

CBER STATISTICAL ANALYSIS OF PRIMARY DATA ON CLINICAL OUTCOMES OF 562 UMBILICAL CORD BLOOD (UCB) TRANSPLANTS

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Introduction

Datasets supplied by the New York Blood Center (NYBC) on 562 recipients of UCB transplants from unrelated donors were analyzed by CBER staff. Results based on this same data have been published earlier (Rubenstein P., Carrier C., Scaradavou A. et al., NEJM, 339: 1565-1577, 1998).

The CBER analysis focused on the association between selected covariates and four clinical outcomes of UCB transplants: the outcomes were neutrophil engraftment, platelet engraftment, acute GVHD [grades III and IV] and disease free survival. The covariates used in the analyses were primarily the age of the recipient, the weight of the UCB recipient, total nucleated cells [TNC] /kg transplanted into the recipient and the number of HLA disparities between donor and recipient.

The purposes of the analyses were to: (1) compare risks (negative clinical outcomes) between very young and adolescents/young adult recipients of UCB transplants; (2) if an increased age-related risk existed, as strongly indicated by published reports, to determine if a gradual cline of increased (with age) or alternatively a sharp breakpoint, characterized the data; (3) distinguish between competing risks; (4) determine if the four clinical endpoints show the same trend.

Methodology of statistical analysis

This is a retrospective analysis of data collected at NYBC from 1992 to 1998. There are limitations on the inferences that can be drawn from the results of retrospective studies as well as the confounding effect of changes over the years in the techniques of transplantation.

Univariate and multivariate analyses identified the covariates which had the highest association with the clinical outcomes. Simple logistic regression was used to analyze the binary outcomes. Time to event analyses utilized Cox regression and Kaplan-Meier analyses. In comparisons, log rank test and Fisher's exact tests were used; all statistical analysis were performed at alpha level of 5%.

Age cohorts as dichotomous groups and as successive/consecutive age groups were assessed to determine if a cutpoint for increase in risk could be identified. Successive age cohorts included recipients within 3 year age brackets except for the last cohort which included recipients ages 21 to 29.

An exploratory approach, CART [classification and regression trees], was applied using the same covariates and clinical outcome endpoints referred to above [Breiman L., Friedman J.H., Olshen R.A and Stone C.J. (1984) , Classification and Regression Trees, Wadsworth International Group, Bemont C.A., Chambers J.M. and Hastie T.J. (1991), Statistical Models in S, pg414]. The tree based modeling applies binary recursive partitioning to construct homogenous subgroups. All statistical analyses were performed with software from JMP (SAS Institute Inc.) and from S-plus (MathSoft, Inc.).

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Covariates

Age is age in years of recipient

Kg is body weight of recipient in kilograms of recipient

TNC is total number of nucleated cells

Abdr are the HLA antigens

Clinical Outcomes

Achieving ANC 500

Time to ANC 500

Achieving Platelet 20,000

Time to platelet 20,000

% Disease free survival are % of patients with disease free survival

% Grade III / IV are % of patients with grade III or IV acute GVHD

Results

Section I. Univariate and multivariate analyses (tables 1, 2 and 3)

Tables 1-3 summarize the significant covariates associated with 3 clinical outcomes (ANC 500, platelet 20,000 and disease-free survival respectively) for all ages and for age 16 years or less. In the univariate analyses, engraftment (achieving ANC 500) correlated significantly with age, weight and TNC/kg for the age cohorts. The covariates were correlated significantly with disease-free survival but were less strongly associated with time of disease free survival. In the multivariate analyses, where complex interactions among covariates were present, the associations of the three covariates(age, weight, and TNC/kg) to clinical outcomes were weaker. Only the level of HLA mismatch remained significantly correlated to disease free survival and successful engraftment of platelets (except for recipients < 16 years).

