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ORIGINAL REPORT

Chemoimmunotherapy With a Modified Hyper-CVAD and Rituximab Regimen Improves Outcome in De Novo Philadelphia Chromosome–Negative Precursor B-Lineage Acute Lymphoblastic Leukemia

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ABSTRACT

Purpose
The adverse prognosis of CD20 expression in adults with de novo precursor B-lineage acute lymphoblastic leukemia (ALL) prompted incorporation of monoclonal antibody therapy with rituximab into the intensive chemotherapy regimen hyper-CVAD (fractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone). Other modifications (irrespective of CD20 expression) included early anthracycline intensification, alterations in number of risk-adapted intrathecal chemotherapy treatments for CNS prophylaxis, additional early and late intensifications, and extension of maintenance phase chemotherapy by 6 months.

Patients and Methods
Two hundred eighty-two adolescents and adults with de novo Philadelphia chromosome (Ph)–negative precursor B-lineage ALL were treated with standard or modified hyper-CVAD regimens. The latter incorporated standard-dose rituximab if CD20 expression ≥ 20%.

Results
The complete remission (CR) rate was 95% with 3-year rates of CR duration (CRD) and survival (OS) of 60% and 50%, respectively. In the younger (age < 60 years) CD20-positive subset, rates of CRD and OS were superior with the modified hyper-CVAD and rituximab regimens compared with standard hyper-CVAD (70% *v* 38%; $P < .001$% and 75% *v* 47%, $P = .003$). In contrast, rates of CRD and OS for CD20-negative counterparts treated with modified versus standard hyper-CVAD regimens were similar (72% *v* 68%, P = not significant [NS] and 64% *v* 65%, P = NS, respectively). Older patients with CD20-positive ALL did not benefit from rituximab-based chemoimmunotherapy (rates of CRD 45% *v* 50%, P = NS and OS 28% *v* 32%, P = NS, respectively), related in part to deaths in CR.

Conclusion
The incorporation of rituximab into the hyper-CVAD regimen appears to improve outcome for younger patients with CD20-positive Ph-negative precursor B-lineage ALL.

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INTRODUCTION

The prognostic relevance of immunophenotypic classification of acute lymphoblastic leukemia (ALL) relates to associations with cytogenetic and molecular aberrancies. While detection of surface antigens (eg, CD19, CD20, CD22, CD33, CD52) on lymphoblasts by flow cytometry (FC) identifies targets for monoclonal antibody (MoAb) therapy, expression of particular antigens may have prognostic implications. CD20 is a B-lineage antigen expressed on normal and malignant cells during nearly all stages of differentiation (except early B-cell precursors or plasma cells). Heterogeneity in CD20 expression among B-cell malignancies has been well-described.[1] It ranges from 40% to 50% in precursor B-lineage ALL compared with 80% to 90% in mature B-cell or Burkitt-type leukemia/lymphoma.

CD20 functions as a calcium channel that influences cell cycle progression and differentiation via downstream signaling pathways, modulating levels of proapoptosis proteins, such as sarco/endoplasmic reticulum Ca^{2+} (SERCA3) and Bax/Bak.[2] Constitutive activation of survival pathways involving nuclear factor-κB and extracellular receptor kinase (ERK1/2) results in overexpression of antiapoptotic

Bcl-2 proteins and associated *Bcl-2* genes.[3] Expression of CD20 likely confers drug resistance via these mechanisms, resulting in persistence of leukemia subclones which eventually re-emerge.

The prognostic significance of CD20 expression in de novo precursor B-lineage ALL was initially evaluated in the pediatric setting with conflicting results. The Pediatric Oncology Group assessed CD20 expression by the traditional 20% cut point and mean fluorescence intensity.[4] CD20 expression and increasing mean fluorescence intensity were independently associated with inferior event-free survival rates irrespective of known prognostic factors such as age and karyotype. In contrast, the St Jude experience suggested that CD20 expression was associated with slightly more favorable prognosis.[5] It was postulated that these disparate results could be accounted for by differences in intensity of regimens and/or application of risk-adapted strategies.

The influence of CD20 expression on outcome for adults with de novo precursor B-lineage ALL was studied in the context of conventional (vincristine, doxorubicin, dexamethasone [VAD][6]) or intensive (fractionated cyclophosphamide plus VAD [hyper-CVAD][7,8]) chemotherapy.[9] Complete remission (CR) rates were similar regardless of CD20 status (positive/negative by 20% cut point). However, CD20 expression was associated with significantly higher relapse rates (61% *v* 37%; $P < .01$) and lower 3-year CR duration (CRD) and survival (OS) rates (22% *v* 58%; $P < .001$ and 27% *v* 60%, $P < .01$, respectively) after hyper-CVAD therapy. These findings were particularly significant for the younger subsets, whereas CRD and OS rates were uniformly poor for the older group (age $\geq$ 60 years). Association of CD20 expression with higher cumulative incidence of relapse was subsequently confirmed in the Group for Research in Adult Acute Lymphoblastic Leukemia (GRAALL) 2003 trial, which applied a pediatric regimen to younger adults with de novo Philadelphia chromosome (Ph) –negative ALL.[10]

Rituximab, a chimerical MoAb directed at surface CD20, induces apoptosis, antibody-dependent cell-mediated cytotoxicity, and complement-mediated cytolysis.[11] Incorporation of rituximab into first-line chemotherapy regimens has significantly improved outcome for subsets of non-Hodgkin's lymphoma such as Burkitt-type leukemia/lymphoma and mantle-cell lymphoma/leukemia.[12-14] The favorable impact of chemoimmunotherapy has even extended to chronic lymphocytic leukemia, where CD20 expression of the malignant clone is lower than normal B lymphocytes.[15,16]

