo test dosing with key drugs of the MM armamentarium as a prelude for individualized therapy; (b) studies in remission for the development of a reliable minimal residual disease (MRD) assay, assuming that residual MM creates an abnormal bone-marrow microenvironment, towards rationally based consolidation and maintenance therapies; (c) comparisons of MM and stroma cells from random bone marrow and MRI-FL sites toward identification of a crucial (stem-cell-like?) MM compartment accounting for disease relapse; (d) comparisons of relapse and baseline MM-cell signatures toward understanding the genetic mechanisms of progression and resistance
- develop alternative clinical endpoints such as MRI-CR and 2-year CR with the promise of advancing success in high-risk MM
- together with proteomics research, identify more suitable therapeutic targets
- develop a focus on epigenetics toward improving treatment toxicity profiles
- exploit long-term follow-up of large patient population toward the development of individual prognostic index parameters to be shared with the scientific community

## SUMMARY

Through incorporation of new agents with salvage potential in end-stage disease, the TT approach has increased stringently defined CR rates to levels of at least 60% and extended median survival beyond 8 years. GEP analysis identifies 15% of patients with short survival times not exceeding 2–3 years. As this high-risk signature may be a common end-stage in MM evolution, therapeutic progress in this group may benefit all patients. Whereas induction of CR does not assure long-term success of therapy, sustaining CR for at least 2 years emerged as a powerful new survival surrogate. The next 5 years are likely to see additional new agents and more powerful combinations. We envision the possibility of individualizing therapy according to pre-treatment GEP data that may be further enhanced by information procured after drug test doses. When examined in remission, a normalized microenvironment signature may be a valuable early surrogate for prolonged disease control.

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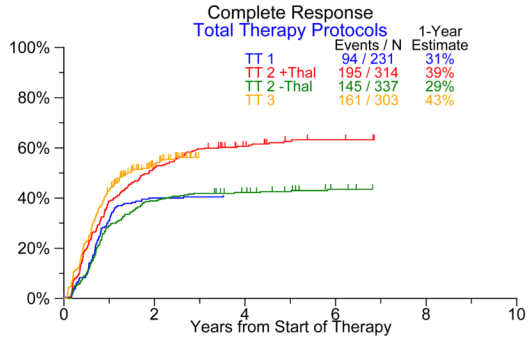
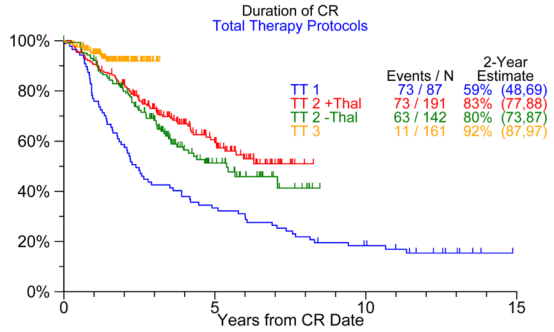
**<Figure 1a>**




**<Figure 1b>**

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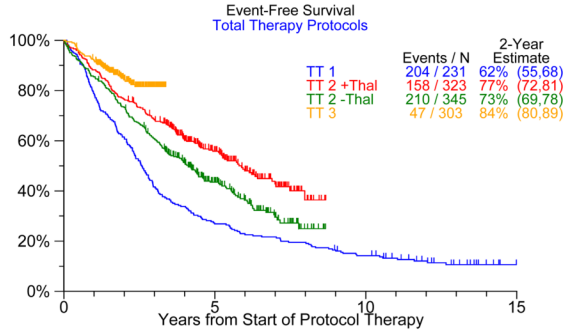

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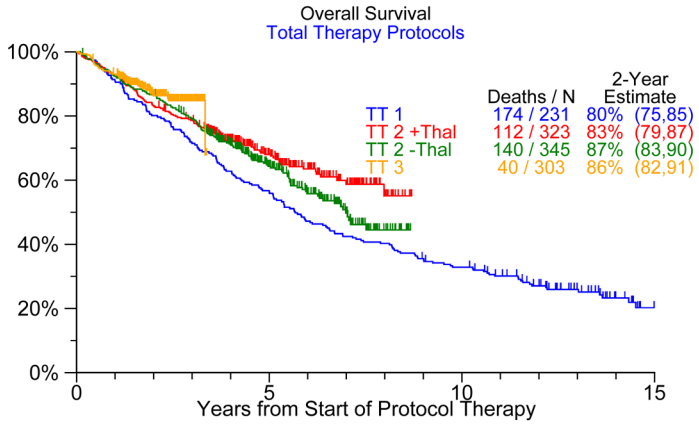


