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Safety and efficacy of risk adapted cyclophosphamide, thalidomide and dexamethasone in systemic AL amyloidosis

Short title: CTD CHEMOTHERAPY IN SYSTEMIC AL AMYLOIDOSIS

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High dose melphalan with stem cell transplantation is believed to the most effective treatment for systemic AL amyloidosis, but many patients are ineligible due to the extent of their disease and treatment-related mortality (TRM) remains substantial. We report the use of a risk adapted oral regimen of cyclophosphamide, thalidomide and dexamethasone (CTD) or attenuated CTD (CTDa) in 75 patients with advanced AL amyloidosis including 44 patients with clonal relapse after prior therapy. Fifty-one (68%) patients received CTD and 24 (32%) received CTDa. A hematological response occurred in 48 (74%) of 65 evaluable patients including complete responses in 14 (21%) and partial responses in 34 (53%) cases. Median estimated overall survival (OS) from commencement of treatment was 41 months and from diagnosis median not reached with a median follow up 22 months. Three year estimated OS was 100% and 82% among complete and partial hematological responders respectively. Toxicity necessitating cessation of therapy occurred in 8%, and was ≥ grade 2 in 52% of patients. TRM was 4%. The clonal response rates to CTD reported here are higher than any previously reported non-transplant regimen in AL amyloidosis and risk adaptation allows its use in poorer risk patients. CTD merits prospective randomized study.

Introduction

Monoclonal immunoglobulin light chain amyloidosis (AL) is the commonest systemic type with an age adjusted incidence of 5.1-12.8 per million patient years.[1] Treatment comprises chemotherapy regimens derived from use in myeloma, and the prognosis remains poor for patients with AL amyloidosis whose underlying plasma cell dyscrasia (PCD) cannot be suppressed or relapses after therapy. Outcomes following treatment with conventional alkylator based oral regimens have been poor with less than one in five patients responding to treatment, and the median overall survival being less than 2 years.[2] Although response rates are much greater following high dose melphalan and autologous stem cell transplantation (SCT),[3] such therapy is limited by high treatment-related mortality[4] (TRM), even among the ~ 50% patients who are considered well enough to be eligible for this intensive approach. Furthermore, the role of SCT in relapsed disease is not well studied. Recent data from ours and other centers suggest equivalent outcomes can be obtained with intermediate dose chemotherapy regimens such as vincristine, Adriamycin and dexamethasone (VAD)[5] or intermediate dose infusional melphalan,[6] and modified conventional regimens like oral melphalan and dexamethasone,[7] although there have been very few direct comparisons. There is certainly no consensus at present on the best treatment for patients with AL amyloidosis.

The discovery of thalidomide as an effective agent in myeloma has ushered a new era of combination therapies for PCDs.[8] In amyloidosis, single agent thalidomide in 'standard' higher doses is tolerated poorly with most patients discontinuing treatment within 3 months.[9,10] Lower doses are better tolerated but adequate hematological responses occur rarely when thalidomide is administered alone.[11] Addition of dexamethasone to thalidomide improves the hematological response rate to about 50% but at the cost of increased toxicity affecting some 60% of patients.[12] In myeloma the oral combination of cyclophosphamide, thalidomide and dexamethasone (CTD) has been evaluated in a number of small studies in relapsed and newly diagnosed cases, in which it has

produced hematological response rates of 61-71%, and has been relatively well tolerated.[13,14] Different versions of this regimen have been reported using cyclophosphamide either daily or weekly with similar response rates. Growing optimism has led to CTD being studied as induction therapy for newly diagnosed myeloma in the current UK MRC Myeloma IX trial including a dose attenuated version (CTDa) for elderly or poor risk patients.

We report here the outcome of 75 patients with systemic AL amyloidosis who were treated with CTD. Despite advanced amyloid in many cases, TRM was very low whereas clonal response rates were higher than any non-transplant regimen described to date in this disease.

Materials and methods

Patients, diagnosis and protocol

The analysis was carried out at the National Amyloidosis Centre (NAC), UK. In accordance with UK guidelines on the treatment of AL amyloidosis,[15] the vast majority of patients attending the NAC since 2000 have received intermediate dose non-transplant chemotherapy regimens. Since such time, cyclophosphamide, thalidomide and dexamethasone (CTD) regimen adapted from the current UK MRC Myeloma IX trial has been included among the offered treatment options. The patients reported here comprise of all patients with AL amyloidosis who were treated with CTD or attenuated CTD (CTDa) between 2000 and 2005, and who underwent systematic prospective evaluation at the NAC. CTD was given as first line therapy in 31 patients, and for refractory or relapsed underlying clonal disease in 44 cases. Only patients with predominant and severe autonomic or peripheral neuropathy were not deemed eligible to receive this regimen due to the potential for exacerbation by thalidomide.

The presence of amyloid was confirmed by characteristic birefringence after Congo red staining of a tissue biopsy and/or by a diagnostic SAP scan. AL type amyloidosis was confirmed by

immunohistochemical staining where possible and otherwise by characteristic clinical and scintigraphic appearances, supported by demonstration of a plasma cell dyscrasia and, where necessary, by exclusion of hereditary amyloidosis by demonstration of wild-type sequence for the genes encoding known hereditary amyloidogenic proteins.[16] Patients attended the NAC for their initial diagnostic evaluation and were followed up at 6 monthly intervals for evaluation of clonal disease and organ responses, and for whole body amyloid load by SAP scintigraphy. Blood samples were requested at monthly intervals during CTD treatment and two monthly thereafter for monoclonal immunoglobulin measurements, which included the serum free light chain assay (FLC) (*Freelite™, The Binding Site, Birmingham, UK*). The novel medical care described here was performed with informed consent from each patient in accordance with the Declaration of Helsinki, and the study was performed with institutional review board approval by the Royal Free Hospital ethics committee.