Table 1. Covariates Identified for ANC 500

	Achieving ANC 500		Time to ANC 500	
	All ages	Age = or < 16 years	All ages	Age = or < 16 years
Univariate model	Significant covariates			
age	Age	Age	Age(marginal)	Age
kg	Kg	Kg	Kg	Kg
tnc	Tnc	Tnc	Tnc	Tnc
abdr	abdr	-	-	-
2 variables in model				
age,kg	kg	-	kg	-
age, tnc	tnc	age (marginal)	tnc	tnc
kg, tnc	Kg	-	kg	tnc
tnc, abdr	tnc	tnc	tnc,abdr	tnc,abdr
age, abdr	age	age	age	age
kg,abdr	kg	kg	kg	kg
3 variables in model				
age, kg, tnc	-	-	tnc	tnc
age,kg, abdr	-	-	kg	-
age,tnc,abdr	tnc	-	tnc,abdr	tnc,abdr
kg, tnc,abdr	-	-	tnc,abdr	tnc, abdr
4 variables in model				
age,kg,tnc,abdr	-	-	tnc,abdr	tnc, abdr
Number of patients	555	445	395	330

tnc = TNC/kg

Table 2. Covariates Identified for Platelet 20,000

	Achieving Platelets 20,00		Time to Platelets 20,000	
	All ages	Age = or < 16 years	All ages	Age = or < 16 years
Univariate model	Significant covariates			
age	age	age	age	age
kg	kg	kg	kg	kg
tnc	tnc	tnc	tnc	tnc
abdr	abdr	abdr	-	-
2 variables in model				
age,kg	-	-	kg	-
age, tnc	age	age	tnc	tnc
kg, tnc	kg	kg	tnc	-
tnc, abdr	tnc,abdr	tnc,abdr	tnc	tnc
age, abdr	age,abdr	age,abdr	age	age
kg,abdr	kg, abdr	kg,abdr	kg	kg
3 variable in model				
age, kg, tnc	-	-	tnc	-
age,kg, abdr	abdr	abdr	kg	-
age,tnc,abdr	age,abdr	age,abdr	tnc	age (marginal)
kg, tnc,abdr	kg,abdr	kg,abdr	tnc	tnc(marginal)
4 variables in model				
age,kg,tnc,abdr	abdr,	abdr	tnc	-
Number of patients	517	410	230	199

Table 3. Covariates Identified for Disease Free Survival

	Achieving Disease Free Survival		Time to Disease Free Survival	
	All ages	Age = or < 16 years	All ages	Age = or < 16 years
Univariate model	Significant covariates			
age	age	age	-	age
kg	kg	kg	-	kg
tnc	tnc	tnc	-	-
abdr	abdr	abdr	-	-
2 variables in model				
age,kg	age	age	-	-
age, tnc	age	age	-	age
kg, tnc	kg	-	-	kg
tnc, abdr	tnc,abdr	tnc,abdr	-	-
age, abdr	age,abdr	age	abdr(marginal)	age
kg,abdr	kg,abdr	kg,abdr	kg,abdr	kg
3 variable in model				
age, kg, tnc	age	-	-	-
age,kg, abdr	age,abdr(marginal)	-	abdr	-
age,tnc,abdr	age,abdr	abdr	abdr	age
kg, tnc,abdr	kg,abdr	tnc(marginal)	abdr	kg
4 variables in model				
age,kg,tnc,abdr	age,abdr	abdr	abdr	-
Number of patients	562	451	197	179

Section II. Clinical Outcomes in 3 Year Age Cohorts

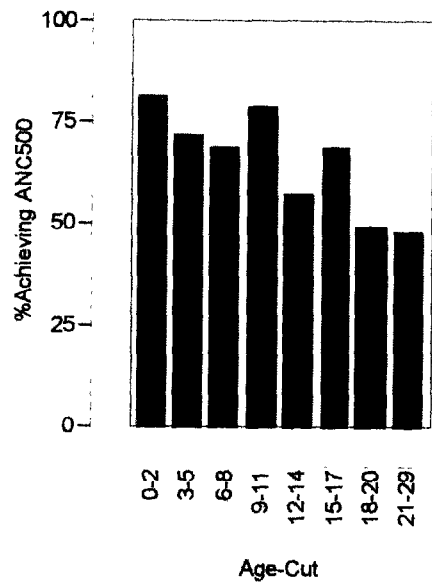
The results for the four clinical outcomes in each of the three-year age cohorts are summarized in Table 4. Graphic representation of the same data is seen in figures 1 through 4 with the numbers of patients in each study group shown below the graph.