The hyper-CVAD program has proven to be an effective first-line therapy for adults with de novo ALL and lymphoblastic lymphoma (LL).[7,8,17] Modifications to the regimen were implemented in order to improve on the results. Early anthracycline intensification was initially incorporated based on earlier reports suggesting that this therapeutic strategy improved relapse-free survival.[18] Maintenance therapy was extended by 6 months with additional early and late intensifications to avoid relapses in close proximity to completion of therapy. Interventions targeting certain subsets included administration of induction chemotherapy in a protective environment if older to reduce early infection-related mortality; alteration in number of intrathecal chemotherapy treatments (IT) for CNS prophylaxis from four to six if classified as low CNS risk and from 16 to 8 if high CNS risk since prior isolated CNS relapse rates were 6% and 1%, respectively; and incorporation of rituximab for CD20 expression $\geq$ 20%. Herein, we summarize the results of modified hyper-CVAD regimens for de novo Ph-negative precursor B-lineage ALL with emphasis on outcomes by CD20 expression.

PATIENTS AND METHODS

Study Group

The diagnosis of ALL was established according to WHO criteria.[19] Eligibility criteria included age $\geq$ 10 years without other active malignancy with expected consequent death within 12 months or known positivity for HIV 1. Adequate hepatorenal function was required unless attributable to leukemia. Protocols were approved by the institutional review board at M. D. Anderson Cancer Center. Informed consent for participation was obtained in accordance with institutional guidelines and Declaration of Helsinki. Mature B-cell ALL or Burkitt-type leukemia/lymphoma and Ph-positive ALL were treated on separate protocols with details reported elsewhere.[13,20-22] Since October 2006, patients with de novo Ph-negative precursor B-lineage ALL younger than 31 years of age were allocated to first-line therapy with an augmented Berlin-Frankfurt-Münster regimen modeled after an established pediatric program.[23]

Therapy

Comparative details of the standard and modified hyper-CVAD 1 or 2 regimens are delineated in Table 1 and online-only Appendix.[7,13,17,24] Treatment included eight or nine induction-consolidation courses of hyper-CVAD, liposomal daunorubicin with cytarabine (only if anthracycline intensification [modified hyper-CVAD 1[25]]), and high-dose methotrexate with cytarabine. Intensive cycles were administered every 21 days or earlier (at least 14 days apart) on recovery (absolute neutrophil count [ANC] $\geq 1 \times 10^9$/L after granulocyte colony-stimulating factor discontinued for $\geq$ 24 hours and untransfused platelet [PLT] count 50-60 $\times 10^9$/L). If marrow lymphoblasts were CD20-positive ($\geq$ 20%), rituximab 375 mg/m^2 was given on days 1 and 11 of hyper-CVAD cycles and on days 1 and 8 of liposomal daunorubicin and cytarabine or high-dose methotrexate and cytabarine cycles, for eight total doses over the first four courses. Rituximab was given with early and late hyper-CVAD intensifications during months 6 and 18 of maintenance therapy.

Methods

FC was performed on diagnostic bone marrow aspirates (BMA) to establish lineage and CD20 expression as previously described.[9] BMA specimens collected at the time of morphological CR (approximately day 21 of induction therapy) were assessed for minimal residual disease (MRD) when feasible (modified hyper-CVAD 2 cohort). BMA cells were stained with four-color antibody panel (multiparameter FC [MFC], sensitivity $< 10^{-4}$) including markers for CD9, CD10, CD13, CD15, CD19, CD20, CD22, CD33, CD34, CD38, CD58, CD66c, and cytoplasmic-terminal deoxynucleotide transferase by methodology previously described (Appendix).[26,27] Total DNA was extracted from BMA samples using automated methods (Autopure; Genta, Minneapolis, MN). B-cell clonality was determined using nonquantitative polymerase chain reaction (PCR) method with V consensus primers (cPCR) derived from framework 1 (FR1), framework 2 (FR2), and framework 3 (FR3) regions, in combination with mixture of fluorescently labeled J primers; sensitivity ranged from 10^{-2} to 10^{-4} depending on number of polyclonal B-cells.[28]

Response Criteria

CR was defined as $\leq$ 5% blasts in normocellular or hypercellular marrow with ANC $\geq 1 \times 10^9$/L, PLT $\geq 100 \times 10^9$/L, and resolution of extramedullary disease. CR with incomplete PLT recovery included CR criteria except for incomplete PLT recovery. Other outcomes were induction death if occurred after start of therapy without meeting definitions of CR or resistant disease, and resistant disease if survived the induction period but leukemia persisted. Relapse was defined as disease recurrence at any site after achievement of CR. Toxicity was graded according to National Cancer Institute Common Toxicity Criteria (version 3.0).

Statistical Considerations

The end points of these sequential prospective, open label, single-center, phase II trials of hyper-CVAD and its variants were response and CRD. OS was measured from initiation of therapy until death. CRD was measured from CR until relapse. Differences in CR rates or pretreatment characteristics among

