Published in final edited form as:

Pediatr Blood Cancer. 2012 September ; 59(3): 468–474. doi:10.1002/pbc.23395.

Absolute Lymphocyte Counts Refine Minimal Residual Disease-Based Risk Stratification in Childhood Acute Lymphoblastic Leukemia

Karen R. Rabin, MD, PhD^{1,*}, M. Monica Gramatges, MD^{1,*}, Michael J. Borowitz, MD, PhD², Shana L. Palla, MS³, Xiaodong Shi, MD¹, Judith F. Margolin, MD¹, and Patrick A. Zweidler-McKay, MD, PhD³

¹Division of Pediatric Hematology/Oncology, Texas Children's Cancer Center, Baylor College of Medicine, Houston, TX

²Department of Pathology, Johns Hopkins University, Baltimore, MD

³Division of Pediatrics, University of Texas M. D. Anderson Cancer Center, Houston, TX

Abstract

Background—Several studies have demonstrated the prognostic utility of absolute lymphocyte count (ALC) during therapy for a range of malignancies, with low ALC associated with adverse outcome. Here we investigated whether ALC retained independent prognostic significance with respect to minimal residual disease (MRD) status in children with acute lymphoblastic leukemia (ALL).

Procedure—We reviewed 171 cases of pediatric ALL treated on the Children's Oncology Group P9900 series of treatment trials. Variables analyzed included ALC at several time points during Induction, age at diagnosis, cytogenetics, initial white blood cell count, and MRD status at Day 29 of Induction (MRD-29).

Results—We found high ALC at Induction Day 29 (ALC-29) to be an independent, clinically significant predictor of improved relapse-free and overall survival. Patients with ALC-29 >1,500 cells/μL had a superior 6-year relapse-free survival (80±4% vs. 62±8%, $p = 0.018$) and overall survival (96±2% vs. 74±8%, $p = 0.001$). Moreover, ALC-29 identified distinct prognostic subgroups within cases stratified by MRD-29. In subjects with >0.01% MRD, ALC-29 > or < 1,500 cells/μL had a significant 51% difference in 6-year overall survival (92±7% vs. 41±16%, $p = 0.0001$).

Conclusions—ALC, a readily obtainable test, constitutes a significant and independent prognostic factor in childhood ALL that may refine current MRD-based risk stratification algorithms and provide key prognostic information in settings where MRD determination is not feasible.

Correspondence: Patrick A. Zweidler-McKay, MD, PhD, Children's Cancer Hospital, University of Texas M. D. Anderson Cancer, Center 1515 Holcombe Blvd, Unit 853, Houston, Texas 77030, (p) 713-563-5395, (f) 713-563-5407, (e) pzweidler@mdanderson.org.
*Dr. K. Rabin and Dr. M. Gramatges contributed equally to this manuscript.

Conflict of Interest Statement

The authors have no affiliations or financial disclosures to report.

Publisher's Disclaimer: This PDF receipt will only be used as the basis for generating PubMed Central (PMC) documents. PMC documents will be made available for review after conversion (approx. 2–3 weeks time). Any corrections that need to be made will be done at that time. No materials will be released to PMC without the approval of an author. Only the PMC documents will appear on PubMed Central -- this PDF Receipt will not appear on PubMed Central.

Keywords

ALL; minimal residual disease; immunology

Introduction

Acute lymphoblastic leukemia (ALL) is the most common childhood cancer [1]. Though cure rates with modern therapy exceed 80%, salvage rates for those children who do relapse are poor, ranging from 10–60% [2]. Thus, optimizing frontline therapy is essential. Current pediatric ALL protocols assign patients to treatment regimens based on their estimated risk for relapse determined by age at diagnosis, initial white blood cell (WBC) count, specific recurrent favorable and unfavorable cytogenetic features, and response to therapy [3]. The most powerful independent prognostic factor is response to therapy, currently defined as the degree of minimal residual disease (MRD) present at specific time points during therapy, with MRD positivity at the end of Induction predictive of increased risk for both early and late relapse [4–6]. Risk stratification according to these features discriminates subgroups whose event-free-survival ranges from below 30% to over 97% [5]. This approach has led to improved outcomes through augmentation of therapy in higher risk patients, as well as reduction of treatment-related toxicities in lower risk patients [7–12].