Table 4. Clinical Outcomes for Age Cohorts

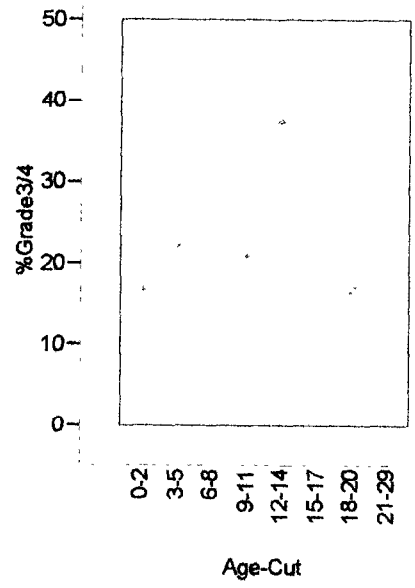
Age Group in years	Achieving ANC 500	Achieving Platelet 2000	% Grade III/IV (aGVHD)	Disease Free Survival
0,1,2	122/148 (82.4%)	80/137 (58.4%)	21/124 (16.9%)	78/151 (51.7%)
3,4,5	63/87 (72.4%)	35/81 (43.2%)	15/68 (22.1%)	32/89 (36.0%)
6,7,8	52/75 (69.3%)	33/69 (47.8%)	12/54 (22.2%)	27/75 (36.0%)
9,10,11	50/63 (79.4%)	34/62 (54.8%)	11/52 (21.2%)	25/64 (39.1%)
12,13,14	26/45 (57.8%)	10/39 (25.6%)	9/24 (37.5%)	11/45 (24.4%)
15,16,17	25/36 (69.4%)	10/35 (28.6%)	6/25 (24.0%)	9/36 (25.0%)
18,19,20	6/12 (50.0%)	4/12 (33.3%)	1/6 (16.7%)	4/12 (33.3%)
21 to 29	18/37 (48.7%)	11/36 (30.6%)	8/20 (40.0%)	5/37 (13.7%)
Total	362/503	217/471	83/373	191/509

aGVHD is acute graft versus Host Disease

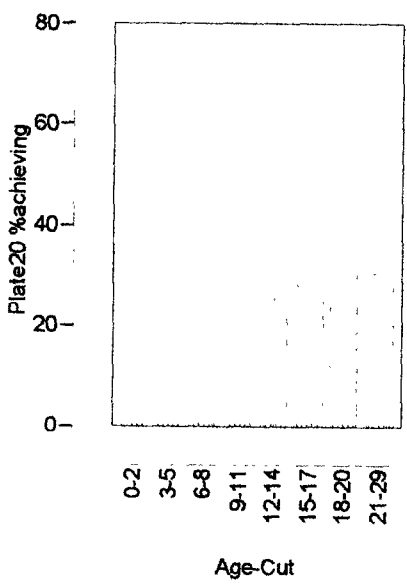
Figures 1-4. Percent of Patients by age cohort for four clinical outcomes



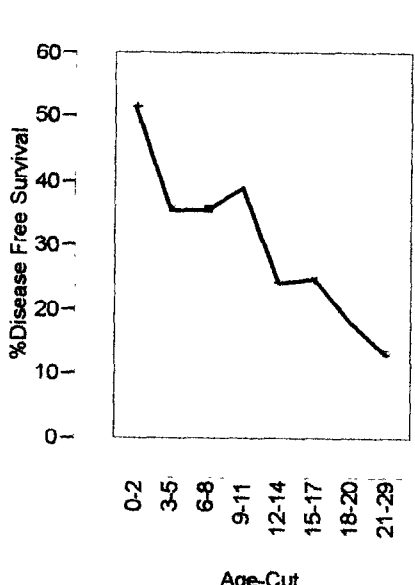
N	148	87	75	6	4	3	1	37
			3	5	6	2		



N	124	6	5	5	2	2	6	2
		8	4	2	4	5	0	0



N	148	87	75	6	4	3	1	37
			3	5	6	2		



N	124	6	5	5	2	2	6	2
		8	4	2	4	5	0	0

Section III. Dichotomous Age Groups-binary data (Table 5)