Table 1. Hyper-CVAD and Modified Hyper-CVAD Chemoimmunotherapy Regimens

Regimen	Modified Hyper-CVAD 1 and 2 (± rituximab)		Standard Hyper-CVAD (1992-1999)
	2: Without Intensification (2001-present)	1: With Intensification (2000-2001)	
Induction			
Hyper-CVAD	Y	Y	Y
Laminar air flow rooms if age ≥ 60 years	Y	Y	N
Rituximab 375 mg/m^2 IV days 1, 11 if CD20 ≥ 20%	Y	Y	N
Consolidation			
Cycle 2 (anthracycline intensification)			
LDNR 150 mg/m^2 IV over 12 h days 1-2	N	Y	N
Cytarabine 1.5 g/m^2 CI IV daily days 1-2			
Prednisone 200 mg PO days 1-5			
Cycles 2, 4, 6, 8 or cycles 3, 5, 7, 9			
MTX 200 mg/m^2 IV over 2 h, then 800 mg/m^2 IV over 22 h day 1	Y	Y	Y
Cytarabine 3 g/m^2 (1 g/m^2 if age ≥ 60) IV over 2 h every 12 h × 4 doses on days 2-3			
Solu-Medrol 50 mg IV every 12 h × 6 doses on days 1-3			
Leucovorin 50 mg IV 12 h after end MTX then 15 mg IV every 6 h × 8 doses or until MTX level < 0.1 μmol/L			
Acetazolamide if urine pH < 7			
Cycles 1, 3, 5, 7 or cycles 1, 4, 6, 8			
Cyclophosphamide 300 mg/m^2 IV over 2 h every 12 h × 6 doses on days 1-3	Y	Y	Y
Mesna 600 mg/m^2 CI IV daily days 1-3			
Dexamethasone 40 mg IV or PO days 1-4, 11-14			
Doxorubicin 50 mg/m^2 CI IV over 2-24 h day 4 (48 h if EF < 50%)			
VCR 2 mg IV days 1,11			
Cycles 1-4			
If CD20 ≥ 20%: 8 doses rituximab 375 mg/m^2 IV	Y	Y	N
Days 1, 11 (hyper-CVAD)			
Days 1, 8 (LDNR- or MTX-cytarabine)			
CNS prophylaxis			
MTX 12 mg (6 mg if Ommaya) day 2	Y		Y
Cytarabine 100 mg day 7 or 8			
No. of ITs			
Liposomal cytarabine in modified hyper-CVAD 2 (n = 32)[24]	Y		N
Risk adapted (LDH ≥ 1,400 U/L, S + G_2M ≥ 14%)			
High (one elevated)	8		16
Indeterminate (one unknown)	8		8
Low	6		4
Maintenance			
Oral POMP (6-mercaptopurine, VCR, MTX, prednisone)	Months 1-5, 8-17, 20-30		Months 1-6, 8-10, 12-24
Intensification			
Hyper-CVAD (plus rituximab 375 mg/m^2 IV days 1, 11 if CD20 ≥ 20%)	Months 6, 18		N
Intensification†			
MTX 100 mg/m^2 IV day 1 weekly × 4	Months 7, 19		Months 7, 11
L-asparaginase 20,000 units IV day 2 weekly × 4			
Supportive care			
IV/oral alkalinzation all courses; rasburicase/allopurinol for induction			
G-CSF 10 μg/kg subcutaneously daily until ANC > 10^9/L; pegfilgastrim 6 mg subcutaneously could be substituted after 2007			
Duration of doxorubicin infusions increased for modified hyper-CVAD regimens for cardioprotection			
Leucovorin rescue: 50-100 mg IV every 4-6 h if MTX levels were elevated at the end of infusion [0 h, confirmed on repeat sample] to greater than 20 μmol/L, > 1 μmol/L at 24 h, or > 0.1 μmol/L at 48 h			

Abbreviations: Hyper-CVAD, fractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone; Modified hyper-CVAD 1, anthracycline intensification with or without rituximab; Modified hyper-CVAD 2, no anthracycline intensification with or without rituximab; Y, yes; N, no; IV, intravenous; LDNR, liposomal daunorubicin; h, hour; CI, continuous infusion; PO, by mouth; MTX, methotrexate; EF, ejection fraction; VCR, vincristine; IT, intrathecal treatments; LDH, lactate dehydrogenase; S + G_2M, proliferative index; POMP, 6-mercaptopurine, VCR, MTX, and prednisone; G-CSF, granulocyte colony-stimulating factor; ANC, absolute neutrophil count.
*Refer to online-only Appendix for further details including guidelines for dosing modifications.
†For standard hyper-CVAD[17] prior to July 2000 etoposide 100 mg/m^2 IV days 1-5, pegylated asparaginase 2,500 U/m^2 day 1 months 9, 12.

Table 2. Characteristics of Adolescents and Adults With De Novo Philadelphia Chromosome–Negative Precursor B-Lineage ALL (n = 282)

