7.1.1 Overview of Level I Evidence in AML.

Seven studies were available for review that provided level I evidence regarding high dose busulfan preparative therapy for transplantation in AML. Three focused on allogeneic transplantation and four on autologous transplantation, and the evidence in each of the transplantation modalities will be summarized separately.

7.1.1.1 Allogeneic Transplantation.

All three allogeneic transplantation studies employed the same Busulfan/Cyclophosphamide regimen (BU/CY2 = Cyclophosphamide 60 mg/kg x 2d), and all made comparisons with a TBI arm. However, the TBI arm in one study (SWOG) was combined with etoposide, while in the other two studies it was combined with cyclophosphamide. Disease eligibility differed among the three studies as well. The study reported by Blaise was the only study that limited inclusion to AML, and patients in this study were further limited to AML in first remission. The other two studies allowed participation of multiple hematologic malignancies. One of those studies, the SWOG study reported by Blume, had eligibility criteria specifically targeting patients with leukemia that had failed at least one prior therapy – a higher risk population. The Nordic BMT group study reported by Ringden stratified its analysis on the basis of "early" or "advanced" disease, allowing participation of a broad spectrum of prognostic categories.

None of these three allogeneic transplantation studies demonstrated superiority of the BU/CY preparative regimen over that of a TBI-based regimen. In fact, in terms of Kaplan-Meier **2 year** probability of survival, patients treated with BU/CY in the Blaise study (all patients had AML in first CR) had statistically significantly inferior survival compared to the TBI group (BU/CY=51% vs. CY/TBI=75%, $p<0.02$). Similarly, patients with a a range of hematologic malignancies that included both "early" and "advanced" disease treated with BU/CY in the Nordic BMT Group trial also had an inferior overall survival, expressed as **3 year** Kaplan-Meier probability of survival (BU/CY=62% vs. CY/TBI=76%, $p<0.03$). Survival in the SWOG study report was expressed as relative risk of mortality, and there was no significant superiority of either preparative regimen when expressed in this manner. The RR of mortality BU/CY:TBI/VP-16 = 0.97 (95% CI = 0.64-1.48). The width of this confidence interval provides no assurance of equivalence of these two regimens. This study employed a different TBI comparative regimen and limited eligibility to advanced hematologic malignancies. Both the Blaise and Nordic BMT Group studies demonstrated statistically significantly higher treatment related mortality associated with the BU/CY arm. This endpoint was not analyzed in the SWOG report.

A subset analysis performed in the Nordic BMT Group study, revealed that the patients with advanced disease treated with the BU/CY preparative regimen had a significantly inferior overall survival and disease free survival than those with advanced disease prepared for transplantation with CY/TBI, (OS, $p=0.002$ and DFS, $p=0.005$). There was no statistically significant difference between treatment arms in these two endpoints in the subset of patients with early disease. In this study, 37 of the 88 patients on the Busulfan arm (42%) had AML, and 25 of those 37 (68%) had early disease. On the TBI arm 32/79 (41%) patients had AML, and 26/32 (81%) had early disease. Multivariate analysis in this study found that improved survival was associated with early leukemia ($p<0.0001$) and TBI ($p=0.02$). A subset analysis of the patients with AML in the Nordic BMT Group trial found no statistically significant difference in Kaplan-Meier probability

of 3y DFS between the two treatment arms (BU/CY=61%, and CY/TBI=64%; p=0.37). There was no such subset analysis reported in the the SWOG study.

7.1.1.2 Autologous Transplantation.

The four studies in autologous transplantation all limited participation to AML in first CR, *and none showed survival advantage for autotransplantation over chemotherapy.* In fact, in the intergroup trial reported by Cassileth, overall survival was significantly higher on the HDAC arm compared to autologous transplantation. The randomized comparator arms were autologous transplantation vs. chemotherapy in all studies, but the chemotherapy regimen intensity varied among the studies. Three of the studies employed a different busulfan + cyclophosphamide conditioning regimen for transplantation than the allogeneic trials – the busulfan dose was the same, but the cyclophosphamide dose was higher, 50 mg/kg/d x 4 (BU/CY4 or BU/CY200). The remaining study, the BGMT 87 study reported by Reiffers, employed a preparative regimen that combined busulfan with melphalan 140 mg/m^2. Three studies employed autologous marrow, and one (the BGMT trial) used either autologous marrow or autologous peripheral stem cells. Marrow purging was performed in two of the studies – Ravindranath's pediatric AML trial (which was also unique in that its entire population was pediatric) and the Cassileth intergroup trial. The Kaplan-Meier probability of overall survival and disease free survival was reported in the reference frame of 3 years in the pediatric trial (Ravindaranath) and the BGMT 87 study. It was reported in a 4-year frame of reference in the studies reported by Harousseau and Cassileth. In all studies there was no statistically significant difference found in these endpoints between randomized treatment groups, except for a significant advantage in overall survival for HDAC compared to autologous transplantation in the Cassileth article. Treatment related mortality was found to be statistically significantly higher on the Auto-BMT arm in the pediatric study (p=0.005). A higher treatment related mortality was reported for Auto-BMT in the Harousseau article (6.5% vs. 3%) and Cassileth article (14% vs. 3%), but was not defined as significantly different between arms.