Treatment

The CTD regimen was adapted from the current UK MRC Myeloma IX trial and consisted of a 21-day cycle oral cyclophosphamide 500 mg once weekly, thalidomide 200 mg/day (starting dose 100 mg/day, increased after 4 weeks if tolerated) continuously and dexamethasone 40 mg days 1-4 and 9-12. This was risk attenuated in elderly patients (> 70 yrs), in those with heart failure exceeding New York Heart Association (NYHA) Class II, and those with significant fluid overload. The attenuated regimen (CTDa) consisted of a 28-day cycle of cyclophosphamide 500 mg days 1, 8 and 15, thalidomide 200 mg/day (starting dose 50 mg/day, 4-weekly 50 mg increments as tolerated), and dexamethasone 20 mg day 1-4 and 15-18. Treatment was given at the referring hospital and antimicrobial and thromboprophylaxis were given according to local protocol. Patients did not receive routine thromboprophylaxis. Treatment was given until a stable clonal response (as defined

below) was achieved on consecutive samples at least four weeks apart or the patient was confirmed as unresponsive to treatment. Thalidomide maintenance therapy was only considered for responders and was decided upon by a combination of patient preference and tolerance to treatment.

Outcome measures

Primary outcome measures were hematological responses and toxicity. Additional outcome variables were overall survival (OS), event free survival (EFS), organ response rates and the course of whole body amyloid burden by serial SAP scintigraphy. Hematological response was assessed by serum and urine electrophoresis and immunofixation and also by FLC. FLC response is a strong predictor of survival in AL amyloidosis[17] and a reliable early marker in myeloma which correlates with clonal plasma cell burden.[18] Conventional response and relapse was defined according to the Bladè criteria.[19] FLC's were considered interpretable for assessing response if the pre-treatment FLC κ/λ ratio was outside the 95% reference range (0.3-1.2)[20] and the concentration of the light chain class (i.e. κ or λ) containing the monoclonal component (also called monoclonal class) was ≥ twice the upper limit of 95% reference range for that class (except in renal failure where only the κ/λ ratio was used). A FLC partial response (PR) was defined as a ≥ 50% fall in the monoclonal class; a FLC complete response (CR) was defined as normalization of the FLC ratio and both light chain classes, unless there was renal failure causing polyclonal retention of FLC, in which case the ratio alone was used. Minor response has not been defined for FLC; hence any change which could not be classed as FLC-PR or FLC-CR and patients with clonal disease progression (as defined below) were together labeled as non-responders. Clonal relapse or progression was classified as an event (EFS) and defined as per the Bladè criteria for conventional serum and urine monoclonal protein measurements and for FLC as follows: from FLC-CR as a newly abnormal ratio with a doubling of the monoclonal class, and from FLC-PR as a ≥ 50% rise in the monoclonal class. The

response was assessed as the best achieved response at least 3 months after completion of CTD therapy. Among patients who subsequently received maintenance therapy with single agent thalidomide, clonal response was assessed after the last cycle of CTD. Progression free survival was defined as the time to clonal relapse or death due to progressive amyloidosis.

Toxicity was recorded according to the National Cancer Institute Cancer Therapy Evaluation Program, Common Terminology Criteria for Adverse Events (Version 3.0)[21].

Amyloidotic organ involvement and responses were defined according to the international consensus criteria.[22] Responses were assessed 6 months following CTD therapy, and before further treatment was delivered. Performance status was assessed as described by the Eastern Cooperative Oncology Group (ECOG) criteria.[23]

All patients underwent ^{123}I-labeled serum amyloid P component (SAP) scintigraphy, and serial studies were used to quantitatively measure the whole body amyloid load, as previously described.[24] Prior to commencement of CTD chemotherapy additional organ involvement that is not encompassed by the amyloidosis consensus criteria was identified by SAP scintigraphy in 22 (29%) cases. Labeled SAP studies were interpreted by a single physician (PNH) with experience of over 4000 SAP scans.

Statistics

Statistical analysis was undertaken using the SPSS 14 software package (SPSS, Chicago, USA). Survival was assessed by the method of Kaplan and Meier and compared by log rank test. Categorical variables were compared with Chi squared or Fishers tests as appropriate. All p values were 2-sided with a significance level of 0.05. Multivariate analysis was by Cox or binary logistic regression as appropriate.

Results

Patients

Seventy five patients received CTD or CTDa between January 2000 and August 2005, among a total of 1577 patients with confirmed AL amyloidosis evaluated at the NAC during this period. Features at the time of commencement of CTD or CTDa therapy are summarized in Table 1. Median age was 60 yrs (range 30-81). Twelve patients (16%) were dialysis dependent. Twenty-five (33%) patients had interventricular septal thickness of ≥ 15 mm and typical amyloid echocardiograms, among whom 8 (10%) had heart failure ≥ NYHA Class III. More than two organs were involved in 25 (33%) patients. Forty-six (61%) patients had ECOG performance status of ≥ 2. Among those receiving CTD as first line therapy, the median (range) performance score was 2 (1-3) and the median (range) number of organs involved by amyloid at commencement of therapy was 2 (1-3). Thirty-nine (52%) of the 75 patients would not have been eligible for SCT according to the criteria published by the Mayo Group.[25]

Treatment and response

Fifty-one (68%) patients received CTD and 24 (32%) received CTDa. The median time from diagnosis to treatment with full-dose or attenuated CTD for all patients was 4.3 months (1.7-104); it was 1.6 months in newly diagnosed patients, and 13.6 months in relapsed patients. The number of prior regimens was: none in 31 (41%) patients, one in 31 (41%) cases, two in 10 (13 %) cases, three in 3 (4%) cases. Ten (13%) patients had undergone a prior autologous stem cell transplant. The median dose of thalidomide was 100 mg (50-200 mg), dexamethasone was 20 mg (2-40 mg) and cyclophosphamide was 500 mg (300-500 mg). Median follow-up from the start of treatment was 18 months (1.4-48) and from diagnosis was 22 months (4-112). Patients received a median of 4 cycles (1-12 cycles) of CTD or CTDa. Nineteen (27%) patients continued with thalidomide maintenance.