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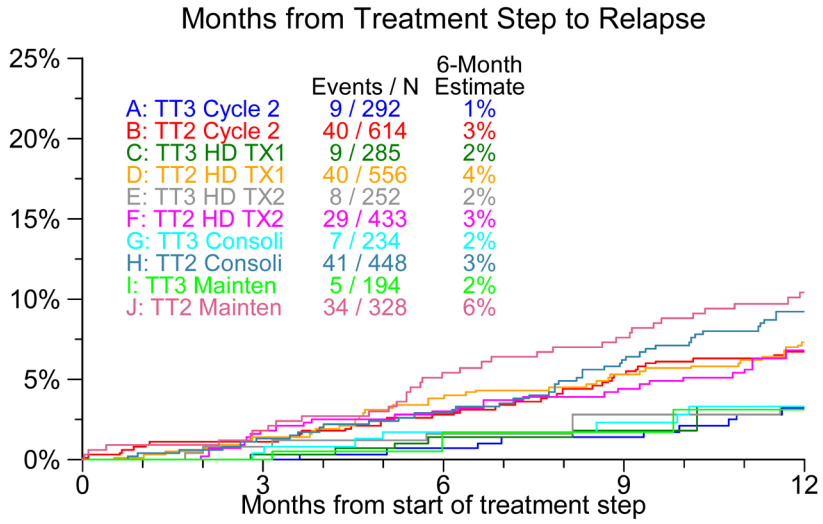



**Figure 1.**

(a) Cumulative proportions of patients achieving immunofixation-negative complete remission (CR) on Total Therapy (TT) regimens. Similar results were observed with TT1 and TT2 without thalidomide (TT2 – Thal) regimens ($P = 0.71$) and also for TT3 and TT2 + Thal regimens ($P = 0.56$); TT2 + Thal was superior to TT2 – Thal ($P = 0.0012$). (b) Comparison of CR durations among TT regimens. Outcomes with TT2 + Thal and TT2 – Thal ($P = 0.74$) were similar; TT2 – Thal was superior to TT1 ($P = 0.01$); TT3 was markedly superior to TT2 + Thal ($P < 0.0001$). (c) Event-free survival (EFS) comparison between TT regimens. TT2 – Thal was superior to TT1 ($P = 0.0002$); TT2 + Thal was superior to TT2 – Thal ($P = 0.002$); TT3 was superior to TT2 + Thal ($P = 0.004$). (d) Overall survival (OS) comparison between TT regimens. TT2 – Thal was borderline superior to TT1 ($P = 0.0626$); TT2 + Thal and TT2 – Thal were equivalent

($P = 0.1687$). Survival on TT3 appears promising versus TT2 + Thal ($P = 0.17$). (e) Higher cumulative relapse rates after each protocol step of TT2 versus TT3.