Because the 4 randomized studies comparing autologous transplantation to intensive consolidation or maintenance chemotherapy reported by Cassileth, Reiffers, Ravindranath, and Harrouseau all started with a population allowed to "drop-out" to allogeneic transplantation if a suitable donor was available and age criteria were met, these 4 studies also reported a comparative analysis of their non-randomized allogeneic transplant arm and the randomized autologous and chemotherapy arms.

The pediatric study reported by Ravindranath included 89 patients who were eligible for and went on to select allogeneic transplantation. Although a BU/CY preparative regimen was recommended for these transplants, study centers could use whatever regimen they thought appropriate, and "a variety of regimens" that were not otherwise defined were actually employed. Ravindranath reported a Kaplan-Meier estimated 3 year EFS of 52% ± 8%SE (or ±16% = 95% CI) for that pediatric population undergoing allogeneic transplantation, and found no significant difference in EFS between allogeneic transplantation and intensive consolidation chemotherapy (36%±5.8%SE, or ±11% CI), p=0.06. However, estimated EFS and OS at 3 years were found to be superior on the allogeneic arm compared to the autologous arm, p=0.01 and 0=0.007, respectively. This was despite no statistically significant difference found between the randomized autologous transplant arm and the ICC arm in both estimated EFS and OS. The 115 pediatric patients who underwent autologous transplantation had a Kaplan-Meier estimated 3y actuarial EFS of 38% ± 6.4% SE (or ±13% CI). *Reviewer note: The Kaplan-Meier probabilities were given with standard errors in this paper and have been reported here both as given in the*

paper, and with 95% confidence intervals as calculated by the reviewer. Review of the definition of EFS provided in the paper suggests that the definition is similar to that of DFS. The article defined EFS as time to death, relapse, or documentation of failure to enter remission.

The randomized, controlled study reported by Harrouseau had a cutoff age of eligibility for allogeneic transplantation of 40 yo. The 73 patients eligible for allogeneic transplantation in the Harrouseau article had a Kaplan-Meier probability of 4y DFS of 49.5% ± 6% SE (or ±12% = 95% CI). A subset analysis of the patients randomized to ICC in the Harrouseau study post-remission who were also ≤40 yo (age range on this study was 15-50 yo) found no significant difference in estimated 4y survival between allogeneic transplantation and ICC. A comparison was not reported between patients undergoing autologous transplantation (with unpurged marrow) and allogeneic transplantation in this trial, however, no significant difference in estimated 4y overall and disease free survival was found between the randomized arms – ICC and autologous transplantation. The 4y Kaplan-Meier estimate of DFS in the 86 patients who were auto-transplanted in this study was 44% ± 5.5% SE (or ±11% = 95% CI). *Reviewer note: This article reported standard errors and are reported here both as standard errors and with 95% confidence intervals calculated by the reviewer.*

The upper age limit for allotransplant in the Reiffers study was 45 yo. There was no purging performed, and some of the auto-transplants were derived from peripheral blood stem cells. The estimated 3y DFS for the 33 patients undergoing allotransplant on this study was 66.5% ±16, while the 3 year estimated DFS of the 39 patients in the autotransplant arm was 51% ± 17%. Subset comparisons were made between the allogeneic patients on study and those patients ≤45 yo on the two randomized arms – autotransplant and maintenance chemotherapy. No patients from the maintenance chemotherapy group had to be dropped from the analysis when this age limitation was applied, but 6/39 patients randomized to autotransplant had to be excluded. A significant difference in estimated 3y DFS between allo-transplantation and maintenance chemotherapy favoring allotransplant ($p<0.02$) was found, but no significant difference in Kaplan-Meier estimated 3y DFS was detected between auto- and allo-transplantation. The comparison between the maintenance chemotherapy and autotransplantation groups, without excluding patients older than 45 years of age, revealed no significant difference between arms in these endpoints.

Finally, the most recent report was the intergroup post-remission therapy trial (ECOG, SWOG, and CALGB) published by Cassileth, et al. Eligibility criteria for age included patients 16-55 yo. All patients who entered the trial were required to have no illness that would preclude transplantation. After achieving CR with induction therapy (idarubicin and cytarabine), patients who remained physically able to undergo allotransplantation and had a genotypically or phenotypically HLA-matched or single-antigen-mismatched related donor were offered allotransplantation. The remaining responders were randomized between autotransplantation and consolidation with HDAC ($3g/m^2$ over 3h q 12h x 12). All patients who entered CR received a post-remission course of attenuated dose of idarubicin (2d) and cytarabine (5d). Patients were required to have bone marrow reassessment to confirm continued remission marrow before transplantation, but not before HDAC. All transplants were performed with BU/CY200 as the conditioning regimen. As is usual in these study designs, there were a significant number of patients who did not receive their intended treatment. Only 54% of the patients randomized to autologous transplant actually underwent auto-BMT, while 91% of the patients randomized to HDAC and 81% who were eligible for allotransplant did receive their intended therapy. The most common reasons for not going on to auto-BMT as planned were refusal (21), relapse before

receiving therapy (15), and inadequate marrow stem-cell harvest (9). Five patients randomized to HDAC refused therapy and 3 patients eligible for allo-transplant refused therapy.