In order to compare clinical outcomes in younger vs. older recipients using different age cutoffs, five sets of dichotomous age groups (age equal to or < 8 vs > 8 years, age equal to or < 12 vs. >12 years, age equal to or < 16 vs. > 16 years, age equal to or < 18 vs. > 18 years, age equal to or < 22 vs. > 22 years were constructed. Table 5 below summarizes the data . No significant difference was found between the age groups. However the results of time to event analyses (see Section IV) for these 5 sets showed significant differences for the set of age equal to or < than 8 vs. age > 8 years and the set of age equal to or < 12 vs. age > 12 years.

Table 5. Dichotomous Age Groups

	Age ≤8 vs. >8		Age ≤12 vs. >12		Age ≤16 vs. >16		Age ≤18 vs. >18		Age ≤22 vs. >22	
Achieving ANC 500	<u>237</u> 310 (76%)	<u>158</u> 245 (64%)	<u>298</u> 389 (77%)	<u>97</u> 166 (58%)	<u>330</u> 445 (74%)	<u>65</u> 110 (59%)	<u>340</u> 460 (74%)	<u>55</u> 95 (58%)	<u>351</u> 477 (74%)	<u>44</u> 78 (56%)
Achieving platelet 20,000	<u>148</u> 287 (52%)	<u>82</u> 236 (35%)	<u>187</u> 364 (51%)	<u>43</u> 159 (27%)	<u>199</u> 414 (48%)	<u>31</u> 109 (28%)	<u>203</u> 429 (47%)	<u>27</u> 94 (29%)	<u>210</u> 445 (47%)	<u>20</u> 78 (26%)
Achieving platelet 50,000	<u>157</u> 300 (52%)	<u>76</u> 236 (32%)	<u>196</u> 378 (52%)	<u>37</u> 158 (23%)	<u>209</u> 431 (48%)	<u>24</u> 105 (23%)	<u>212</u> 445 (48%)	<u>21</u> 91 (23%)	<u>217</u> 460 (47%)	<u>16</u> 76 (21%)
Disease-free Survival	<u>137</u> 315 (43%)	<u>60</u> 247 (24%)	<u>165</u> 395 (42%)	<u>32</u> 167 (19%)	<u>179</u> 451 (40%)	<u>18</u> 111 (16%)	<u>183</u> 466 (39%)	<u>14</u> 96 (15%)	<u>187</u> 483 (39%)	<u>10</u> 79 (13%)
Acute GVHD										
Grade 0-1	<u>132</u> 246 (54%)	<u>82</u> 161 (51%)	<u>169</u> 308 (55%)	<u>45</u> 99 (45%)	<u>186</u> 339 (55%)	<u>28</u> 68 (41%)	<u>193</u> 349 (55%)	<u>21</u> 58 (36%)	<u>198</u> 361 (55%)	<u>16</u> 46 (35%)
Grade 2	<u>66</u> 246 (27%)	<u>27</u> 161 (17%)	<u>76</u> 308 (25%)	<u>17</u> 99 (17%)	<u>80</u> 339 (24%)	<u>13</u> 68 (19%)	<u>82</u> 349 (24%)	<u>11</u> 58 (19%)	<u>84</u> 361 (24%)	<u>9</u> 46 (20%)
Grade 3-4	<u>48</u> 246 (20%)	<u>52</u> 161 (32%)	<u>63</u> 308 (21%)	<u>37</u> 99 (37%)	<u>73</u> 339 (22%)	<u>27</u> 68 (40%)	<u>74</u> 349 (21%)	<u>26</u> 58 (45%)	<u>79</u> 361 (22%)	<u>21</u> 46 (46%)

Section IV. Dichotomous Age Groups-time to event data (Table 6). The study population was divided into two dichotomous age groups: they were ages 0 to 12 years and ages 13 years or older. The odds ratio for the clinical outcomes and the Kaplan-Meier proportion were analyzed .