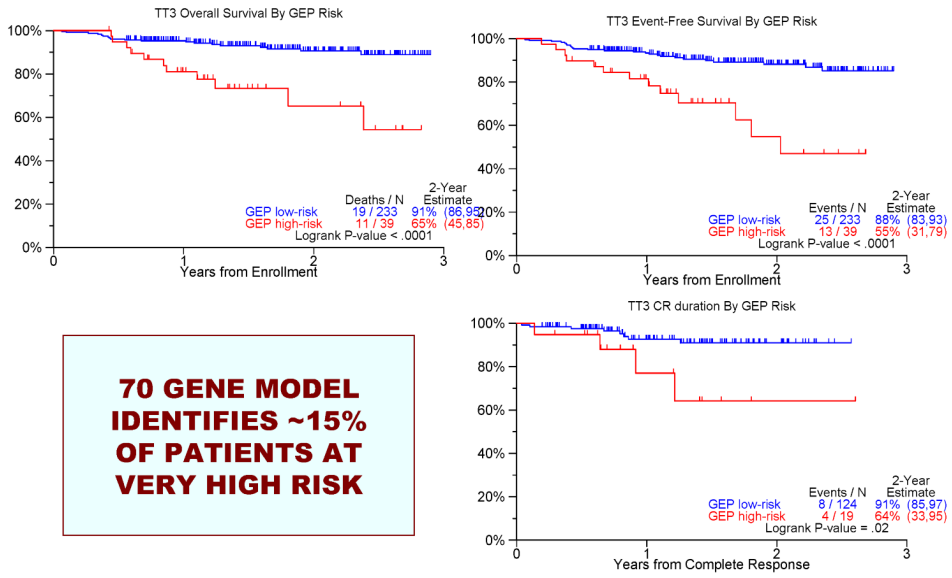
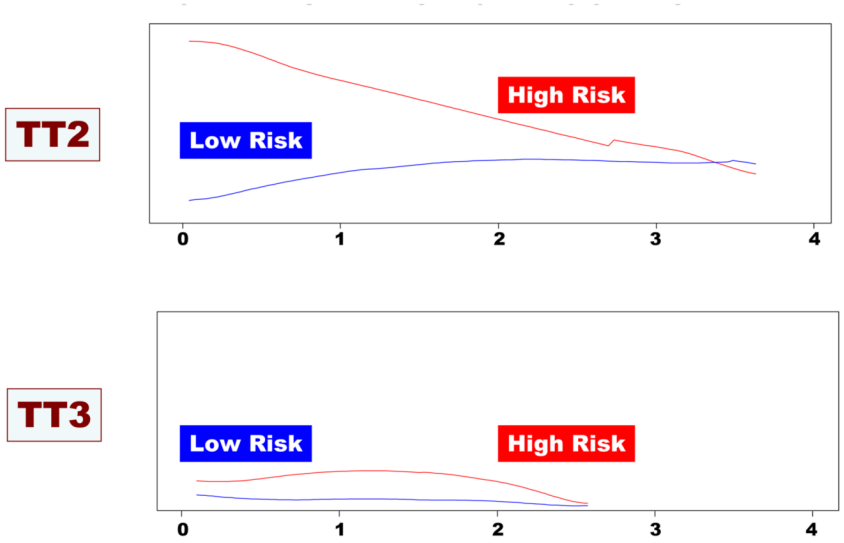



**Figure 2.**

Gene expression profiling (GEP-)defined high-risk group of 15% of patients has markedly inferior clinical outcomes with Total Therapy 3 (TT3).

<Figure 3a>

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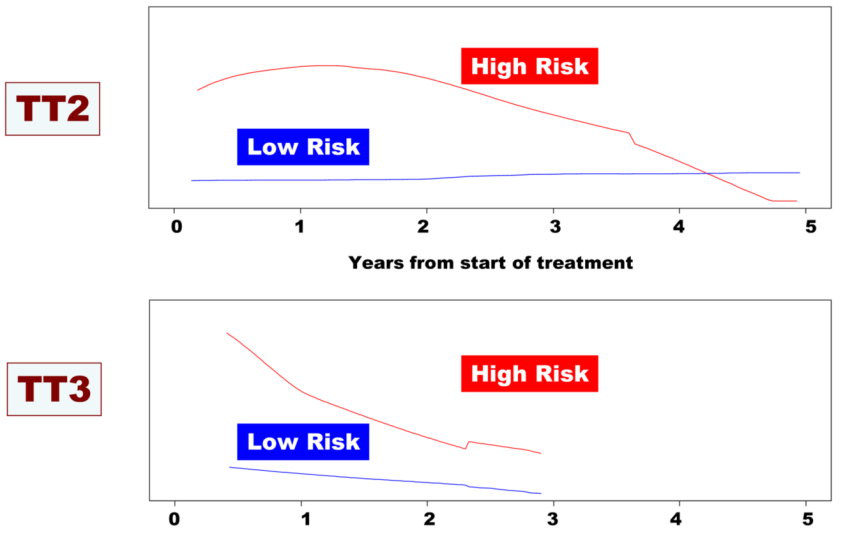



**Figure 3.**

(a) Hazard rates of relapse over time in Total Therapy 2 and 3 (TT2 and TT3) according to gene expression profiling (GEP-)defined risk category. (b) Hazard rates of death over time in TT2 and TT3 according to GEP-defined risk category.