The life-table estimates of DFS at 4y were both 35%±9% on the autologous and HDAC arms. The median DFS was 14 months on the autotransplant arm, and 18 months on HDAC. The median DFS was 32 months on the allogeneic transplant arm, and the life-table estimate of DFS at 4y was 43%±10%. Life table estimates of overall survival at 4 years were 52%±9% for HDAC, 43%±9% for autologous transplantation, and 46%±10% for allotransplantation. The comparison of overall survival estimates for HDAC vs. autologous transplantation favored HDAC, p=0.05. The comparison of the overall survival estimates between HDAC and allotransplant found that survival was favored with HDAC as well, p=0.04. Relapses occurred most frequently on HDAC, followed by autologous transplantation. Treatment related mortality at 100d was highest on allotransplant, followed by autologous transplantation.

The outcomes of these autologous transplantation studies are similar. No definite superiority of autologous transplantation over post-remission chemotherapy was demonstrated. Superiority of allogeneic transplantation over autotransplant was only seen in the pediatric AML study. This comparison was not addressed in the Harrouseau study, which found no significant difference in its comparison of ICC to allogeneic transplantation, and found no difference in DFS between the randomized arms of ICC vs. autologous transplantation.

7.1.2 Non-Level I Supportive Studies in AML.

The sponsor's 43 article dataset and the reviewer's literature search were examined for any potentially supportive studies that, though not level I evidence, had a cohort control or historic control design. Three articles from the sponsor's "core dataset" and one article from the reviewer's literature search were identified (all derived from Tables and) and summarized below.

Table 22 Summary of Non-Level I Supportive Studies in AML

Citation	Design and Dose	Disease	BMT Type	% Engrafted Median Days	%Relapse	%Survival Median Survival	Adverse Events
Cassileth,P. JCO. 1993 Feb; 11(2): 314. Autologous Bone Marrow Transplant in AML in First Remission	Prospective, Cohort Control ECOG study P-C 486. Patients in CR after induction chemotherapy were offered alloBMT if <41 yo and HLA-identical sibling In CR but ≥ 41 yo or no donor offered autoBMT Preparative regimen Busulfan 4 mg/kg x 4d + Cyclophosphamide 50 mg/kg/d x 4d	AML Previously untreated Age:17-54 AutoBMT median age = 36 AlloBMT median age = 32	Autologous marrow was purged with 4-hydroperoxycyclop hosphamide AutoBMT=39 AlloBMT=19	ANC ≥ 500: AutoBMT=31d (median) AlloBMT= not given Plt's >20,000: AutoBMT=47 d (median)		Median f/u=31 months <u>Actuarial DFS at 3y:</u> AutoBMT=54% ± 16% AlloBMT=42% ± 22%	TRM: AutoBMT=6% Grade ¾ hepatoxicity "probably VOD" = 26% AlloBMT = 30%
Dusenbery, KE. Int J. Radiat. Oncol. Biol. Phys. 1996; 36(2): 335. Autologous BMT in AML: the University of Minnesota Experience	Retrospective, Historical Control All patients presenting with AML in CR without HLA compatible donor were given autologous BMT. Two preparative regimens were used. Assignment was based on 1) participation in a clinical trial comparing BU/CY to CY/TBI 2)Not eligible or refused clinical trial 3) BU/CY on CCG protocols 4)On a pilot study and transplanted with CY/TBI 5)Non-randomly assigned to BU/CY on the basis of age ≤ 2 yo BU/CY = Cyclophosphamide 50 mg/kg x 4d	AML Includes CR1 and >CR1	Autologous BMT Marrow purged with 4-hydroperoxycyclop hosphamide in all but 6 pt's BU/CY=46 CY/TBI=29	ANC > 500 BU/CY=42 d median CY/TBI=41 d Median One patient on each arm required reinfusion of marrow for non-engraftment	2 year actuarial Relapse Rate: BU/CY=55% (95% CI=40-71) CY/TBI=44% (95% CI=25-63) p=0.45	<u>2 year actuarial Overall Survival:</u> BU/CY=46% (95% CI = 24%-67%) CY/TBI=51% (95% CI = 36%-66%) p=0.96 <u>2 year actuarial DFS:</u> BU/CY=39% (95% CI = 25%-53%) CY/TBI=52% (95% CI = 33%-70%) p=0.35 Subset Analysis of DFS on patients in CR>1 (BU/CY=15 CY/TBI=16) BU/CY=7% CY/TBI=38% P=0.04	VOD: BU/CY = 7/46 Fatal in 3/7 CY/TBI=0/29 P=0.04 TRM (non-relapse deaths) BU/CY = 5/42 CY/TBI= 2/29 P=0.03