Table 6. Mean Number of Days Compare between Dichotomous Age Groups

	Age ≤8 vs. >8 years	Age ≤12 vs. >12 years	Age ≤16 vs. >16 years	Age ≤18 vs. >18 years	Age ≤22 vs. >22 years
Time to Achieving ANC500	24.9 vs. 28.0 Significant difference	25.4 vs. 28.2 Significant difference	26.1 vs. 26.2 NS	26.1 vs. 26.1 NS	26.1 vs. 26.3 NS
Time to Achieving Platelet 20,000	59.0 vs. 74.8 Significant difference	62.0 vs. 75.5 Significant difference	62.9 vs. 74.9 NS	63.6 vs. 71.5 NS	64.6 vs. 64.6 NS
Time to Disease-free Survival	1057 vs. 962 NS	1054 vs. 898 Marginal difference	1031 vs. 1003 NS	1027 vs. 1041 NS	1025 vs. 1096 NS

NS is no significant difference

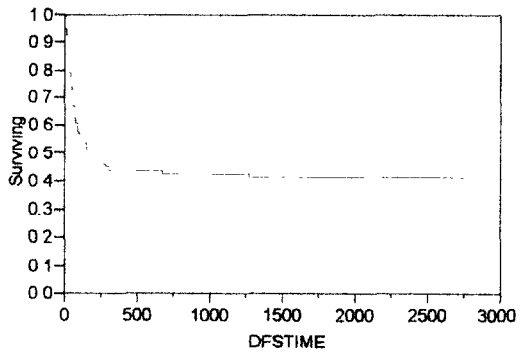
Section V. Disease Free Survival Curves for Consecutive /Successive Age Groups (Figure 5)

Consecutive 3 year age cohorts were compared using Kaplan-Meier analyses (figure 5). The largest difference is between the cohort groups 10 (ages 9, 10,11 years) and 13 (ages 12, 13, and 14 years), & cohort groups groups 16 vs. 19 and cohort groups 1 vs. 4. The 16 vs.19 comparison has the lowest number of study subjects. The difference between the cohort groups 10 and 13 and 1 and 4 were statistically significant.

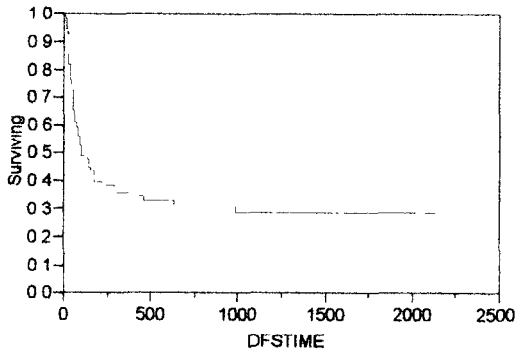
In figure 5 the following groups correspond to the following ages:

<u>Cohort group</u>	<u>Ages in years</u>
1	0,1,2
4	3,4,5
7	6,7,8
10	9,10,11
13	12,13,14
16	15,16,17
19	18,19,20

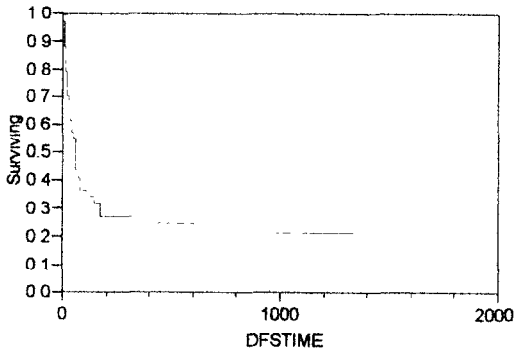
Figure 5. Disease Free Survival Curves for Two Consecutive Age Groups