# <Figure 4>

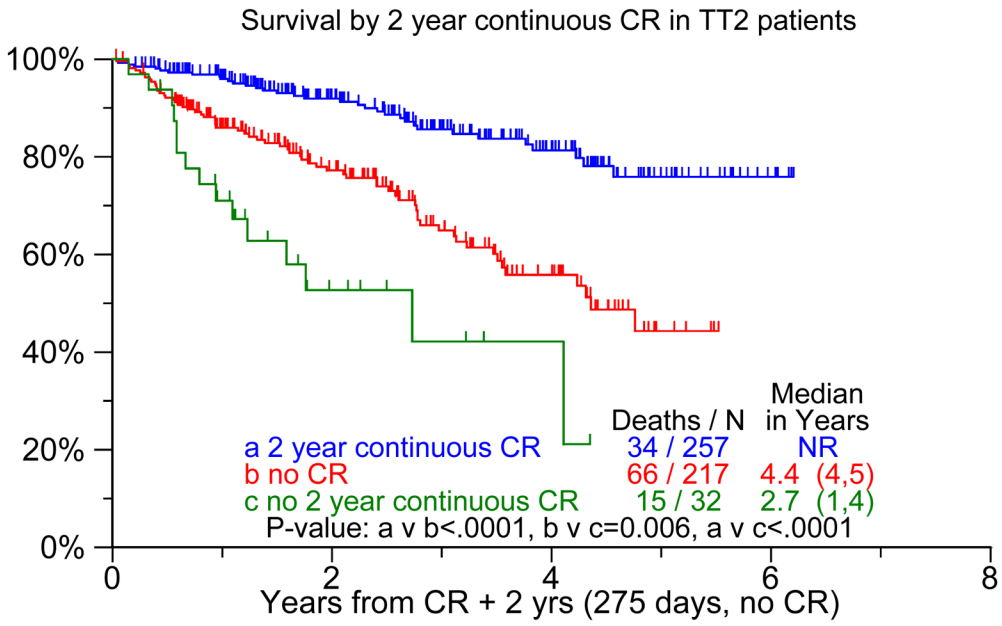
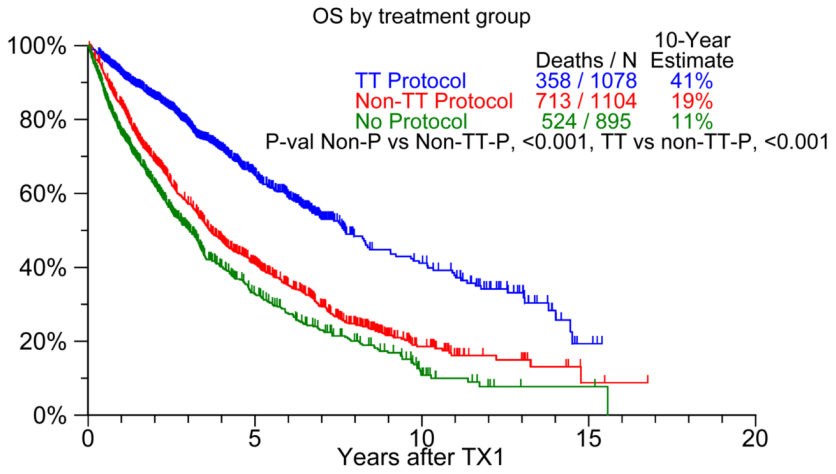



**Figure 4.**
Superior survival in patients on Total Therapy 2 (TT2) with complete response (CR) sustained at 2 years compared to those never achieving CR, and especially patients achieving CR but experiencing a relapse within 2 years. Survival is measured from a 2-year landmark plus 275 days as the median time to CR.