Citation	Design and Dose	Disease	BMT Type	% Engrafted Median Days	%Relapse	%Survival Median Survival	Adverse Events
		Age: BU/CY: Median=10.8 (0.6-53.2) CY/TBI Median=32.4 (1.6-57.5)					
Vaughan, WP. Bone Marrow Transplant. 1991; 8:489. Improved Results of Allogeneic BMT for Advanced Hematologic Malignancy Using Busulfan, Cyclophosphamide and Etoposide as Cytoreductive and Immunosuppressive Therapy.	Retrospective Historical Control Patients treated with a BU/CY/VP preparative regimen were compared to a control group prepared using "various TBI regimens during an overlapping time period". BU/CY/VP-16= BU 4 mg/kg x 4 d + CY 60 mg/kg x 2d + VP-16 60 mg/kg x 1 TBI : Ara-C + CY 90 mg/kg (9) or CY 60 mg/kg x 2 (2) or Ara-C 3 g/m2 q 12h x 12d (1)	AML=10 ALL=3 NHL=8 HD=1 CML=1 AUL=1 Disease primary refractory, relapsed, reinduction failure, CR≥3, or >second chronic phase CML Age: BU/CY/VP: Median=27.5y TBI: Median=9y	Allogeneic HLA-matched sibling BU/CY/VP N=24 TBI "control" N=12	ANC > 500: BU/CY/VP = 11-26 d TBI = 11-20d Plt's > 20,000: BU/CY/TBI= 14-108 d TBI= 14-27 d	K-M Estimated Relapse at 50 weeks: BU/CY/TBI=20% TBI=67% P<0.02	K-M Estimated DFS at 50 weeks: BU/CY/VP=40% TBI=17% (not statistically significant)	VOD: BU/CY/VP = 17% TBI = 0% H. Cystitis: BU/CY/VP = 2/24 (8%) TBI = 0% Pumonary failure: Early= BU/CY/VP=1/24 (4%) TBI = 1/12 (8%) Late= BU/CY/VP = 1/24 (4%) TBI= 2/12 (17%)

Citation	Design and Dose	Disease	BMT Type	% Engrafted Median Days	%Relapse	%Survival Median Survival	Adverse Events
Michel, G. JCO. 1994 June; 12(6): 1217. Allogeneic BMT for Children with AML in First CR: Impact of Conditioning Regimen Without TBI – A Report from the Societe Francaise de Greffe de Moelle. Update: Michel, G. JCO. 1997 June; 15(6):2238. The Effects of Allogeneic BMT for Children with AML in First Remission: The Impact of Conditioning Regimen Without TBI – A Report from the Societe Francaise de Greffe de Moelle	Retrospective Cohort Control Patients selected from a French BMT registry of childhood AML that included 113 children ≤16 yo who received HLA-identical BMT in first CR (6/79-8/92) 45/113 were prepared with a BU/CY regimen 32/113 were prepared with TBI. A comparison between TBI and non-TBI regimens was performed on these patients. BU/CY = Cyclophosphamide 50 mg/kg x 4 in 19 Cyclophosphamide 60 mg/kg x 2 in 23 TBI was combined with: Cyclophosphamide 60 mg/kg x 2 in 24 Cyclophosphamide+other drugs in 4 Melphelan in 4.	AML, first CR Age: BU/CY120: Mean Age=8.3 BU/CY200: Mean Age=5.6 TBI: Mean Age=11.2	HLA identical Related allogeneic BMT BU/CY120 N = 23 BU/CY200 N = 19 TBI N = 32	No Mention	5 year K-M estimate: BU/CY120 = 54% BU/CY200 = 13% TBI = 10% Multivariate analysis BU/CY120 had significantly higher risk of relapse, p=0.02. <u>Update:</u> BU/CY120: 7y K-M = 40.5%±19 BU/CY200: 7y K-M = 16%±16% TBI: 7y K-M = 23%± 16% <u>No significant difference on multivariate analysis</u>.	<u>K-M Estimated Event Free Survival at 28 mo:</u> BU/CY120=46% ± 24% K-M EFS at 31 mo: BU/CY200= 82% ± 18% K-M EFS at 48 mo: TBI= 80% ± 14% No statistical significant difference. Update: BU/CY120: 5y and 7y K-M EFS=59.5% ± 19% BU/CY200: 5y and 7y K-M EFS=79% ± 17% TBI: 5y K-M EFS=73.5%±15 7y K-M =63% ± 18% No statistically significant difference	TRM: BU/CY120=0 BU/CY200=1/19 TBI= 3/32