	Modified Hyper-CVAD ± Rituximab											
	2: Without Intensification (n = 126)				1: With Intensification* (n = 47)				Hyper-CVAD Without Rituximab (n = 109)			
	CD20 Negative		CD20 Positive		CD20 Negative		CD20 Positive		CD20 Negative		CD20 Positive	
Characteristic	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
No.	53		73		23		24		56		53	
Age, years												
≤ 30	13	25	25	34	8	35	6	25	25	45	19	36
31-59	30	57	29	40	9	39	9	38	26	46	25	47
≥ 60	10	19	19	26	6	26	9	38	5	9	9	17
Median	42		47		40		53		32		43	
Range	15-75		15-79		18-80		19-83		13-70		16-78	
Sex												
Female	31	49	22	35	10	43	14	58	21	38	17	32
Zubrod performance status												
0-1	48	91	60	82	19	83	15	63	44	79	33	62
2-3	5	9	13	18	4	17	9	38	12	21	20	38
Leukocyte count, $\times 10^9$/L												
< 5	26	49	42	58	11	48	18	75	20	36	25	47
5-29.9	17	32	22	30	4	17	4	17	24	43	20	38
≥ 30	10	19	9	12	8	35	2	8	12	21	8	15
Hemoglobin, g/dL												
< 10	36	68	62	85	19	83	20	83	41	73	41	77
Platelet count, $\times 10^9$/L												
< 100	35	66	64	88	15	65	18	75	40	71	42	79
β-2 microglobulin, mg/dL												
≥ 3	17/47	36	35/61	57	12/23	52	8/21	38	20/45	44	12/35	34
Lactate dehydrogenase, U/L												
≥ 1,400	25	47	32	44	7	30	6	25	29	52	22	42
% peripheral blasts												
Any	41	77	61	84	16	70	19	79	53	95	45	85
Karyotype (except IM; n = 50;58;21;23;48;45)												
Diploid	22	44	30	52	12	54	12	52	18	38	19	42
Hyperdiploid	9	18	5	9	—		5	22	4	8	6	13
Hypodiploid	1	2	5	9	2	9	—		2	4	4	9
Translocation/other	18	36	18	31	7	33	6	26	24	50	16	36
CNS leukemia												
Yes	3	6	9	12	—		1	4	1	2	3	6
Splenomegaly												
Yes	9	17	15	21	2	9	3	12	11	20	18	34
Lymphadenopathy												
Yes	12	23	21	29	8	35	5	21	18	32	14	26
Hepatomegaly												
Yes	4	7	10	14	1	4	2	8	6	11	8	15
CNS relapse risk (n = 56;60;23;24;44;45)												
Low	17	30	24	40	6	26	5	21	12	27	18	40
Indeterminate	6	10	6	10	7	30	12	50	1	2	1	2
High	33	59	30	50	10	43	7	29	31	70	26	58
Systemic risk classification												
Low	13	25	26	36	2	9	6	25	18	32	16	30
High	40	75	46	64	21	91	18	75	38	68	37	70

NOTE. Includes precursor B-cell lineage ALL with data for CD20 expression (missing data for 31 cases treated with hyper-CVAD).

Abbreviations: ALL, acute lymphoblastic leukemia; Modified hyper-CVAD 1, anthracycline intensification with or without rituximab; Modified hyper-CVAD 2, no anthracycline intensification with or without rituximab; Hyper-CVAD, fractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone; Negative, < 20% CD20 expression; Positive, ≥ 20% CD20 expression; IM, insufficient metaphases; CNS relapse risk (does not include CNS disease): low, proliferative index (S + G_2M) < 14% and LDH ≤ 1,400 U/L; High, S + G_2M ≥ 14% or LDH ≥ 1,400 U/L; Indeterminate, value unknown; Systemic risk classification: high risk, WBC > 5 × 10^9/L, > 1 course to CR, Philadelphia chromosome or CNS disease; Low risk, none of these features.

*Anthracycline intensification with cycle 2 of liposomal daunorubicin and cytarabine.

Table 3. Outcome for Adults With De Novo Philadelphia Chromosome–Negative Precursor B-Lineage Acute Lymphoblastic Leukemia by Pretreatment Characteristics (n = 282)

Characteristic	No.	%	% CR	P (χ^2)	% CRD			% OS		
					3 Year	5 Year	P	3 Year	5 Year	P (χ^2)
Age, years										
Overall	282		95	—	60	50	—	58	50	—
≤ 30	96	34	99	.02	63	50	NS	70	64	< .001
31-59	128	45	98		66	55		60	50	
≥ 60	58	21	88		53	47		29	21	
Sex										
Male	282	57	96	NS	57	60	.1	57	48	NS
Female	120	43	96		69	45		57	50	
Performance status										
0-1	219	78	96	NS	65	55	.04	60	50	NS
2-3	63	22	97		50	40		50	44	
Leukocyte count, $\times 10^9$/L										
< 5	142	50	96	NS	65	54	< .001	60	49	< .01
5-29.9	91	32	98		65	54		64	54	
≥ 30	49	17	94		47	44		42	35	
Hemoglobin, g/dL										
< 10	219	78	97	NS	60	62	NS	55	45	NS
≥ 10	63	22	94		67	48		59	55	
Platelet count, $\times 10^9$/L										
< 100	214	76	95	NS	60	48	.03	53	43	< .01
≥ 100	68	24	98		68	61		69	67	
Serum creatinine, mg/dL										
< 1.3	252	89	97	< .01	64	54	.06	60	56	< .01
≥ 1.3	30	11	87		51	35		43	25	
Serum bilirubin, mg/dL										
< 1.3	254	91	96	NS	64	50	NS	59	50	NS
≥ 1.3	27	10	93		45	45		46	41	
Serum albumin, g/dL										
< 3	232	83	94	NS	42	39	.01	45	40	.04
≥ 3	49	17	97		65	53		60	50	
β–2 microglobulin, mg/dL (n = 232)										
< 3	128	55	98	NS	70	55	NS	65	55	.05
≥ 3	104	45	95		53	55		52	46	
Lactate dehydrogenase, U/L										
< 1,400	161	57	95	NS	60	48	NS	57	50	NS
≥ 1,400	121	43	98		60	52		57	48	
Systemic risk classification										
Low	81	29	99	NS	64	52	NS	72	65	< .001
High	200	71	95		63	50		51	43	
Karyotype (n = 275)										
Diploid	113	40	96	NS	68	55	NS	60	50	.06
Hyperdiploid	29	10	97		75	75		65	65	
Hypodiploid	14	5	100		48	39		55	42	
t(1;19)	8	3	100		85	68		62	62	
del 9p	13	5	92		67	45		69	61	
t(4;11), t(11;19), 11q	18	6	94		14	14		20	10	
Other	44	16	93		64	56		59	48	
Insufficient metaphases	36	13	94		49	39		50	42	
CD20 expression										
Positive	150	53	94	NS	53	48	.002*	60	52	NS
Without rituximab	55		93		40	40		45	39	
With rituximab	95		96		67	53		61	49	
Negative	132	47	96		69	52		54	46	
Myeloid marker expression (n = 269)										
Positive	125	46	94	NS	64	50	NS	55	45	NS
Negative	144	54	97		53	50		57	52	
Regimen										
Hyper-CVAD	109	39	96	NS	53	46	< .01	55	49	NS
Modified hyper-CVAD 1	47	17	99		54	44		55	44	
Modified hyper-CVAD 2	126	45	94		78	56		60	50	