**<Figure 5a>**

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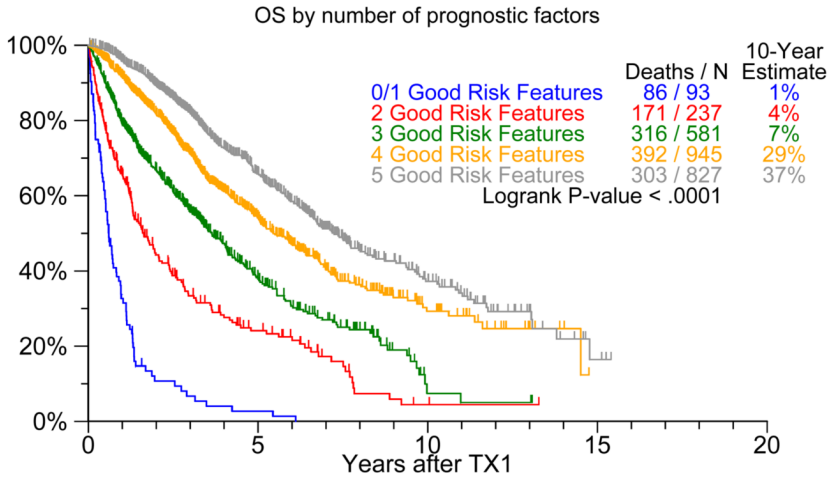

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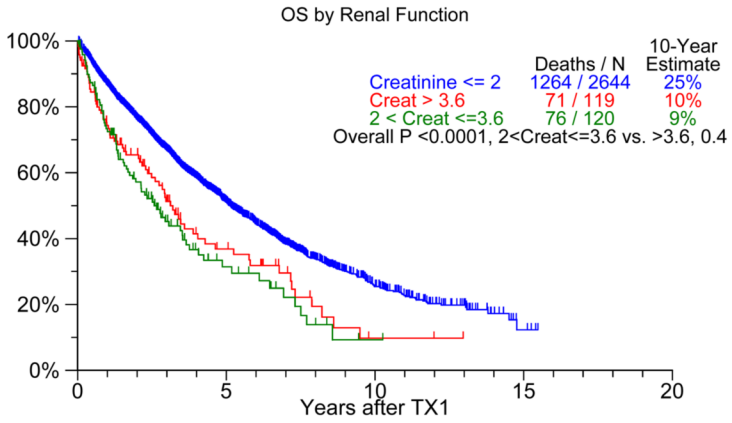


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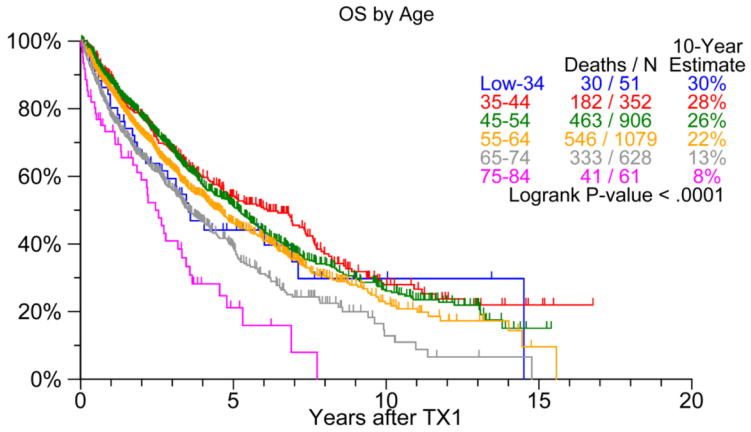


**Figure 5.**

(a) Survival from first transplantation among the 'Arkansas 3000' group of patients according to whether they were treated on Total Therapy (TT) protocols (TT-P), other protocols for previously treated patients (non-TT-P), or off-protocol (non-P). (b) Survival according to the number of favorable parameters present prior to first transplant. Favorable features included absence of cytogenetic abnormalities (CA), low $\beta_2$-microglobulin (B2M), low C-reactive protein (CRP), high albumin, high platelet count. (c) Survival outcomes according to renal function (among the creatinine >2 mg/dL group, median cut-point of 3.6 mg/dL was considered). (d) Survival outcomes by decade of age in ARK3000 (age range 15–85 years).

**Table 1**

Characteristics of patients and response to high-dose dexamethasone (DEX), thalidomide (THAL), cisplatin, doxorubicin, cyclophosphamide, etoposide (HD-DTPACE). See text for details.

| Cycle # | n | %CA | CR | nCR | PR | Stable | NR |
|---|---|---|---|---|---|---|---|
| 1 | 16 | 87 | 5 | 2 | 8 | 1 | 0 |
| 2 | 8 | 87 | 1 | 3 | 3 | 0 | 1 |
| 3 | 3 | 100 | 0 | 0 | 2 | 1 | 0 |
| 4 | 3 | 100 | 0 | 3 | 0 | 0 | 0 |
| Total | 30 | 90 | 6 (20%) | 8 (27%) | 13 (43%) | 2 (7%) | 1 (3%) |

CA, cytogenetic abnormality; CR, complete response; nCR, near-complete response; PR, partial response; NR, no response.