The most useful supportive study listed in the Table above is the retrospective cohort study reported by Dusenberry. It includes only patients with AML - CR1 and >CR1. Its comparability to the randomized studies discussed above, however, is limited by the fact that all participants underwent autologous transplantation. The preparative regimens in this study were the focus of comparison. BU/CY4 and CY/TBI were retrospectively compared in the context of the autologous setting. Patients varied on whether they received purged marrow, and the non-randomized comparator arms were not balanced in number of participants – 46 vs. 29 (BU/CY vs. CY/TBI). This study found no statistically significant difference between preparative modalities in 2 y K-M estimated relapse rate, overall survival, or disease free survival, although a statistically higher incidence of VOD was reported on the BU/CY arm compared to the CY/TBI arm, and treatment related mortality was significantly higher on the BU/CY arm. This echoes the results of the AML randomized controlled studies examining the comparability of BU/CY vs. CY/TBI, but those studies were in the allogeneic setting (Blume, Ringden, and Blaise). A subset of 35 patients reported within this study had been part of a randomized trial comparing conditioning with BU/CY to CY/TBI for autotransplantation in AML. This study report[10] was not included in the level 1 patient discussion because the total number of patients treated on each arm was small – BU/CY=17 and CY/TBI=18. Patients in this study were stratified by remission status. Six in each arm were in first CR. Two in each arm were in first relapse. Eight BU/CY and 9 CY/TBI patients were in CR2. One CY/TBI patient was in second relapse and one BU/CY patient was in CR3. TBI was fractionated (twice a day x 4 days). Autologous marrow was purged with 4-hyroperoxycyclophosphamide. No statistically significant difference in 2 year probability of overall survival was detected between the two regimens – BU/CY=35% (95% CI=12-58%) and CY/TBI=46% (95% CI= 20-71%), p=0.41. The 2 year estimate of DFS was not found to be statistically different either – BU/CY=24% (95% CI=3-44%) and CY/TBI=50% (95% CI=26-74%), p=0.12. VOD was limited to the BU/CY arm and was fatal in 2/3 cases.

The Cassileth study, which at best qualifies as supportive evidence, was included in the sponsor's 43 article "core dataset". This article includes high dose busulfan preparative regimens in both arms of a non-randomized, cohort control study that compared autologous transplantation and allogeneic transplantation in de novo AML. This study is most comparable to the 4 randomized studies comparing autologous transplantation to intensive consolidation or maintenance chemotherapy reported by Reiffers, Ravindranath, Harrouseau, and the 1998 NEJM article by Cassileth discussed above. For the purposes of examining whether the data from Cassileth's cohort control study support the data derived from the 4 randomized studies, the reviewer compared the efficacy results reported for its treatment cohorts to the allogeneic and autologous efficacy results reported in those three randomized studies. That comparison follows.

The level I study reported by Ravindranath was limited to pediatric AML, while those reported by Cassileth, Reiffers, and Harrouseau were not. EFS was reported as 3-year probabilities on both the autologous and allogeneic arms of the Ravindranath (level I) and supportive Cassileth studies. Ravindranath reported a Kaplan-Meier estimated 3 year EFS of 52% ±8%SE (or ±16% = 95% CI) in its 89 patient pediatric population that underwent allogeneic transplantation with various preparative regimens, compared to the DFS of 42% ± 22% reported in 19 adults undergoing allogeneic transplantation in the Cassileth article. The 115 pediatric patients reported by Ravindranath who underwent autologous transplantation had a Kaplan-Meier estimated 3y actuarial EFS of 38%±6.4% SE (or ±13% = 95% CI), compared to a DFS of 54%±16% in the 39 patients who had autologous transplantation in the Cassileth study. Marrow was purged in both studies.

The randomized, controlled study reported by Harrouseau is similar to that of the supportive Cassileth study in that the cutoff age for eligibility for allogeneic transplantation was 40 yo.

However, the survival analysis in the Harrouseau study was a Kaplan-Meier estimated 4y analysis, and that of Cassileth's was a 3y analysis. The 73 patients eligible for allogeneic transplantation in the Harrouseau article had a Kaplan-Meier 4y DFS probability of 49.5% ± 6 % SE (or ±12% = 95% CI) compared to 42% ± 22% at 3y in the Cassileth study. The 4y Kaplan-Meier estimate of DFS in the 86 patients who were autotransplanted in the study reported by Harrouseau was 44% ± 5.5% SE (or ±11% = 95% CI), compared to 54% ± 16% in the Cassileth article. Marrow was not purged in the Harrouseau trial, while it was in Cassileth's.

The age eligibility criteria for allogeneic transplantation differed between the studies reported by Reiffers and Cassileth in the supportive study. In the level I Reiffers study, 45 yo was the age cutoff for allotransplantation, autografts were performed without purging, and some autografts were peripheral blood stem cells. The estimated 3y DFS for the 33 patients undergoing allotransplant on this study was 66.5%±16% compared to 42%±22% in the non-level I Cassileth study. The 3-year estimated DFS of the 39 patients in the autotransplant arm from the Reiffers study was 56%±16%, compared to 54%±16% for the 36 patients autotransplanted in the Cassileth supportive study.

In comparing the results from the 1998 level I Cassileth study and the non-level I supportive study by Cassileth, one must again recognize that the survival probabilities were reported from differing time frames – 3 year estimates in the supportive study and 4 year life table estimates in the level I study. In the most recent report by Cassileth the 4y Life Table DFS was 35%±9% for autologous BMT, compared to the 3y estimate of DFS of 54%±16% in the supportive study. For allo-BMT the 4y Life Table DFS was 43%±10% in the level I study compared to a 3y estimate of 42%±22%.