Abbreviations: CRD, complete remission duration; OS, overall survival; CR, complete remission; NS, not significant; Systemic risk classification: High risk, WBC > 5×10^9/L, > 1 course to CR, Philadelphia chromosome or CNS disease; Low risk, none of these features; Hyper-CVAD, fractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone; Modified hyper-CVAD 1, anthracycline intensification with or without rituximab; Modified hyper-CVAD 2, no anthracycline intensification with or without rituximab.

*P value represents comparison with or without rituximab within the CD20-positive group. No differences in rates of CR, CRD, or OS by French-American-British subtype; presence or absence of peripheral blasts; CNS disease at presentation; CNS risk classification; or presence or absence of lymphadenopathy, splenomegaly, and/or hepatomegaly.

subgroups were analyzed by χ^2 or Fisher's exact tests. Unadjusted CRD and OS analyses were performed using Kaplan-Meier plots[29] with differences among them analyzed by the log-rank test.[30] Goodness-of-fit was assessed by Martingale residual plots.[31] The Cox proportional hazards model[32] was used to assess treatment and characteristics predicting CR, CRD, and OS. Factors significant for CR, CRD, and OS outcomes by univariate analysis were analyzed further by stepwise regression using assumption of proportional hazards as suggested by Cox.[32]

RESULTS

Study Group

From July 1992 to August 2009, 282 adolescents and adults with de novo Ph-negative precursor B-lineage ALL were treated with standard (n = 109) or modified hyper-CVAD regimens (with [n = 47] or without [n = 126] anthracycline intensification; Table 2). Median age was 41 years (range, 13 to 83); 21% were 60 years or older and 57% were males. There were no significant differences in distribution among treatment categories by CD20 status (positive or negative), except for higher incidence of CD20 expression in the older group.

Response

The overall CR rate was 95% (Table 2). Lower CR rates were observed in the older group (88% *v* 97%; $P = .02$, with failures equally divided between resistant disease and induction death). CR rates by pretreatment characteristics are provided in Table 3. There were no significant differences in CR rates by regimen or CD20 expression. Assessments of MRD were performed on BMAs collected at the time of CR in 93 of 126 patients (74%) treated with modified hyper-CVAD 2. The incidence of MRD-negativity by MFC (< 0.01%) was higher (81% *v* 58%; $P = .02$) for the CD20-positive subset (n = 57) treated

Table 4. Outcome With Hyper-CVAD and Modified Hyper-CVAD Regimens in De Novo Philadelphia Chromosome–Negative Precursor B-Lineage Acute Lymphoblastic Leukemia by Therapy and CD20 Status

Characteristic	Modified Hyper-CVAD ± Rituximab								Hyper-CVAD Without Rituximab (n = 109)			
	2: Without Intensification (n = 126)				1: With Intensification (n = 47)							
	CD20 Negative		CD20 Positive		CD20 Negative		CD20 Positive		CD20 Negative		CD20 Positive	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
No.	53		73		23		24		56		53	
Response, overall												
CR	50	94	67	92	22	96	24	100	55	98	50	94
CRp	1	2	1	1	1	4	—		—		—	
NR	1	2	2	3	—		—		1		—	
ED	1	2	3	4	—		—		—		3	6
Follow-up, months												
Median	41				90				132			
Range	4-81+				24-106+				6-200+			
Relapse rate	10	19	20	28	12	52	11	46	31	56	30	60
Time to relapse, months												
Median	30		27		10		25		30		11	
Range	4-72		2-75		2-47		7-38		5-140		2-124	
Deaths in CR												
Overall	4	8	13	18	4	17	3	13	5	9	2	4
No. age ≥ 60 years	0		9		4		3		2		1	
% 3-year CRD												
Overall by therapy	78				53				54			
% 3-year CRD												
Overall	84		73		50		54		68		37	
Age, years												
≤ 30	84		75		62*		40*		75		26	
31-59	89		75		45*		67*		62		48	
≥60	71		65		0*		45*		50*		50*	
% 3-year OS												
Overall by therapy	60				55				55			
% 3-year OS												
Overall	63		57		44		65		65		45	
Age, years												
≤ 30	77		75		63*		80*		84		47	
31-59	56		66		55*		78*		58		48	
≥ 60	60*		15		0*		45*		20*		34*	

Abbreviations: Hyper-CVAD, fractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone; Modified hyper-CVAD 1, anthracycline intensification with or without rituximab; Modified hyper-CVAD 2, no anthracycline intensification with or without rituximab; CR, complete remission; CRp, CR with incomplete platelet recovery; NR, no response; ED, death during induction; CRD, CR duration; OS, overall survival.
*Ten or fewer patients in cohort.

with rituximab compared with the CD20-negative group (n = 36). The respective rates for MRD-negativity by cPCR for immunoglobulin heavy chain gene rearrangements in 91 evaluable patients were 85% versus 70% ($P = .1$). Discordance between MFC and cPCR was observed in 21 of 81 BMAs with concurrent assessments (5 of 56 [9%] MRD-negative by MFC were positive by cPCR whereas 16 of 25 [64%] MRD-positive by MFC were negative by cPCR).