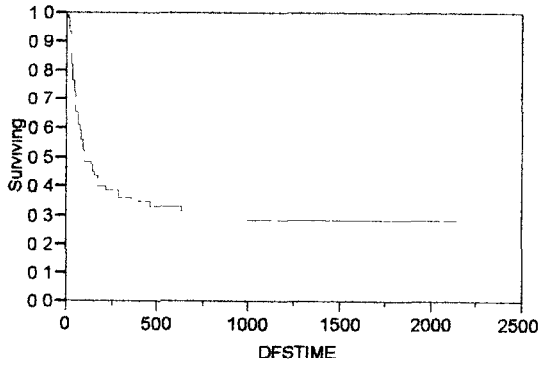
1
4



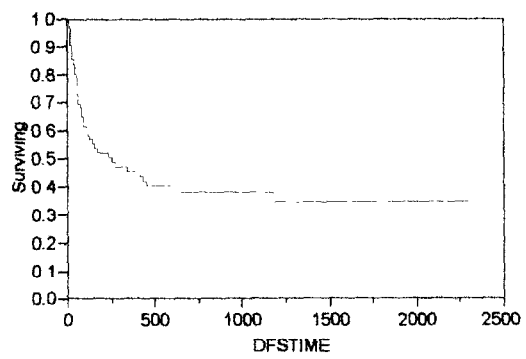
7
10



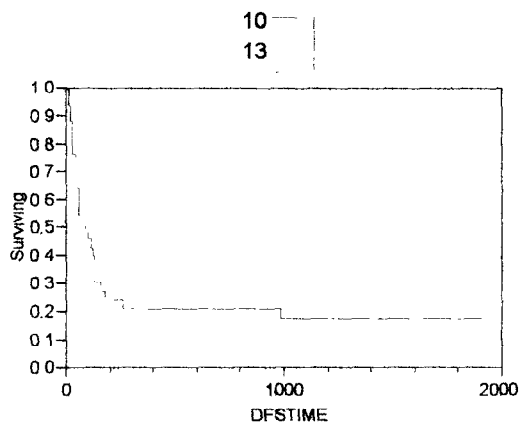
13
16



4
7



10 (red) vs 13 (green on bottom)



16 -
19

Section VI. Odds Ratio and Kaplan-Meier Analysis for DFS in Dichotomous Age Groups. The study population was divided into two dichotomous age groups; they were ages 0 to 12 years and ages 13 years or older. The odds ratio for the clinical outcomes and the Kaplan-Meier proportion were analyzed (table 7). The estimated odds ratios from the data indicate that the children of age 12 or younger presented better clinical outcomes for successful engraftment of ANC and platelets, lower risk of GVHD grade III/IV, and a higher survival rate. Survival curves for the dichotomous cohorts are shown in Figure 6. Kaplan-Meier survival probability of children aged 12 or less is significantly higher than those ages 13-29 ($p < .001$).

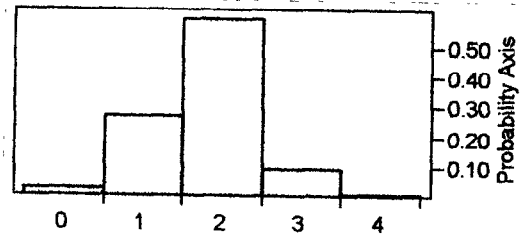
Table 7. Odds Ratios and K-M Proportions			
Prognostic factors	Proportions Ages 0-12	Proportions Ages 13-29	Odds Ratio (Confidence intervals) age (0-12)/age (13-29)
ANC 500	76.6%	56.1%	2.56 (1.61,4.05)
Platelet 20,000	51.4%	28.0%	2.71 (1.66,4.49)
aGVHD grade III/IV	20.5%	30.8%	0.58 (0.31,1.11)
Disease Free Survival	41.8%	22.8%	2.43 (1.47, 4.09)