The remaining three "supportive studies" in Table 20 are heterogeneous in comparator arms, patient populations, disease inclusion criteria, and transplantation methods. They contribute little pertinent information to support this review. The study reported by Michel appears comparable to the level I trial reported by Ravindranath as it also enrolled only a pediatric population, but the focus of the latter study was a comparison of autologous transplantation and ICC, (although there was a non-randomized allogeneic transplantation group). The study reported by Michel focuses on allogeneic transplantation, examining differences among 3 preparative regimens – two busulfan/cyclophosphamide regimens and TBI (in combination with various chemotherapy drugs) conditioning. The Kaplan-Meier probability of EFS for each of the 3 arms in the earlier Michel study report is expressed in differing time frames, and all differ from the reference reported for EFS in the non-randomized allogeneic transplant patients in the Ravindranath study, making comparisons between the studies difficult. The Kaplan-Meier probability of event free survival reported in these studies are, however, summarized below:

Ravindranath Allogeneic (non-randomized arm) 3y EFS = 52% ± 8% SE (or ±16% CI)

Michel Allogeneic BU/CY120 28 mo. EFS = 46% ± 24%
5y EFS = 59.5% ± 19%
7y EFS = 59.5% ± 19%

Michel Allogeneic BU/CY200 31 mo. EFS = 82% ± 18%
5y EFS = 79% ± 17%
7y EFS = 79% ± 17%

Michel Allogeneic TBI-based 48 mo. EFS = 80% ± 14%
5y EFS = 73.5%± 15%
7y EFS = 63% ± 18%

No significant difference was detected among the conditioning regimens in EFS in this study reported by Michel, but this was not a randomized study.

Finally, the retrospective historical control study reported by Vaughan is problematic for use as supportive data for a number of reasons. There were a variety of hematologic malignancies represented in the study, the control group has a median age of 9 yo while that on the busulfan study arm was 27.5 yo, and the probability of DFS in the study was reported at 50 weeks. The busulfan comparator arm was a three drug combination of busulfan + cyclophosphamide + etoposide, rather than the two-drug busulfan + cyclophosphamide regimen.

7.1.3 Summary and Conclusion – Allogeneic Transplantation in AML.

Allogeneic bone marrow transplantation has become an accepted treatment modality for AML. A retrospective comparison of patients treated with chemotherapy on EORTC protocols and patients who underwent allotransplantation by the European Cooperative Group for BMT found that the leukemia free survival was improved in patients who underwent transplantation in first CR (age-matched controls).[11] In 1993, Christiansen reviewed the available prospective trials comparing allogeneic transplantation to chemotherapy in AML, and noted that the majority of these studies – which did not involve randomization to allotransplant, but rather assignment to transplantation based on donor availability – found no significant difference in DFS between arms, although there were some that did. The 3 studies that demonstrated benefit employed CY/TBI conditioning and were reported by Appelbaum, Conde, and Reiffers (1989). The study reported by Reiffers[12] (1989) was different from the Reiffers study discussed earlier for level I evidence in autologous transplantation. The Reiffers 1996 study was larger and employed busulfan conditioning (and revealed significantly higher probability of DFS at 3y associated with allo-BMT than with chemotherapy). The earlier Reiffers 1989 study had 3 arms – allotransplantation (n=20), chemotherapy (n=20), and autotransplantation (n=12). The 30 month probability of DFS after allo-BMT was 66% (95% CI=45-87%), after auto-BMT 41% (95% CI=4-78%) and after chemotherapy 16% (95% CI=0-31%). Only the comparison between allo-BMT and chemotherapy was found to be statistically significant, $p<0.002$. The Conde study found a significant difference in DFS between allo-BMT (n=14) and chemotherapy (n=25), with a minimum follow-up of 3 months. The study reported by Applebaum had a minimum follow-up of >60 months and found a significant difference in DFS between allo-BMT (n=33) and chemotherapy (n= 43).

Those studies reviewed by Christiansen that utilized high dose cytarabine in their chemotherapy arms, found no statistically significant difference in the DFS between arms. The importance of factoring dose intensity of chemotherapy into comparative outcome assessments of chemotherapy vs. allotransplantation in this disease is exemplified by a subsequent publication of a CALGB study that examined varying the dose intensity of post-remission chemotherapy. That trial found that the 187 patients enrolled on the high dose cytarabine arm ($3g/m^2$) had a 4 year probability of DFS of 39% (95% CI=32-46%).[13] The subset of patients in this arm who were ≤ 60 had a 4y probability of DFS of 44% (95%CI = 36-51%). The 4 year probability of overall survival (OS) was 46% in the high dose cytarabine arm, and in the ≤ 60 yo subset it was 52% - percentages that rival those reported for allogeneic transplantation. These probabilities were found to be