The underlying PCD was evaluable for response in 65 (87%) patients. Sixty (80%) were evaluable by FLC measurements, and 30 by conventional monoclonal protein measurements. In 10 cases the pre-treatment FLC concentration, though abnormal, was less than twice the upper limit of normal, although 5 of these patients were evaluable by conventional criteria. Five further patients were not evaluable either because data were incomplete or because insufficient time had elapsed after completing CTD therapy. Responses are shown in Table 2. Forty-eight (74%) evaluable patients responded, with CR in 14 (21%) and PR in 34 (53%) cases. An FLC response was seen in 43/60 (72%) patients, with a CR by FLC criteria in 19 (32%) and PR in 24 (40%). A conventional paraprotein response was seen in 22 of 30 (73%) evaluable patients, with a CR in 1 (3%) and a PR in 21 (70%) cases. Among 5 patients with CR by FLC assessment who had a response assessable by conventional criteria, 4 had a PR by conventional criteria. There was no discrepancy between non-responders by either criteria. Of the patients given CTD, 76% responded as compared to 61% of those given CTDa ($p = 0.331$). Among the 34 responders in whom samples were provided for monthly FLC assays, a response (at least PR) occurred by the end of the first, second and third months of treatment in 50%, 76% and 100% of cases respectively. On univariate analysis, receiving treatment for more than 2 months was a significant positive factor for hematological response ($p = 0.022$; OR 2.6). On multivariate analysis, the only independent factor (negatively) affecting hematological response was performance status ($p = 0.02$; odds ratio (OR) 0.005) whilst other factors including organ involvement, light chain isotype, CTD dose attenuation, or development of toxicity were not significant. There was no significant difference in hematological response rate between newly diagnosed and relapsed patients.

Sixty patients were evaluable for organ responses. Organ responses occurred in 15 of 48 (31%) hematological responders compared to 1 (5%) hematological non-responder ($p < 0.0001$), a patient who had improvement of serum FLC but < 50%. Among 11 cases in whom renal function improved, the median decrease in proteinuria from the onset of treatment was 76% (range 50-95%).

Hepatic function improved in 5 cases, including complete normalization of the liver function tests including alkaline phosphatase in 2 patients and the function of other organ systems improved in 6 cases, with some patients showing improvement in more than 1 organ. No patient with end-stage renal failure became dialysis independent following CTD chemotherapy and no objective cardiac responses were observed. Regression of amyloid deposits by serial SAP scintigraphy was recorded in 16% (7 of 43) of hematological responders but was not in any non-responder. Regression of amyloid from the liver (Figure 1) was observed in every patient in whom there was improvement in liver function, whereas regression from the kidneys was only observed in 2 of 11 patients with improvement in renal function, the remainder showing stable deposits.

Survival

At censor, the Kaplan-Meier estimated median OS for the whole cohort from diagnosis was not reached (Figure 2A). The median OS from commencement of CTD chemotherapy was 41 months (Figure 2B). Patients who had a FLC response had markedly better OS than patients who had < 50% FLC response (median not reached vs. 17 months; $p < 0.0001$). The estimated 3-year survival (Figure 2C) was 100% for patients achieving a CR (by both conventional and FLC criteria), 82% for those achieving a PR and nil (median 17 months) for hematological non-responders (log rank $p < 0.0001$). The median OS in patients not eligible for SCT (Figure 2D) by the Mayo Group criteria[25] was 17 months for non-responders and not reached among responders ($p < 0.0001$). Factors affecting OS from the end of treatment are shown in Table 3. The only independent factors affecting OS from the end of treatment were ECOG performance status and hematological response to treatment. Previous treatment(s) was not a significant factor affecting OS. Median EFS from the end of CTD therapy was 21 months. There was no significant difference in EFS between patients with a hematological CR or PR. The only significant factors affecting EFS on multivariate analysis

was receiving thalidomide maintenance ($p = 0.032$; OR 3.6) (Table 3). There was no significant impact of thalidomide maintenance on OS.

Toxicity

Toxicities of ≥ grade 2 were observed in 39 (52%) patients with all patients reporting grade 1 toxicities. Toxicities are summarized in Table 4. Fatigue and constipation (grade 1) were reported by all patients. Most patients needed an increase in the diuretic dosage during treatment but this was most often needed during the dexamethasone component of the regime. Symptomatic bradycardia or other treatment related cardiac arrhythmias were not seen in this cohort. Toxicity occurred more frequently in patients receiving CTD (60%) than CTDa (50%) ($p = 0.02$). On multivariate analysis, receiving full dose CTD was the only significant independent factor in development of ≥ grade 2 toxicity ($p = 0.029$). Interestingly, there was no correlation between involvement of a particular organ by amyloid and associated toxicity. Toxicity of ≥ grade 3, needing dose reduction or regime discontinuation, was seen in 24 (32%) patients and led to omission of dexamethasone in 11 (15%), cyclophosphamide in 4 (6%) and thalidomide in 3 (4%) cases. The chemotherapy regimen was discontinued in 6 (8%) patients within 8 weeks. Two (3%) patients had non-fatal but serious thrombotic complications during treatment (both pulmonary emboli). One such patient discontinued thalidomide while the other continued treatment with adequate anticoagulation and remains on thalidomide maintenance. There were three (4%) possible treatment-related deaths – one with multi-organ failure after an infection and two with massive gastrointestinal bleeding (one patient with proven and another with unproven but almost certain gastro-intestinal amyloid).