Remission Duration and Survival Outcomes

With a median follow-up of 64 months (range, 4 to 200), the overall 3-year CRD and OS rates were 60% and 58%, respectively (Table 3). Favorable predictors of longer CRD included better performance status, lower WBC, higher PLT, and higher serum albumin before initiation of therapy. Worse OS rates were noted for older age, higher WBC, lower PLT, elevated serum creatinine levels, lower albumin levels, elevated β-2 microglobulin levels, and high systemic risk classification. Multivariable analysis for OS identified younger age, lower WBC, higher PLT, and therapy with rituximab as independent predictors of favorable outcome.

Treatment with modified hyper-CVAD 2 was associated with improvement in 3-year CRD rates compared with modified hyper-CVAD 1 or hyper-CVAD (78%, 54%, and 53%, respectively; $P < .01$). When analyzing the entire CD20-positive precursor B-lineage ALL group, incorporation of rituximab into the modified hyper-CVAD regimens was associated with significant improvement in 3-year CRD rates (67% *v* 40%; $P = .002$), but not OS rates (61% *v* 45%; $P =$ not significant [NS]). Relapse rates declined from 60% to 37% ($P = .003$; Table 4). In the CD20-positive group treated with modified hyper-CVAD 2 (n = 57), absence of detectable MRD by MFC at the time of CR was associated with significantly better 3-year CRD rates (82% *v* 24%; $P = .002$) but not OS rates (70% *v* 27%; $P =$ NS), in part related to deaths in CR (n = 10) for the MRD-negative group.

Since our prior analysis established the lack of prognostic significance of CD20 expression in the older group,[9] a subset analysis excluding these patients was conducted to assess influence of rituximab therapy in the younger CD20-positive subsets (Table 4; Figs 1A, 1B, 1C). Significant improvements in 3-year CRD (70% *v* 38%; $P < .001$; Fig 1A) and OS (75% *v* 47%; $P = .003$; Fig 1B) rates favoring rituximab

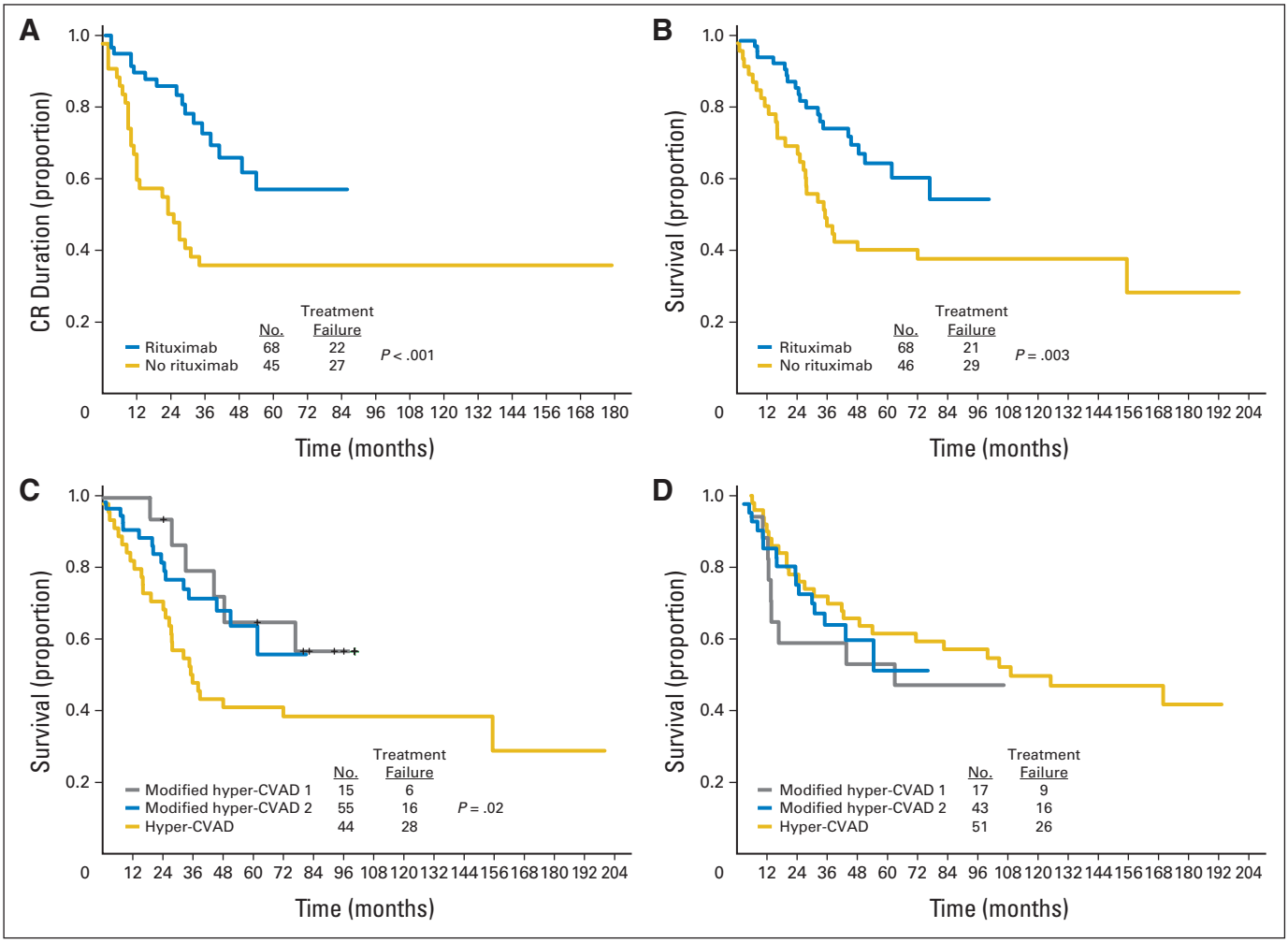

Fig 1. Outcomes by therapy for younger patients (age younger than 60 years) with Philadelphia chromosome–negative precursor B-lineage acute lymphoblastic leukemia. In the CD20-positive subset, (A) complete remission (CR) duration and (B) survival by inclusion or exclusion of rituximab therapy, and (C) survival by hyper-CVAD (fractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone) regimen (standard, modified hyper-CVAD 1 with rituximab inclusive of anthracycline intensification, or modified hyper-CVAD 2 with rituximab eliminating anthracycline intensification) are depicted. (D) Survival by regimen (without rituximab) for the CD20-negative group is also depicted.