statistically significantly superior to the other two lower dose cytarabine arms. Another study that has raised the issue of dose intensity in this disease is a recently published report by Stevens[14] (1998) of the results of the pediatric patients participating in the 10th United Kingdom Medical Research Council (MRC) trial in AML, which examined allotransplantation vs. autotransplant vs. intensive post-remission chemotherapy. Although 359 children entered this trial, only 61 underwent allotransplant, and there were only 50 patients evaluated in the other two arms. CY/TBI was the conditioning regimen for transplantation for patients >2 yo. Younger patients were treated with BU/CY. (This trial was not reviewed in the discussion of level 1 evidence for busulfan as the exact number of patients receiving busulfan was not specified in the article.) As usual, patients undergoing allotransplantation were not randomized into this arm, but were treated as such if there was a donor available. The article reports that a Mantel-Byar analysis found no significant difference in survival between patients who underwent allotransplantation and those who did not. The authors of this paper suggested this lack of benefit for allotransplantation could have either reflected a false negative result arising from small patient numbers, or a demonstration of the impact of the intensive chemotherapy delivered post-remission. Another 1998 publication of this trial by Burnett, that examines the entire pediatric+adult patient population participating in this study, focused on the comparative results of the randomized arms – chemotherapy vs. autologous transplantation. It will be discussed in the autologous transplantation summary, but found no statistically significant advantage in probability of overall survival for autotransplantation, unless survival was partitioned into two time periods – in the first two years post treatment vs. beyond two years. Disease free survival was significantly better on the autologous arm, however, when the entire population was examined in this article.

The recent publication of the intergroup study of post-remission dose intensity (Cassileth, 1998), which was discussed at length earlier, must also be considered. Patients were not randomized to the allotransplantation arm in this study, as is usual in these studies. The 4y estimates of overall survival favored HDAC consolidation over allogeneic transplantation and autologous transplantation. The authors did note in their discussion of these findings, that though relapses were higher on the HDAC arm, treatment related mortality was lower on that arm than with autologous or allogeneic transplantation. The fact that 35% of HDAC patients subsequently underwent transplantation (allo- or auto-) when they relapsed after HDAC was something the authors felt should be considered when examining the relative treatment outcomes in these arms.

Not all studies of standard dose vs. high dose cytarabine have demonstrated benefit associated with the use of high dose cytarabine. A SWOG study reported by Weike[15] examined HDAC vs. conventional dose cytarabine in both induction and consolidation, and found no significant difference in OS, although the 4 year probability of relapse free survival favored HDAC – 33% (HDAC) vs. 21% (standard dose cytarabine) in patients <50 yo, p=0.049. The probabilities were 21% vs. 9%, respectively, for patients between 50 and 64 years of age. Two dose levels of high dose cytarabine were utilized in this study secondary to observed toxicity ($2g/m^2$ and $3g/m^2$, both infused over 3 hours every 12 h), and the majority of high dose patients received the lower of the two doses.

In the 8 prospective studies examined by Christiansen as discussed above, all preparative regimens for allotransplantation were TBI based. Five used CY/TBI, one TBI+piperazinedione, and one TBI+cytarabine±cyclophosphamide. CY/TBI is frequently referred to in the literature as the most commonly used preparative regimen in AML.[10,16] It's selection as the comparator arm in the allogeneic transplantation level I studies examined in this review appears to based on its role in the historical development of transplantation as a treatment modality.

Despite conflicting data from randomized, controlled trials, the therapeutic utility of allotransplantation in AML appears to be accepted. Its timing is perhaps subject to greater dispute. Treated with chemotherapy alone, 65-75% of patients with AML will relapse. Alternatively, 25-35% of patients with AML will be cured with chemotherapy alone. These statistics imply that broad application of allogeneic transplantation to all patients with an available donor and who are otherwise medically eligible for transplantation, results in 25-35% undergoing this intensive form of therapy when they are already cured. Studies suggest that a cure can be expected in approximately half of patients who undergo allogeneic transplant for AML in first CR.[16,18] Treatment related mortality associated with this modality is significant, and acute graft vs. host disease of significant grade has been reported in 30-45% of patients undergoing allogeneic transplant, with a case fatality rate reported as high as 50%. Other complications of transplantation, including VOD, interstitial pneumonitis, and infection contribute to treatment failure associated with allo-transplantation. It has been argued that if allogeneic transplantation were reserved until first relapse, the 30% cure rate that has been observed with transplantation at that stage of disease could be added to the approximate 30% cure rate from the original induction chemotherapy, resulting ultimately in an approximate 50% overall cure - similar to that reported for first line application of allogeneic transplant in this disease.[16] This approach would avoid treatment related mortality associated with transplantation in patients already cured with chemotherapy.

This literature review has identified reports from 3 prospective, randomized controlled trials that investigated the use of high dose busulfan-based preparative regimens vs. a TBI based regimen in the setting of allogeneic transplantation for AML. One of those trials was limited to patients with AML, and more specifically, AML in first CR. The other two studies allowed participation of patients with a variety of hematological malignancies. AML patients represented 1/3 of the study population in the Blume trial, which had a unique TBI/VP-16 comparator arm. This trial also limited participation to more advanced stage disease – beyond first line therapy. In the other trial, reported by Ringden, AML patients represented 40% of the study population, and included both patients in first CR and those beyond first CR. Two of these studies found BU/CY to be inferior to TBI/CY in both actuarial overall survival and DFS. One of those trials (Blaise) was limited to AML with early disease. The other was the Nordic BMT Group Study (Ringden) in which 40% of participants had AML - with early and late stage disease represented. The remaining study (Blume), in which a third of the participants had AML (all with disease beyond CR1), found no statistical difference in the relative risk of mortality between the BU/CY and TBI/VP-16 arms. There was not evidence of equivalence. The RR of mortality was 0.97 (BU/CY : TBI/VP-16), and the study was powered (89%) to detect a RR of 2.3. Thus, in allogeneic transplantation for AML, 2/3 studies offering level I evidence argue that BU/CY is inferior to TBI-based therapy.