MRD, as a proxy for the chemosensitivity of the leukemic blasts, serves as a powerful and independent predictor of outcome in various studies [4–6,13–15]. However, given that half of observed relapses in subjects enrolled on Pediatric Oncology Group protocols 9904–9906 occurred in patients who were MRD-negative ($< 0.01\%$) at the end of Induction, [5] other factors must also affect relapse risk. In addition, the complexity, cost, and training required to perform and interpret MRD testing precludes its use in many institutions in the United States and Europe, and certainly in most developing nations [14]. Cooperative groups provide centralized MRD testing on clinical trials, but patients not enrolled on these trials are often unable to benefit from this technology. It is critical to recognize that the vast majority of children diagnosed with ALL live in resource-poor countries without access to MRD, where cure rates average 35%, dramatically inferior to those observed in the United States and Europe [16]. Therefore, there is substantial need for further refinement of current MRD-based risk stratification schema, in addition to a need for an alternative means for risk assessment when MRD testing is not available.

The time required to re-establish normal hematopoiesis following chemotherapy, particularly recovery of lymphocytes, has emerged in recent studies as a powerful predictor of survival in both childhood and adult malignancies, likely due to the integral role of lymphocytes in cancer surveillance. Initial studies demonstrated the prognostic significance of absolute lymphocyte count (ALC) in the post-transplant setting, where early lymphocyte recovery, reflecting the speed of engraftment and potential establishment of a graft-*versus*-tumor immune response, was prognostic of better outcomes in both adults and children [17–19]. Subsequent studies have demonstrated that a high ALC also has a positive prognostic impact in malignancies treated with chemotherapy alone. To date, higher ALC has been associated with improved survival of adults with acute myeloid leukemia (AML), non-Hodgkin lymphoma (NHL), multiple myeloma, sarcomas, and carcinomas [20–26], as well as children with ALL, AML, NHL, osteosarcoma, and Ewing sarcoma [27–30]. The number of independent publications and the diversity of diseases where post-chemotherapy ALC is prognostic provide robust evidence of the significance of host lymphocyte recovery, which may reflect a critical role for the host immune response in combating cancer.

In childhood ALL, the most common pediatric malignancy, there is only one report on the significance of ALC by De Angulo et al., who reported that lower ALC at multiple time points during Induction therapy was associated with elevated hazard ratios for relapse and death [30]. However, this cohort was predominantly composed of adolescents and young adults, nearly one-third were treated with the adult HyperCVAD regimen[31], and the multivariate analysis did not include MRD. Thus, the significance of ALC independent of MRD remains unclear within a representative pediatric population treated on standard pediatric regimens.

Methods

Patients

Patients were included if they were between 1–21 years of age; diagnosed with *de novo* B-precursor ALL; treated at Texas Children's Cancer Center or Lucile Packard Children's Hospital between January 1992–August 2007; enrolled on or treated as per the Children's Oncology Group P9900 series of trials (P9904, P9905, and P9906); and had adequate medical records available for review. Treatment regimens included standard three or four drug induction depending on risk categorization, and have been described elsewhere [5]. The study was approved by the Baylor College of Medicine and Stanford University Medical School institutional review boards, and informed consent was obtained from all subjects.

Analysis

Medical records were reviewed to determine age, gender, initial WBC count, cytogenetics, treatment protocol, relapse and follow-up status, survival, ALC, absolute neutrophil count (ANC), and platelet count on Induction days 1, 15, 22, and 29. ALC values were obtained from the clinical lab record, and were determined either by manual differential (in cases flagged for abnormal values) or by automated cell counters. Day 29 MRD was determined from bone marrow aspirations collected on Day 29 of Induction, with analysis performed in the central reference laboratory of Dr. Borowitz at Johns Hopkins University, using flow cytometry as previously described[32]. Overall survival was defined as the time from the start of treatment to death. Last follow-up was measured from the date of treatment initiation to the date of last follow-up or death in patients without any evidence of relapse. Relapse-free survival was only determined for patients who achieved a complete remission, and was defined as the time from the start of treatment to relapse or last follow-up.

Statistical methods

Relapse-free survival (RFS) and overall survival (OS) were estimated using the methods of Kaplan and Meier [33]. A lowess smoothed plot of the Martingale residuals was used as an outcome-oriented approach to determining the optimal cutpoint [34,35]. Differences between survival curves were assessed for statistical significance using the log-rank statistic. The Cox proportional hazards regression model was used to consider each potential prognostic factor in univariate and multivariate analyses, and hazard ratios (HR) were estimated with 95% confidence intervals [36]. The associations of ALC-29 <1,500 cells/ μ L with relapse and with death were compared using Fisher's exact test.