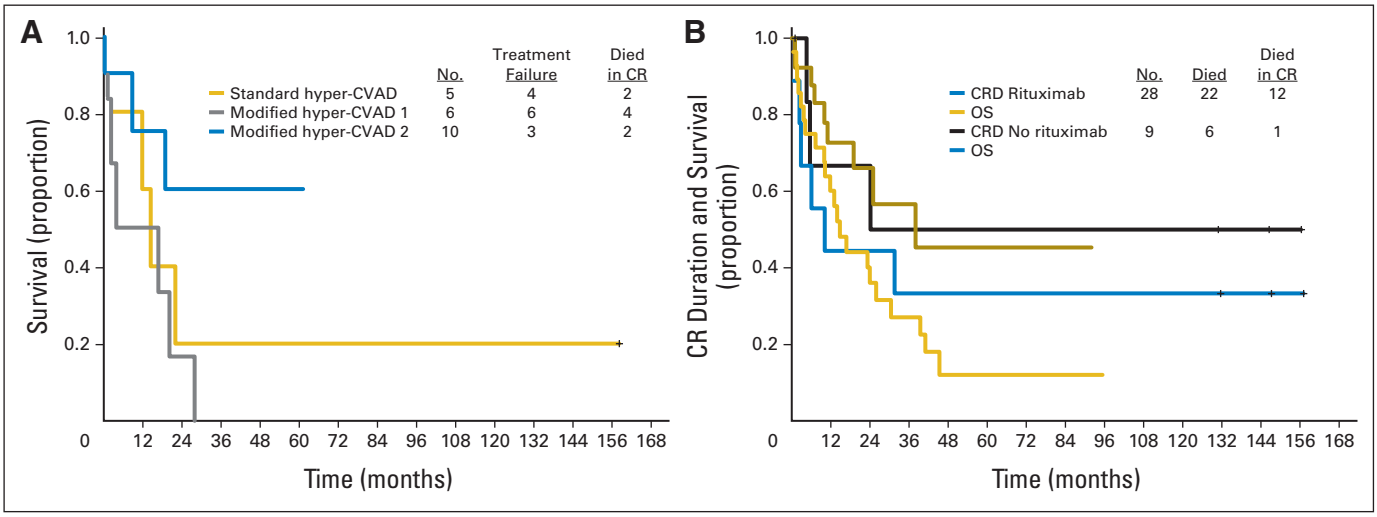

Fig 2. Outcomes by therapy for older patients (age 60 years or older) with Philadelphia chromosome–negative precursor B-lineage acute lymphoblastic leukemia. (A) In the CD20-negative subset, survival by regimen is depicted. (B) In the CD20 positive subset, complete remission (CR) duration (CRD) and survival by rituximab therapy is depicted. OS, overall survival.

were observed. The outcomes were either similar or superior to those for their CD20-negative counterparts within the age subcategories (Table 4). There were no significant differences in rates of OS by regimen for younger CD20-negative precursor B-lineage ALL (Fig 1D), in contrast to better OS rates for modified hyper-CVAD and rituximab regimens within the CD20-positive group (Fig 1C). Notably there was no benefit to anthracycline intensification (modified hyper-CVAD 1[24]), rather there appeared to be an inferior outcome, particularly in the CD20-negative elderly group (Table 4; Fig 2A).

Outcome in Elderly Subset

The overall CR rate for 58 patients age 60 years or older was 88%; 3-year CRD and OS rates were 53% and 29%, respectively (Table 4; Appendix Table A1, online only). Eighteen (62%) of 29 older patients treated with modified hyper-CVAD 2 were assessed for MRD by MFC at the time of CR. Patients in the CD20-positive subset treated with rituximab had a higher rate of MRD-negativity than their CD20-negative counterparts (11 of 12 [92%] *v* three of six [50%]; $P = .05$). However, this did not translate into improved CRD or OS outcomes compared with the older CD20-positive subgroup treated with standard hyper-CVAD, in part related to deaths in CR (Appendix Table A1; Fig 2B; Appendix).

Treatment Delivery and Toxicity

Toxicity profiles of the chemoimmunotherapy regimens were similar to previous reports detailing modified hyper-CVAD 1 for LL and hyper-CVAD and rituximab for Burkitt-type leukemia/lymphoma.[8,13,17,25] There was no difference in time to CR with the addition of rituximab (median 23 *v* 21 days). There was no increase in induction mortality for the CD20-positive subset treated with rituximab, rather, overall early mortality rates in older patients treated in the protective environment declined from 11% to 2% (P = NS).