Age Group above 13

Distributions

ABDRMMHI

(# of HLA-A, -B, -DR mismatches)



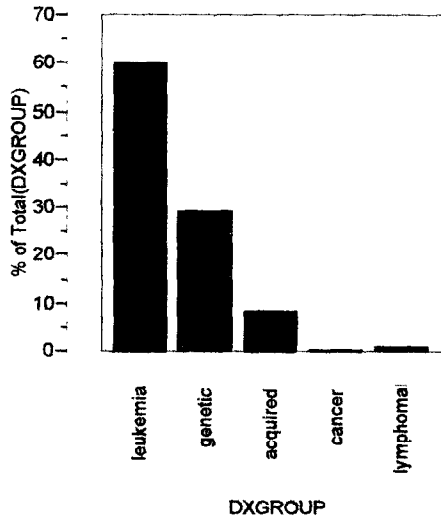
Frequencies

Level	Count	Percent
0	5	3.0%
1	45	27.1%
2	98	59.0%
3	16	9.6%
4	2	1.2%
Total	166	

Fig 6. Distributions of Diagnosis Groups for Two Age Cohorts

Age Group 0-1

Chart

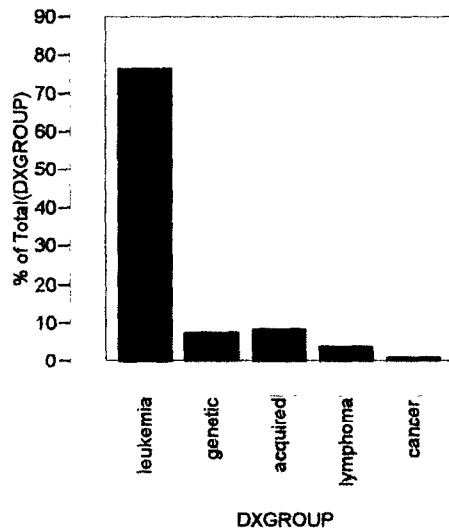


Frequencies

Diganosis	Count	Percent
acquired	34	8.6%
cancer	2	0.5%
genetic	116	29.4%
leukemia	238	60.2%
lymphoma	5	1.3%
Total	395	

Age Group 12 above

Chart



Frequencies

Level	Count	Prob
acquired	15	9.0%
cancer	3	1.8%
genetic	13	7.8%
leukemia	129	77.2%
lymphoma	7	4.2%
Total	167	

VIII. Classification and Regression trees (exploratory analysis)

For each of four outcome endpoints, the results of CART (Classification and Regression analysis) is shown in Tables 9, and 10 and Figures 9 a,b,c,d. Tree partition attempts to construct homogenous subgroups using the maximum reduction in deviance as the basis for the "splits". The variable of first split would be the most important predictor for the classification. Table 9 summarizes the predictors for the first split and the estimated probability of the clinical outcome event for the associated first two subgroups of the first "split".

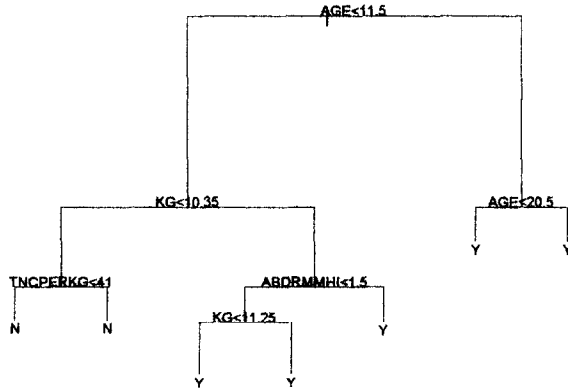
Table 10 only considers age as a variable in the association with clinical outcome endpoints. The age of the first split provides information on the change point for the two subgroups. Based on the results, the important change points on age predictor for the four clinical events are indicated in table 9; they are age 12 for disease free survival and platelet engraftment, age 13 for ANC engraftment and age 21 for GVHD III/IV.