Discussion

This is the first report of the safety and efficacy of CTD or CTDa combination chemotherapy in a series of patients with systemic AL amyloidosis, and the resulting hematological response rates of 74% are higher than for any previously reported non-stem cell transplant regimen in this disease. It is also noteworthy that hematological responses were rapid, with all responses having occurred within three months of commencing chemotherapy. Table 5 summarizes the previously reported clonal response rates and toxicity with standard chemotherapy regimens and following stem cell transplantation in AL amyloidosis.

Hematological response to treatment is a strong predictor of survival in AL amyloidosis. Early series demonstrated prolonged OS among patients with AL amyloidosis who achieved hematological responses by immunofixation electrophoresis (IFE) and bone marrow biopsy.[2,26] Improved hematological response rates and OS compared to historical controls were demonstrated among patients receiving high dose melphalan and stem cell rescue (SCT).[27] A > 50% FLC response after chemotherapy was shown by our group to be associated with significantly prolonged OS in patients with AL amyloidosis, irrespective of the treatment regimen used.[17] More recently, studies have suggested that deeper clonal responses are associated with improved OS.[28,29] The Boston investigators reported that decreases in FLC are more readily detected early after chemotherapy in patients with AL amyloidosis than changes in IFE and that a > 90% reduction in FLC concentration predicts favorable long term outcome after SCT regardless of PR or CR by IFE.[28] More recently, the Mayo Clinic investigators reported that achieving low absolute FLC concentrations after SCT predicts improved OS among patients with AL amyloidosis.[29]

Despite the high clonal response rates with SCT in AL amyloidosis, TRM has consistently remained around 13% in all reported US studies and has been considerably higher in all European series.[30,31] The increased risks of SCT in AL amyloidosis compared to multiple myeloma are

underpinned by the organ dysfunction and reduced functional reserve induced by the presence of amyloid deposits. In the large Boston series[32] (in which the TRM with SCT was 13%) 394 (56%) of 701 consecutive patients with AL amyloidosis were considered eligible for SCT but due to disease progression or patient choice only 312 (45%) initiated treatment. Despite an impressive median OS of 4.6 years among the 45% of patients who initiated treatment, the median OS was only 4 months among the 44% ineligible patients. The median OS of all 701 patients was not specified in this study. It is likely that careful patient selection could reduce TRM[4] but this would also limit SCT to a small proportion of AL amyloidosis patients. The challenge in AL amyloidosis is therefore to achieve, rapidly after commencement of chemotherapy, a high rate and depth of hematological response whilst minimizing TRM.

Conventional chemotherapy regimens like oral melphalan and prednisolone have poor response rates, take a long time to achieve a response and are associated with a poor long term outcome in the majority of treated patients with AL amyloidosis.[2,26] Adding more alkylating agent does not improve survival or responses.[33] A SWOG study of high dose single agent dexamethasone had better responses than the earlier regimens and appeared to show an improvement in outcome.[34] We recently reported in abstract form good responses to VAD[35] and intermediate dose intravenous melphalan,[6] and similar response rates were reported with oral melphalan and dexamethasone.[7] Thalidomide is an oral agent with a mechanism of action that differs from standard chemotherapy agents.[36] Hematological responses occur in 30-50% patients with relapsed multiple myeloma using single agent thalidomide,[37] and are increased by addition of dexamethasone[38] or when used in combination with a variety of other agents.[13,14,39] In AL amyloidosis, single agent thalidomide is not very effective in low doses[11] and very poorly tolerated in higher doses[9] with 50% experiencing grade 3/4 toxicities. Palladini *et al* reported improved rates of hematological response (48%) with combination thalidomide and dexamethasone (thal-dex) but ≥ grade 3 toxicity in 64% patients.[12] A risk adapted strategy has been successful in reducing TRM with SCT in AL amyloidosis and

allowing delivery of high dose treatment to patients who would not otherwise be eligible,[4] and the risk adapted CTD regimen reported here permitted treatment of patients who would not have tolerated full dose thalidomide and/or dexamethasone.

The overall hematological response rate of 74% seen in the current study with CTD or CTDa chemotherapy is higher than any previously reported in AL amyloidosis in a non-transplant setting. A relatively low median dose of thalidomide (100 mg), ineffective as monotherapy,[11] was used in the current study. Importantly, the hematological responses to CTD in this series were rapid with 50% of responses occurring within one month, 76% by two months and the remainder by 3 months from commencement of CTD chemotherapy. Although continuing treatment beyond three months in patients not achieving a response is probably futile, the merit of continuing treatment for longer in responders is not known and needs to be studied since there is some evidence that total thalidomide dose has an impact on survival in myeloma[37] and also possibly in amyloidosis.[12] Performance status was the only independent factor (negatively) affecting the response rate, probably accounted for by the fact that there was a positive correlation between better performance status and continuing treatment for more than 2 months. It is noteworthy that there was no significant difference in response rates, EFS or OS from the end of treatment between newly treated and relapsed patients. The reasons for this remain unknown but are likely to reflect the biologic characteristics of the underlying plasma cell clone and the unique mechanism of actions of thalidomide.

The median OS of the current cohort from diagnosis was not reached and was estimated at 41 months from commencement of CTD chemotherapy; median follow-up was 22 months from diagnosis and 18 months from start of chemotherapy. The specific impact of CTD on survival is difficult to assess and needs to be interpreted with caution due to the possible contribution of prior/subsequent treatments. The only independent factors impacting on OS were performance status and hematological response to treatment. The median OS was significantly better in patients achieving a > 50% reduction in FLC ($p < 0.0001$) and better still for patients in CR with a 100%

estimated 3 year survival. This provides further support to our previously published observations that a > 50% FLC response remains a strong predictor of improvement in survival.[17] Although, there were too few patients in the current series to make a meaningful comparison between patients having 50-90% FLC response versus > 90% FLC response but the excellent survival of patients achieving CR appears to lend support to the observation from SCT data of better outcomes in patients with a > 90% FLC response.[28,29] In contrast with myeloma where patients who do not attain a CR after a first autologous stem cell transplant appear to benefit from a second stem cell transplant and improvement of response,[40] there is no data to answer the question of merit in continuing with therapy until attainment of a CR in patients with amyloidosis, an area that needs addressing as part of a prospective randomized trial.