**Table 2**

Melphalan (MEL) plus Velcade (bortezomib), thalidomide, and dexamethasone (M-VTD) regimen and response.

| **MEL dose mg/m$^2$** | **MEL 150** | **MEL 180** | **MEL 210** | **MEL 240** | **MEL 270** | **MEL 300** |
|---|---|---|---|---|---|---|
| n | 12 | 10 | 25 | 25 | 11 | 11 |
| Parameter | All 94 | MEL300 (n = 11) | | | | |
| CA | 69% | 91% | | | | |
| Albumin <3 g/dL | 67% | 50% | | | | |
| Prior transplant | 70% | 82% | | | | |
| ≥PR | 89% | 82% | | | | |
| nCR/CR | 68% | 73% | | | | |
| Alive | 95 | 91% | | | | |

CA, cytogenetic abnormality; PR, partial response; nCR, near-complete response; CR, complete response.

**Table 3**

Multivariate analysis of pre-transplant parameters and treatment regimen associated with overall survival (OS), event-free survival (EFS) and complete response (CR) duration.

| **Multivariate analysis**<br>**Favorable parameter** | **n/N (%)** | **OS**<br>**HR (95% CI)** | ***P*-value** | **EFS**<br>**HR (95% CI)** | ***P*-value** | **Duration of CR**<br>**n/N (%)** | **HR (95% CI)** | ***P*-value** |
|---|---|---|---|---|---|---|---|---|
| No CA13/hypodiploidy | 2023/2683 (75%) | 0.46 (0.41,0.52) | <0.001 | 0.58 (0.52,0.65) | <0.001 | 1061/1398 (76%) | 0.55 (0.47,0.65) | <0.001 |
| Platelets ≥100,000/μL | 2360/2683 (88%) | 0.56 (0.48,0.66) | <0.001 | 0.63 (0.55,0.73) | <0.001 | 1261/1398 (90%) | 0.61 (0.48,0.77) | <0.001 |
| Albumin ≥3.0 g/dL | 2454/2683 (91%) | 0.64 (0.54,0.76) | <0.001 | 0.58 (0.49,0.68) | <0.001 | 1323/1398 (95%) | 0.64 (0.48,0.85) | 0.002 |
| B2M < 3.0 mg/L | 1737/2683 (65%) | 0.63 (0.56,0.71) | <0.001 | 0.69 (0.62,0.76) | <0.001 | 967/1398 (69%) | 0.68 (0.58,0.80) | <0.001 |
| CRP < 6.0 mg/dL | 1635/2683 (61%) | 0.76 (0.68,0.85) | <0.001 | 0.86 (0.78,0.96) | 0.004 | 862/1398 (62%) | 0.80 (0.69,0.94) | 0.006 |
| TT protocol | 1016/2683 (38%) | 0.54 (0.47,0.61) | <0.001 | 0.61 (0.55,0.69) | <0.001 | 623/1398 (45%) | 0.53 (0.45,0.63) | <0.001 |

HR, hazard ratio; 95% CI, 95% confidence interval; P-value from Wald chi-square test in Cox regression; CA, cytogenetic abnormality; B2M, $\beta_2$-microglobulin; CRP, C-reactive protein; TT, Total Therapy.

**Table 4**

Patient characteristics and response data with Velcade (bortezomib), thalidomide, dexamethasone/low-dose doxorubicin (VTD-ADR) regimen.

| Cycle # | n | %CA | CR | nCR | PR | Stable | NR |
|---|---|---|---|---|---|---|---|
| 1 | 17 | 70 | 3 | 1 | 9 | 1 | 3 |
| 2 | 3 | 33 | | 1 | 2 | | |
| 3 | 1 | 100 | | | 1 | | |
| Total | 21 | 71 | 3 (14%) | 2 (10%) | 12(57%) | 1 (5%) | 3 (14%) |

CA, cytogenetic abnormality; CR, complete response; nCR, near-complete response; PR, partial response; NR, no response.