Outcome Variable	Predictors shown in tree partitions	Predictor For the first split	The separated two subgroups with <u>Estimated probability</u> of outcome event
Survival	AGE, KG TNC, HLA	AGE < 12	(N=375) AGE <12, Pr (survival)=57% (N=181) AGE >12, Pr (survival)=81%
ANC 500	TNC, KG AGE	TNC < 45.13	(N=340) TNC<45.13, Pr (achieving ANC 500)=36% (N=211) TNC>45.13, Pr (achieving ANC 500)=17%
Platelet 20,000	TNC, HLA	TNC < 26.93	(N=186) TNC<26.93, Pr (achieving platelet 20,000)=28% (N=333) TNC<26.93, Pr (achieving platelet 20,000)=47%
GVHD3/4	KG, AGE TNC, HLA	KG < 33.25	(N=264) KG < 33.25, Pr (having GVHD3/4)=18% (N=140) KG > 33.25, Pr (having GVHD3/4)=37%

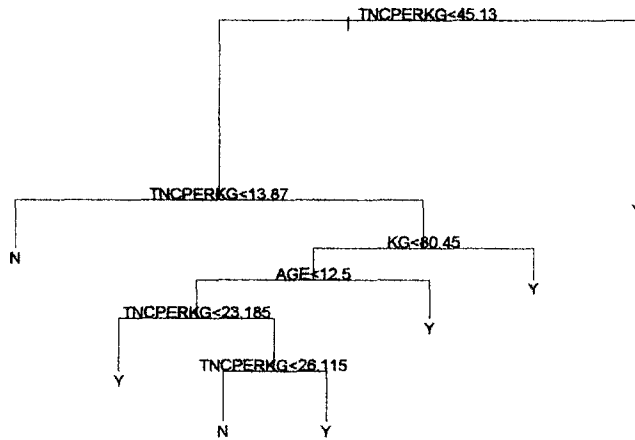
Outcome Variable	Predictor	For the first split	The separated two subgroups with <u>Estimated probability</u> of outcome event
Survival	AGE	AGE < 12	(N=379) AGE <12, Pr(survival)=57% (N=183) AGE >12, Pr(survival)=81%
ANC 500	AGE	AGE < 13	(N=389) AGE < 13, Pr (achieving ANC 500)=23% (N=166) AGE > 13, Pr (achieving ANC 500)=41%
Platelet 20,000	AGE	AGE < 12	(N=349) AGE < 12, Pr (achieving platelet 20,000)=48% (N=174) AGE > 12, Pr (achieving platelet 20,000)=27%
GVHD3/4	AGE	AGE < 21	(N=353) AGE < 21, Pr (having GVHD3/4)=21% (N=54) AGE >21, Pr (having GVHD3/4)=46%

Figure 9. Regression trees for the key clinical outcome endpoints

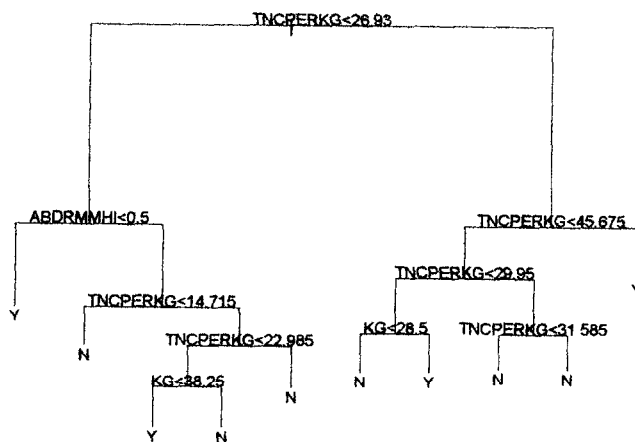
a) Tree partition for disease-free survival



b) Tree partition for ANC engraftment



c) Tree partition for platelet engraftment



d) Tree partition for GVHD III/IV