Results

Established prognostic factors

Demographic features of the 171 cases are summarized in Table I. Mean age at diagnosis, median initial WBC, and gender proportions were indistinguishable from the P9900 cohort as a whole. Distribution on the three P9900 series trials was similar, with a somewhat lower

fraction of the highest-risk patients treated on 9906 (6% vs. 12.6%), and a somewhat shorter mean duration of follow-up (59 vs. 81 months) [8]. As expected, both univariate and multivariate analyses demonstrated the favorable prognostic significance of age <10 years, favorable cytogenetics, and MRD at day 29 (MRD-29) <0.01% for relapse-free survival (Tables II and III). Initial WBC (utilizing the NCI Rome cut-point of 50,000/ μ L [3]) was the only established prognostic factor not found to have prognostic significance. ANC did not exhibit consistent prognostic significance at any time point (Supplemental Table I). As previously identified by De Angulo et al. [27], platelet count <100,000/ μ L at day 29 was associated with poorer RFS and OS (Table II), but prognostic significance of this factor was not maintained in multivariate analysis (Table III).

ALC-29 population distribution

The median ALC at Induction day 29 (ALC-29) in our study population was 2,280 cells/ μ L, with a range of 192–9,890 cells/ μ L (Figure 1). ALC-29 was positively associated with both improved RFS and OS. ALC-29 was significant both as a continuous variable, with each 100 cells/ μ L increasing OS by 4% ($p = 0.014$), and as a categorical variable using multiple cut-points. The cut-point demonstrating the most robust predictive power was found to be 1,500 cells/ μ L, using Martingale residual analysis [34]; approximately 70% of values lie above this cut-point and 30% below it (Figure 1). The significance of ALC in predicting RFS and OS at Induction day 15, either as a continuous or a dichotomized variable, was also examined and did not have a statistically significant effect.

Prognostic impact of ALC-29

Patients with ALC-29 >1,500 cells/ μ L had an 18% higher 6-year RFS (80 $\pm$ 4% vs. 62 $\pm$ 8%, HR 2.2, $p = 0.018$; Figure 2A) and a 22% higher 6-year OS (96 $\pm$ 2% vs. 74 $\pm$ 8%, HR 7.0, $p = 0.001$; Figure 2B) (Table II). In a multivariate analysis that also included additional established risk factors (age at diagnosis, initial WBC, and favorable cytogenetics), ALC-29 retained prognostic significance for OS, however it did not retain significance for RFS (Table III). While the HR for overall survival among patients with ALC-29 <1,500 cells/ μ L was a robust 5.8 (95% CI (1.04, 31.9), $p = 0.04$), unexpectedly the HR for relapse was 1.0 (95% CI (0.40, 2.7), $p = 0.92$). This was not due to low ALC-29 being predictive of deaths in remission, since 12 of 14 (86%) deaths occurred due to relapse. Closer inspection of the data indicated that the majority of patients who died had ALC-29 <1,500 cells/ μ L (10 of 14, or 71%), whereas only one-quarter of patients who relapsed but survived had ALC-29 <1,500 cells/ μ L (6 of 24, or 25%; Fisher's exact $p < 0.008$).

ALC-29 refines MRD-based risk stratification

ALC allowed discrimination of distinct prognostic subgroups within the two groups defined by the MRD-29 cutoff of 0.01%. Among patients with MRD-29 <0.01%, those who also had ALC-29 <1,500 cells/ μ L had poor survival rates, whereas those with ALC-29 $\geq$ 1,500 cells/ μ L fared significantly better (6-year RFS of 69 $\pm$ 9% vs. 85 $\pm$ 4% (16% difference), HR 2.4, $p = 0.05$; and 6-year OS 81 $\pm$ 9% vs. 98 $\pm$ 1% (17% difference), HR 13.2, $p = 0.02$) (Figures 3A and B). More importantly, ALC identified discrete subgroups among those patients with MRD-29 $\geq$ 0.01%. Among patients with MRD-29 $\geq$ 0.01%, those with ALC-29 <1,500 cells/ μ L had dismal RFS and OS rates, but surprisingly, those with ALC-29 $\geq$ 1,500 cells/ μ L did very well, particularly for OS (Figures 3A and B). The 6-year RFS was 33 $\pm$ 15% vs. 59 $\pm$ 14% (26% difference), HR 2.0, $p = 0.24$; and the 6-year OS was 41 $\pm$ 16% vs. 92 $\pm$ 7%, HR 9.1, $p = 0.04$, demonstrating a remarkable 51% difference in OS based upon ALC in patients with MRD-29 $\geq$ 0.01%. Another way of expressing this is that ALC-29 identified additional patients at high risk of relapse and death, despite negative MRD-29 (<0.01%): 61% of relapses and 42% of deaths in our cohort were not predicted by MRD-29 $\geq$ 0.01%,