The estimated median event free survival in this series was 21 months and, unlike OS, was significantly prolonged by thalidomide maintenance, an observation which needs confirmation in a larger series. The durability of hematological responses in AL amyloidosis with intermediate dose chemotherapy regimens such as VAD and CTD may well be reduced when compared to (higher dose) melphalan-based chemotherapy regimens, another area for investigation by a prospective randomized study. Organ responses were observed in 27% evaluable patients in this series with renal improvement being most frequent. Objective cardiac improvement was not seen in this study. The relatively poor rate of organ responses is likely to reflect, in part, the short duration of follow up, since improvements are often substantially delayed after chemotherapy in patients with AL amyloidosis.

Despite the poor risk cohort, it was encouraging that 68% patients were able to continue with the regimen without dose modification. All patients reported grade 1 side effects. Side effects (≥ grade 2 toxicity) were seen in 52% patients with 32% developing ≥ grade 3 toxicity despite the risk adapted strategy. This is favorable compared to the reported toxicity profile of single agent dexamethasone (52% ≥ grade 3 toxicity)[34] or thal-dex (64% ≥ grade 3 toxicity),[12] and is probably

explained by the use of higher doses of dexamethasone (median 40 mg) and thalidomide (median 300 mg) in these respective studies compared to the current study (20 mg and 100 mg respectively). Fatigue and lethargy were most commonly reported with fluid retention or worsening of congestive cardiac failure being the next most common problems. Most patients needed an increase in diuretic dosage during treatment. Dexamethasone was discontinued more frequently than thalidomide. Peripheral neuropathy was not a major problem in this cohort in keeping with other thalidomide series, although no patients with a pre-existing amyloid peripheral neuropathy were included. Routine thromboprophylaxis was not administered since amyloid patients are predisposed to bleeding. Four percent of patients in the current study had a serious thrombotic event whilst on chemotherapy treatment, none of whom had received thromboprophylaxis. This number is less than comparative data for thalidomide when used as part of combination chemotherapy in myeloma,[37] including CTD.[41] Due to the serious nature of the adverse events, prophylaxis with either aspirin, low molecular heparin or warfarin is now routinely recommended for high risk patients receiving thalidomide, such as those with nephrotic syndrome. However, thromboprophylaxis has to be individually tailored according to bleeding risk, taking into consideration presence of gastro-intestinal or liver amyloid and/or clotting factor deficiencies. The TRM of 4% with CTD was comparable to that with VAD,[5] and significantly lower than that with intermediate dose melphalan (12%).[6] Two patients, neither of whom were taking anti-coagulants or had an overt bleeding diathesis, died due to massive gastrointestinal bleeding. Amyloidosis patients have previously been reported to have a high risk of gastrointestinal toxicity (9% patients $\geq$ grade 3 toxicity) with single agent dexamethasone.[34] Dexamethasone is a critical component of the CTD regimen but neither the minimum effective dose nor the role of early dose reduction have been studied. Use of stool occult blood screening prior to each chemotherapy cycle may be useful and needs further study in this context. Routine prophylaxis with a proton pump inhibitor is recommended for all amyloid patients

receiving high dose dexamethasone. A single patient developed multi-organ failure and died following an infection in the absence of significant cytopenia.

In conclusion, CTD appears to be a highly effective initial chemotherapy regimen for the treatment of systemic AL amyloidosis. A risk adapted strategy permitted the use of this regimen in patients with advanced disease, where few studies have shown benefit, and although toxicity was common, thalidomide-related side effects were less apparent than in single agent studies. More stringent use of risk adaptation may improve the tolerability of the regimen. At present, the long term durability of the responses is unknown and remains to be determined. Only SCT achieves higher FLC response rates of 83-88% in patients with amyloidosis but whether this advantage is counterbalanced by the requirement for careful patient selection and the increased risk of TRM with SCT compared to risk adapted CTD merits further prospective randomized study.

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Table 1. Patient characteristics at commencement of CTD chemotherapy

	Median (range)	Number of patients (%)
Age in yrs (Median)	60 (29 - 81)	
Gender (Male:Female ratio)	0.9:1	
Monoclonal light chain:		
κ		23 (31%)
λ		52 (69%)
Hemoglobin (g/L)	12.0 (8.2 - 18.9)	
Platelets (x 10^9/L)	248 (89 – 875)	
Creatinine (µmol/L)	96 (56 – ESRD)	
Albumin (g/L)	38 (14 – 55)	
Bilirubin (mMol/L)	8 (4 – 64)	
Alkaline phosphatase (IU/L)	110 (43 – 1357)	
24 hour proteinuria (g/24 hrs)	1.8 (<0.1 – 11)	
Creatinine clearance (ml/min)	58 (ESRD – 140)	
Organ Involvement		
Liver (Consensus criteria)		18 (24%)
Liver (SAP scintigraphy)		32 (43%)
Renal (Consensus Criteria)		48 (64%)
Renal (SAP scintigraphy)		56 (75%)
Cardiac (Any involvement)		44 (59%)
Cardiac (IVS ≥15mm)		25 (33%)
Peripheral neuropathy		0
Total number of organs (international consensus criteria)		
1 organ		16 (22%)
2 organs		34 (45%)
3 or more organs		25 (33%)
ECOG performance status		
≤1		29 (39%)
2		36 (48%)
≥3		10 (13%)

Table 2. Clonal response to chemotherapy by serum free light chain (FLC) assay, by conventional immunofixation electrophoresis (IFE) and combined FLC plus IFE

Response	FLC response (n = 60)	Conventional response (n = 30)	Combined Response (n = 65)
CR	19	1	14 (21%)
PR	24	21	34 (53%)
Total response	43 (72%)	22 (73%)	48 (74%)

Numbers in rows do not add up as patients are overlapping or in different response categories for FLC and conventional responses.