Treatment realization for the intensive phase of chemotherapy was reviewed for 121 (96%) of 126 patients treated with modified hyper-CVAD 2. Seventy-eight of 121 patients (64%) completed eight cycles of chemotherapy within a median time (start of induction to start of cycle 8) of 5.8 months (range, 4.4 to 10 months). Nineteen patients (16%) received four or fewer cycles; 12 (63%) were CD20-positive and 10 (53%) were 60 years or older. Reasons for early discontinuation of intensive chemotherapy (after four cycles or less in 19, after five cycles in eight, after six cycles in 13, after seven cycles in three patients) included relapse (n = 9), deaths in CR (n = 7; 6 were CD20-positive), or infectious-related toxicity (n = 2). Five patients underwent allogeneic stem-cell transplantation (SCT) in first CR; four for t(4;11)(q21;q23) karyotype and one for persistent MRD. An additional 20 patients were transitioned to 6-mercaptopurine, vincristine, methotrexate, and prednisone maintenance phase chemotherapy early owing to recurrent life-threatening infections (n = 13), persistent cytopenias (n = 2), CNS toxicity (n = 2, ventriculoperitoneal shunt; cerebrovascular accident), and myocardial infarction (n = 1).

Despite similar toxicity profiles and recovery times from myelosuppression (data not shown), the number of deaths in CR was higher for the CD20-positive subset treated with modified hyper-CVAD 2, predominantly related to infections with multidrug resistant organisms in the older group (n = 6) during consolidation chemotherapy, before implementation of rotating antibacterial antibiotic prophylaxis (online-only Appendix). Other causes of death in this subgroup included complications related to secondary myelodysplastic syndrome (n = 2), cardiovascular events (n = 2), or seizure-related anoxic encephalopathy (n = 1); two others were not on active therapy at the time of their demise (Appendix Table A1).

DISCUSSION

The hyper-CVAD regimen, modeled after a pediatric treatment designed for childhood Burkitt lymphoma, has proven to be an effective program for de novo ALL and LL.[7,8,17,33] Further improvements in outcome have been observed with incorporation of targeted agents such as tyrosine kinase inhibitors (eg, imatinib) for Ph-positive ALL and MoAb therapy (eg, rituximab) for Burkitt-type leukemia/lymphoma.[13,20]

The results of our study suggest that the addition of rituximab to hyper-CVAD-based regimens for CD20-positive precursor B-lineage ALL significantly improved 3-year rates of CRD (70% *v* 38%;

$P < .001$) and OS (75% *v* 47%; $P = .003$). In striking contrast to the Burkitt-type leukemia/lymphoma experience,[13] where outcome was significantly improved for the older subset, the benefit of rituximab in precursor B-lineage ALL appears to be confined to the younger group (3-year rates for CRD 45% *v* 50%; OS 28% *v* 32% if 60 years or older). These results are not inconsistent with our previous findings that outcome was uniformly poor in the elderly subset treated with hyper-CVAD in the prerituximab era, regardless of CD20 expression. It is plausible that alternative dosing schemas using rituximab in conjunction with lower intensity therapy in the postinduction phase (to avoid infectious complications of myelosuppression) may yield better survival outcomes for this older group.

Although these prospective analyses are comparative in nature (ie, nonrandomized), the absence of significant differences in outcome among the standard or modified hyper-CVAD regimens in the contemporaneously treated CD20-negative precursor B-lineage ALL subset suggests that other factors such as improvements in supportive care cannot account for these findings.

Preliminary results of other regimens incorporating rituximab for de novo CD20-positive precursor B-lineage ALL have been presented. In 26 elderly patients (> 55 years) treated with chemoimmunotherapy (eight applications of standard-dose rituximab) according to the German Multicenter Study Group for Adult ALL (GMALL) 2002 protocol, the CR rate was 63% with 1-year OS rate of 54%.[34] Younger patients were treated per GMALL 07/2003 according to risk group. If high-risk (HR; WBC $> 30 \times 10^9$/L or late CR), standard-dose rituximab was administered with three phases of induction (I, II) and first consolidation chemotherapy followed by allogeneic SCT. Patients with standard risk (SR) received eight treatments with standard-dose rituximab before induction (phase I, II), reinduction, and six consolidation chemotherapy cycles. Preliminary results were presented for 185 patients (133 SR; 52 HR) with CD20-positive precursor B-lineage ALL; 117 (63%) received rituximab and were compared with 70 treated with the same chemotherapy regimen without rituximab.[35] In the SR subset, addition of rituximab improved 3-year CRD and OS rates (64% *v* 48%, $P = .009$ and 75% *v* 54%, $P =$ not reported, respectively). In the HR group, allogeneic SCT was performed in 66% of cases with improvement in 3-year OS rates after rituximab (75% *v* 40%; $P =$ not reported). Rates of death in first CR in this younger group were similar (4% *v* 3%).

Prospective randomized clinical trials incorporating rituximab into first-line therapy for precursor B-lineage ALL (some irrespective of CD20 expression) are planned. In our prior analysis, lower cut points of CD20 expression (eg, 10%) were discriminatory in predicting outcome.[9] Upregulation of CD20 expression during induction chemotherapy in ALL cases deemed CD20-negative at baseline suggests that anti-CD20 MoAb therapy may be beneficial even with low levels of expression.[36-38] Similar to the non-Hodgkin's lymphoma experience, extended dosing of rituximab during 6-mercaptopurine, vincristine, methotrexate, and prednisone maintenance therapy may further reduce relapses. Incorporation of novel agents which not only have antileukemic activity but also counter mechanisms of resistance to rituximab (eg, anti-Bcl-2 agents, mammalian target of rapamycin inhibitors, hypomethylating agents) should be explored.[36] Higher-affinity novel antibodies directed against different CD20 epitopes (eg, ofatumumab) or other lymphoblast surface antigens such as epratuzumab (targeting CD22) and alemtuzumab (targeting CD52) may further improve outcome.[39-43]

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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