Certainly the Blaise study, which limited participation to patients with AML in first CR, offers the most clear cut evidence among these three studies. Study participation was limited to "adults", as eligibility criteria specified that participants had to be of age > 14 yo. All patients received the same GvH prophylaxis, except for a small subset who were evenly split between study arms and were participating in an investigation of anti-p55 MoAb. The publication of this study reported not only superior Kaplan-Meier estimated overall survival and DFS for the TBI arm, but also a statistically significantly superior probability of relapse associated with TBI (14%± 5% SE vs. 34% ±6% SE on BU/CY; $p<0.04$). There was a trend toward higher incidence of treatment related mortality on the BU/CY arm as well, but this was not found to be statistically significant (27% vs. 8%; $p<0.06$). The results of this study, however, have been criticized in the literature[10,17] because the efficacy results on the CY/TBI arm seemed unusually high (OS *at 2 years* =75% ± 7% SE and DFS at *2 years* =73% ±7% SE), and the relapse rate with BU/CY was higher than

reported in prior studies (34%). Copelan reported, based on personal communication, that in the International Bone Marrow Transplant Registry, the 2 year leukemia-free survival for patients with AML in first remission transplanted with a TBI/CY preparative regimen (n=296) was 61%± 6%. The 2 year LFS with BU/CY in that registry (n=97) was 62%±12%. Another specific criticism of the results from the Blaise study is that the TBI arm's treatment related mortality of <10% seemed unusually low. A counter-argument could be made that this observed improved treatment mortality compared to historical results may reflect improved supportive care measures developed by the time this study was conducted, and its use of fractionated TBI with lung shielding.

The Nordic BMT Group study also reported a similar high probability of survival associated with CY/TBI - 76% at 3 years compared to 75% at 2 years in the Blaise study. This study also found the TRM on the TBI arm to be less than 10% (9%) and the majority of the centers used fractionated TBI with lung shielding. On this study the superiority of treatment related mortality was found to be statistically significant for the TBI arm – 9% vs. 28% on the BU/CY arm, p=0.006. This study did involve multiple hematologic malignancies and the 40% of participants who had AML had both early and more advanced disease. When a subset analysis of DFS was performed in this study on the AML patients as a group (n=69), there was no significant difference between arms in 3y probability of DFS – 61% on BU/CY vs. 64% on TBI, p=0.37. The AML subset in this study with early disease, would be the subset most comparable to the Blaise study. Ringden reports that this small subset of AML patients treated with BU/CY (n=25) had a 3y probability of DFS of 83% compared to 58% in the 26 patients on the TBI arm (p=0.22). The findings for busulfan in this subset appear superior to the 51 patients treated with busulfan in the Blaise study – 2y DFS of 47%. The authors of the Nordic BMT study concluded the discrepancy in these results between trials, albeit derived from subset analysis, supported the need for additional randomized trials to put this issue to rest.

A subset analysis of early, low risk disease vs. advanced, high risk disease that included all the hematological malignancy subtypes in the Nordic BMT Group study found that those with advanced disease had a significantly lower DFS treated with BU/CY than with TBI (p=0.005). On the busulfan arm 12/47 participants with AML (26%) had advanced disease compared to 6/32 (19%) on the TBI arm. So this analysis was based on small numbers of patients. On multivariate analysis, survival was significantly associated with early leukemia – all types - (p<0.0001) and preparative regimen (TBI superior), p=0.02. An additional imbalance was in distribution of M4/M5 subtypes between arms. The M4/M5 subtypes have been reported to be associated with lower leukemia-free survival in the transplantation setting.[17] There were 10 on the busulfan arm and only 7 on the TBI arm.

Thus, available level I evidence suggests BU/CY is inferior preparative therapy for allogeneic transplantation in AML when compared to CY/TBI. However, this evidence has been criticized in the literature as problematic, as discussed above. Can one establish that the CY/TBI comparator arm is in itself effective conditioning for transplantation in AML? Historically, it was the earliest established preparative regimen and it has been the regimen most commonly used. As discussed above, comparisons of chemotherapy to transplantation after TBI preparative therapy in AML (CR1) have not clearly established its superiority to dose intensive chemotherapy. This hampers any potential attempt at defining possible equivalence of BU/CY with CY/TBI, if one accepted the objections to the validity of the inferior results found in the two pertinent level 1 studies. Despite the presence of the level I evidence that suggests that BU/CY is inferior to CY/TBI in this setting, the volume of uncontrolled phase 2 studies reported in the literature suggests the BU/CY regimen is accepted and commonly used in the allogeneic setting.