Table 3. Factors affecting overall survival from the end of CTD chemotherapy

Factor	Univariate Significance(Odds ratio; 95% CI)	Multivariate significance (Odds ratio; 95% CI)
Overall Survival (from the end of treatment)		
Number of organs	0.69 (1.1; 0.5-2.1)	
Performance Status	**<0.0001**(5.2; 2.2-12.4)	**0.01**(9.6; 1.9-16.2)
Cardiac involvement	0.30 (0.5; 0.1-1.8)	
Liver involvement	**0.025** (0.2; 0.083-0.89)	ns*
Renal involvement	0.42 (1.5; 0.5 -4.5)	
Amyloid load on SAP scan	0.082(0.79; 0.61-1)	
Any previous treatment	0.30 (1.7; 0.58-5.4)	
≥ 2 months on treatment	**0.002**(6.22; 1.9 -19.4)	ns*
CTD or CTDa	0.071 (1.4; 0.97-2.1)	
Organ response	0.14 (1.8; 0.81-4.22)	
Any toxicity	0.33 (1.7; 0.5-5.1)	
Hematologic Response	**0.002**(12.6; 2.6-60.3)	**0.048**(6.3; 1.1-39.2)
Event Free Survival		
Any previous treatment	0.99 (0.99; 0.42-2.3)	
No. of prior treatments	0.87 (0.95; 0.56-1.5)	
CTD or CTDa	0.15 (1.2; 0.92-1.6)	
Thalidomide maintenance	**0.033**(3.2; 1–9.7)	**0.032**(3.6; 1.1-11.5)
Toxicity	0.98 (1; 0.44-2.2)	

* ns - not significant

Table 4. Toxicity of CTD chemotherapy

Side Effect (≥ grade 2)	Number of patients (%)
Fluid retention or worsening congestive heart failure	16 (21%)
Tiredness or sleepiness	30 (40%)
Peripheral neuropathy	4 (5%)
Tremor	2 (3%)
Cytopenia	4 (5%)
Infections	5 (7%)
Constipation	6 (8%)
Dizziness	2 (3%)
Thrombosis	2 (3%)
Gastrointestinal bleeding	3 (4%)

Table 5. Hematological response rates and toxicity of CTD, thalidomide and intermediate dose chemotherapy regimens in AL amyloidosis

Study	Regimen	Number of patients	Hematological summated partial and complete response rates	Grade 3 or greater toxicity	Treatment-related mortality
Dispenzieri et al* (2004)[11]	Low dose thalidomide	18	Nil	17%	NR
Dispenzieri et al* (2003)[9]	Thalidomide (full dose)	12	Nil	50%	NR
Seldin et al* (2003)[10]	Thalidomide (full dose)	16	25%	25%	NR
Palladini et al (2004)[12]	Thalidomide / dexamethasone	31	48%	65%	NR
Goodman et al (2005)[5]	VAD[+]	229	61%	-	5%
Goodman et al (2004)[6]	IDM[**]	144	54%	-	12%
Sanchorwala et al (2005)[28]	SCT[***]	66	88%	-	14%
Present study	**CTD or CTDa**	**75**	**74%**	**32%**	**4%**

NR – not reported
* FLC responses not assessed
[+] Vincristine, Adriamycin, Dexamethasone
** IV intermediate dose melphalan (25mg/m^2)
*** Autologous stem cell transplantation

Figure legends

Figure 1. Serial ^{123}I-labeled anterior whole body SAP scintigraphy showing visceral amyloid deposits in the spleen and liver pre-treatment (left). Six months post-CTD treatment (right), which resulted in a complete clonal response, marked regression of amyloid from the liver was evident.

Figure 2. Overall Survival and impact of pre-treatment status and hematological response on survival. A) Overall survival for all patients from diagnosis of amyloidosis. B) Overall survival from beginning of CTD chemotherapy stratified by newly diagnosed and relapsed/refractory patients. C) Overall survival from end of CTD chemotherapy according to degree of hematological response. D) Impact of pre-treatment eligibility for stem cell transplantation (by Mayo Group criteria) on overall survival from the end of CTD chemotherapy, stratified by hematological response.

Figure 1.

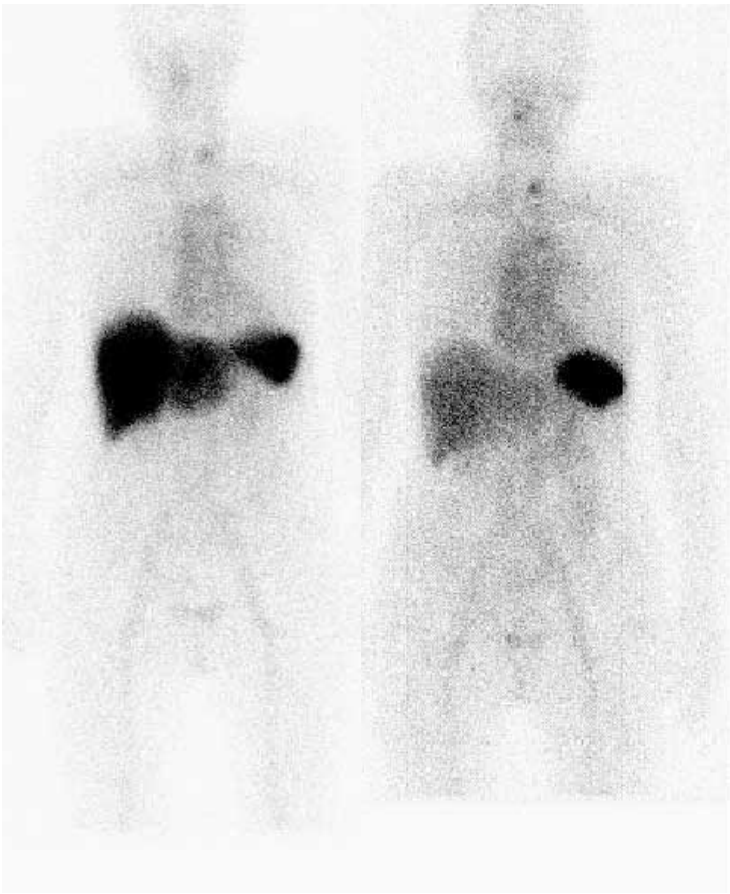


Figure 2A.