but ALC-29 $<1,500$ cells/ μ L predicted a significant number of these events within this MRD-29 negative group: 8 of 19 relapses (42%) and 4 of 5 deaths (80%).

Three prognostic groups identified by ALC-29 and MRD-29

From these data, we identified three clinically relevant prognostic groups based on the combined information provided by both ALC-29 and MRD-29. The favorable group, consisting of 60% of patients, was defined as possessing both ALC-29 $<1,500$ cells/ μ L and MRD-29 $<0.01\%$. This group demonstrated an $85\pm4\%$ 6-year RFS, and a striking $98\pm1\%$ 6-year OS. The intermediate group, comprising 32% of patients, consisted of both patients with a low ALC-29 and low MRD-29, and patients with a high ALC-29 and high MRD-29. This intermediate group exhibited a 6-year RFS of 59–69%, and a 6-year OS of 81–92%. Finally, the combination of low ALC-29 and high MRD-29 identified a very unfavorable subgroup with 6-year RFS of $33\pm15\%$ and 6-year OS of $41\pm16\%$.

These results demonstrated that adding ALC to current MRD-based risk stratification may improve both the sensitivity and specificity of risk groups. As indicated above, by using MRD and ALC together in this cohort, 92% of patients can be grouped as favorable and intermediate risk with OS $>90\%$, while just 8% of patients are identified as a high risk group with very poor outcome.

Discussion

In this analysis of 171 pediatric ALL patients enrolled in the COG P9900 series, ALC was a novel prognostic indicator independent of MRD and other established risk factors. Subjects with high ALC and negative MRD at Induction day 29 demonstrated a 98% 6-year OS, compared with subjects having a low ALC and positive MRD, who demonstrated a 41% 6-year OS. Most importantly, amongst those subjects who were MRD positive, those with elevated ALC fared significantly better, with a surprisingly favorable 6-year overall survival of 92%, a highly relevant 51% difference in outcome. Our results indicated that ALC-29 can add specificity to an MRD-based risk stratification system, permitting a refined stratification that utilizes both features to more precisely categorize subjects into groups with very good, intermediate, and very poor prognosis. This study validates and extends the findings of a prior study of ALC in ALL [30], demonstrating its significance in a larger, more representative and uniformly treated pediatric ALL cohort, and suggesting its utility in a risk stratification schema incorporating MRD. While early recovery of ALC has been confirmed as a favorable prognostic factor in a variety of malignancies, the numeric cutoff value and time point in therapy when ALC is measured varies between studies. The difference between the present study (reporting significance of ALC-29 greater or less than 1,500) and the prior ALL study by De Angulo et al. (reporting significance of ALC-15 greater or less than 350) may be explained by differences in the patient population. Most patients in the present study were under 10 years of age and received a relatively non-myelosuppressive three-drug induction, whereas most patients in the study by De Angulo et al. were above 10 years of age and/or received high-risk therapy which included an anthracycline. The combination of an older patient population and the intensity of anthracycline-based induction regimens may have provided a stronger divergence of lymphocyte values at the day 15 timepoint. Indeed, ALC cutoff values will likely need to be set for a specific population depending on factors such as age and intensity of induction therapy. These parameters will be tested prospectively in the upcoming Children's Oncology Group therapeutic trial for newly diagnosed high-risk ALL (AALL1131).

Another marker of hematopoietic recovery, platelet count $>100,000$ cells/ μ L at day 29, also demonstrated adverse prognostic significance for both RFS (trend, $p = 0.053$) and OS ($p < 0.001$) in univariate analysis, and has been reported previously in adult patients with newly

diagnosed ALL [37]. The lack of prognostic significance in multivariate analysis could be either due to limited power of the present study, or due to covariation of platelet and ALC recovery. A future large-scale prospective study may shed further light on this issue.