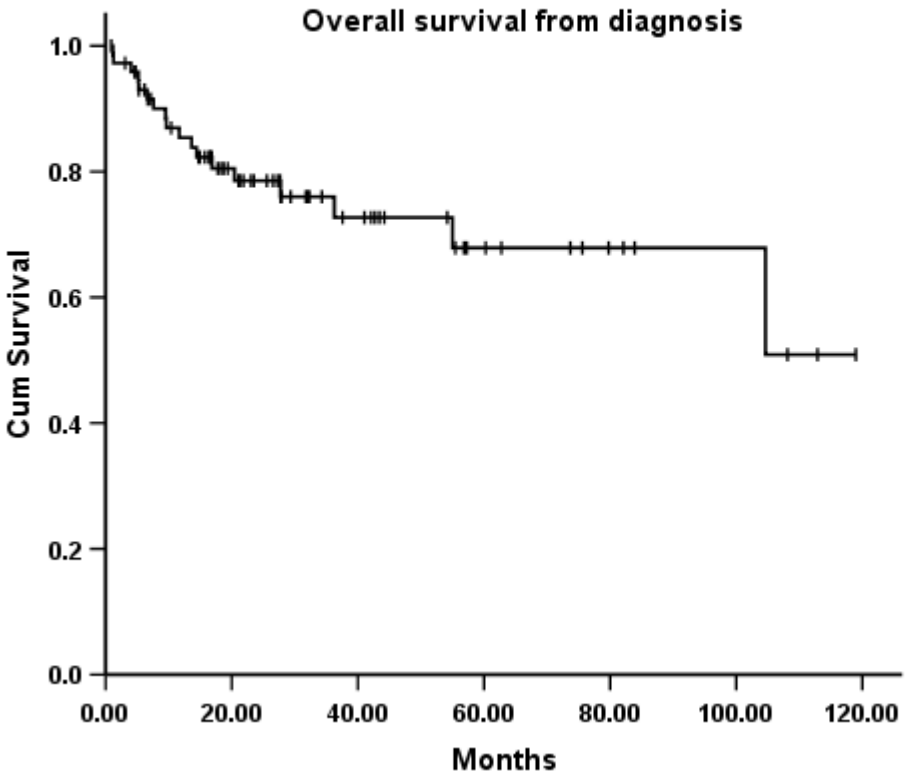
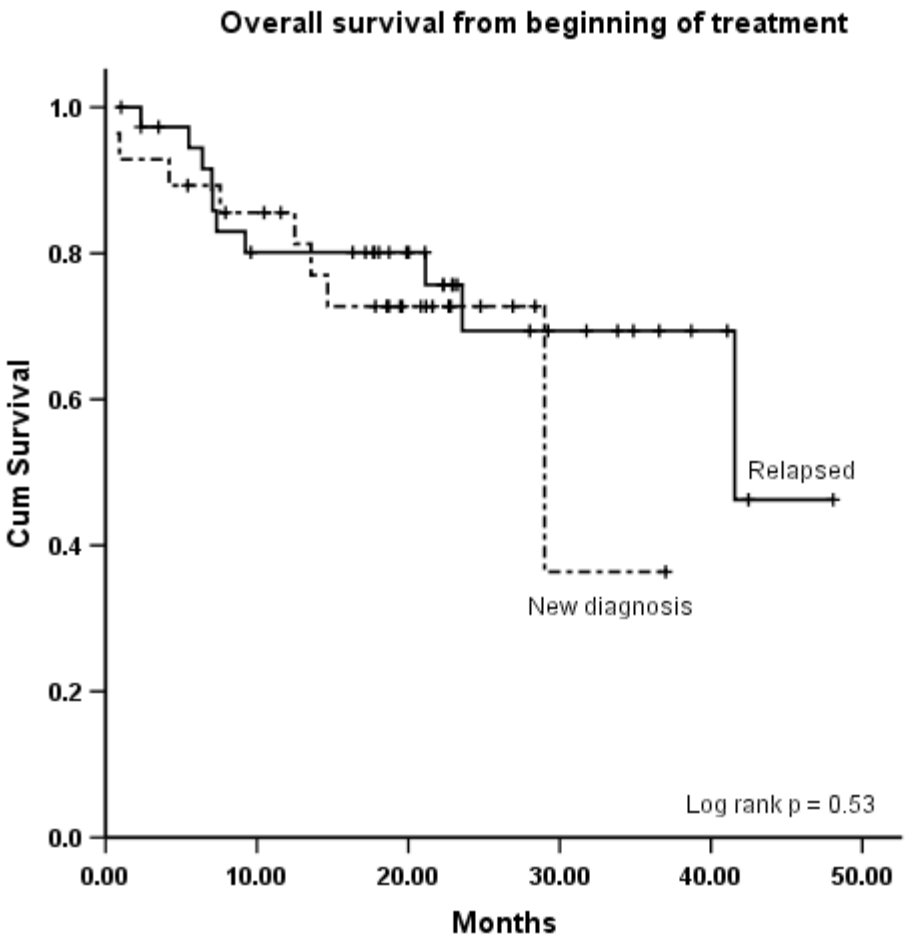

Overall survival from diagnosis
Cum Survival
1.0
0.8
0.6
0.4
0.2
0.0
0.00
20.00
40.00
60.00
80.00
100.00
120.00
Months

Figure 2B.


Overall survival from beginning of treatment
Cum Survival
1.0
0.8
0.6
0.4
0.2
0.0
Relapsed
New diagnosis
Log rank p = 0.53
0.00
10.00
20.00
30.00
40.00
50.00
Months

—

Figure 2C.

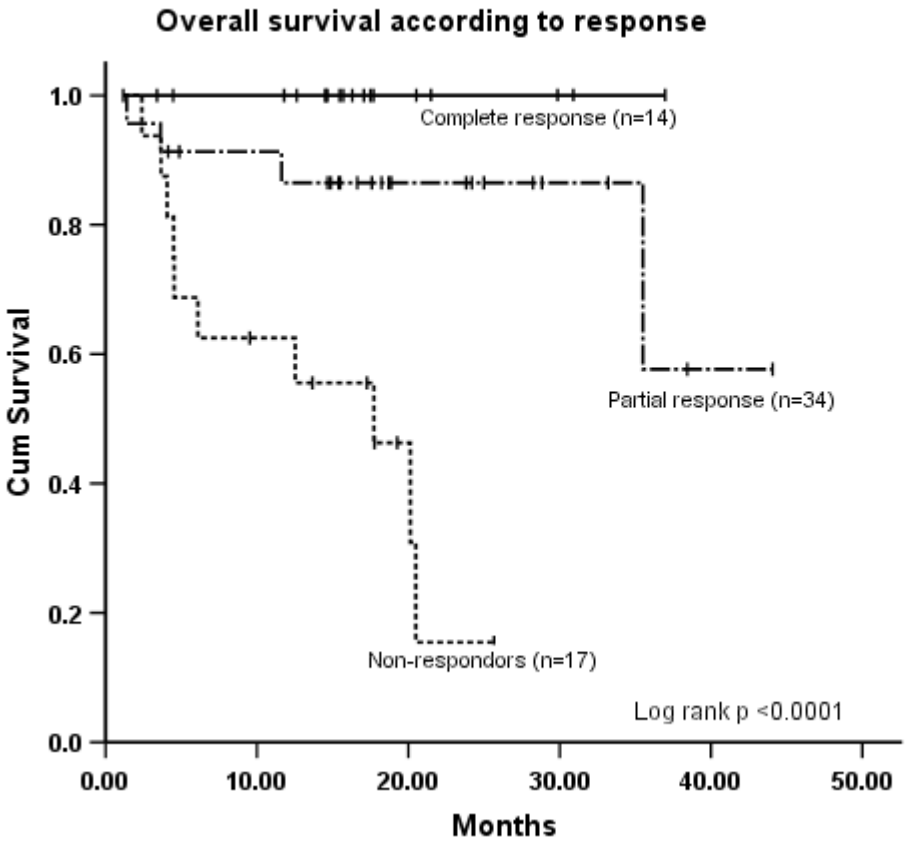
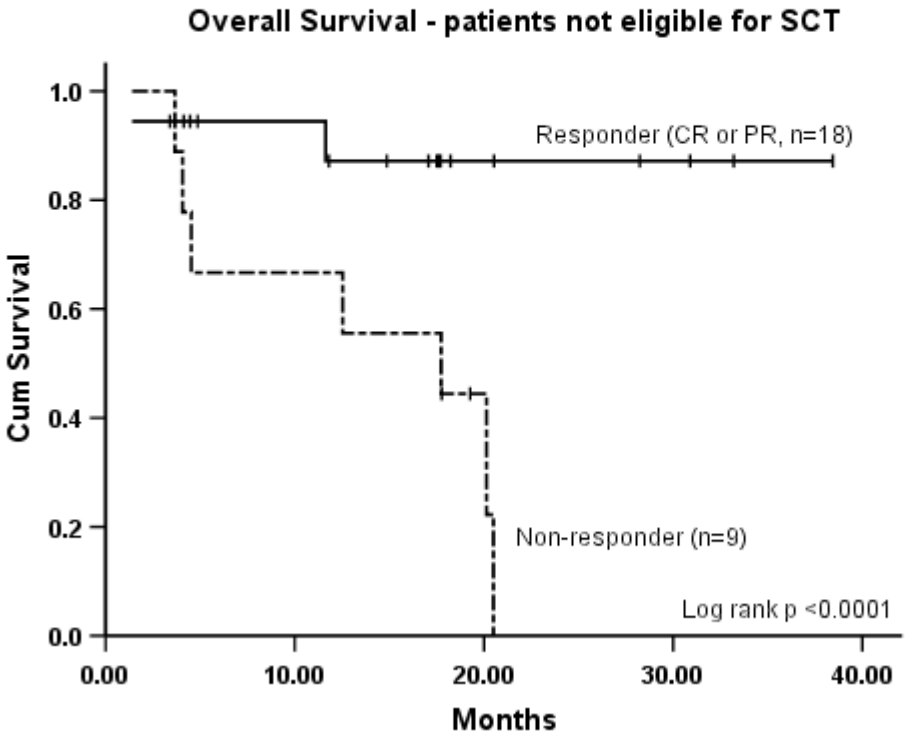

Overall survival according to response
Cum Survival
1.0
0.8
0.6
0.4
0.2
0.0
Complete response (n=14)
Partial response (n=34)
Non-respondors (n=17)
Log rank p <0.0001
0.00
10.00
20.00
30.00
40.00
50.00
Months

Figure 2D.


Overall Survival - patients not eligible for SCT
Cum Survival
1.0
0.8
0.6
0.4
0.2
0.0
Responder (CR or PR, n=18)
Non-responder (n=9)
Log rank p <0.0001
0.00
10.00
20.00
30.00
40.00
Months