Our data set was generally representative of B-precursor pediatric ALL as treated on the P9900 series of trials, except for the relative lower-representation of the highest-risk cases (11/171 cases), which likely explains why initial WBC count did not demonstrate statistical significance in multivariate analysis. As expected, patients treated on P9906, the highest risk regimen, had a dismal outcome, with 8 of 11 (73%) suffering relapse and 6 of 11 (55%) dying, with 5 of 6 (83%) of the latter patients having an ALC-29 <1,500 cells/ μ L. The majority of remaining relapses and all of the deaths occurred in P9905 patients. The fact that ALC retained strong and independent prognostic impact is all the more impressive given the relative paucity of high-risk patients (P9906) in our study population, and weaker prognostic ability in low-risk patients (P9904).

In a multivariate analysis that included additional established risk factors, the prognostic value of ALC-29 remained significant for OS, but not for RFS. Interestingly, this difference was not accounted for by increased deaths in remission, since these constituted only 2 of 14 deaths. While low ALC was a risk factor for relapse, it was an even stronger risk factor for death once relapse occurred. This finding has intriguing implications since all 12 patients who died in relapse had undergone allogeneic transplant; apparently features of the original host immune system still affected their outcome.

The significance of ALC in pediatric ALL and many other pediatric and adult malignancies suggests an important role for the host in mediating cancer survival. Lymphopenia may result either from a pre-existing immunologic dysfunction, or be related to lympholytic cytokines produced by the malignant cell population itself [20], and may particularly affect leukemia surveillance and destruction. There is increasing evidence that some patients are able to mount an adaptive immune response directed toward tumor-specific antigens and associated with a cytotoxic T cell expansion [38]. For example, in leukemia patients treated with chemotherapy, NK cell anti-leukemia cytotoxic activity has been directly correlated with disease-free-survival [39]. Several studies have also demonstrated the dynamic changes that occur in both quantitative and qualitative measures of humoral and cellular immunity during leukemia therapy, with effects extending from months to years following completion of therapy [39–42]. Two reports have identified an expanded oligoclonal population of activated regulatory T cells following hematopoietic recovery from intensive therapy in AML[43,44]. Future studies are needed to characterize the lymphocyte subsets responsible for survival benefit in ALL using both quantitative and functional assays. In addition to augmentation of conventional chemotherapy, an approach used generically for high-risk patients, therapies designed to enhance immune anti-tumor effects might be specifically beneficial for low-ALC patients. Numerous such interventions exist in the post-transplant setting, and several are under study in the pre-transplant setting as well, including beta-glucan [45] and Bacillus Calmette-Guerin with thymic stromal lymphopoietin. [46]

Our results indicated that ALC at the end of Induction refined MRD-based risk stratification and may help guide in risk stratification when MRD testing is not available. ALC is a readily obtainable, simple, relatively inexpensive, and easy to interpret value. Where available, MRD has become an essential tool integral to making treatment-related decisions. However, MRD is not always available nor is it predictive of all relapses. In our analysis, ALC-29 refined risk stratification, identifying a subset of MRD-positive individuals with relatively good prognosis, and MRD-negative patients at higher risk for relapse. A confirmatory cohort in a large prospective trial will be needed to confirm the results presented herein. As mentioned above, ALC-29 data will be prospectively collected in the

next Children's Oncology Group therapeutic trial for newly diagnosed high-risk ALL (AALL1131), which may serve to validate the prognostic significance of ALC-29, determine whether ALC-29 is predictive of response to different treatment arms, and support the future incorporation of ALC into ALL risk stratification algorithms. In particular, MRD-negative patients with a low ALC-29 may warrant more intensive therapy similar to other high-risk patients who are MRD-positive. Furthermore, additional studies are needed to evaluate the mechanism underlying the prognostic significance of ALC, particularly which lymphokines and lymphocyte subsets are most important for effective immune surveillance, both in pediatric ALL and across a spectrum of hematologic and oncologic malignancies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors would like to thank Gary Dahl, Stanford University, for his mentorship on this project, and Meenakshi Devidas, Children's Oncology Group Data Center, for providing demographic data for patients enrolled on the P9900 trial series. This work was supported by the Rachleff Family, who generously provided research support to Dr. M. Gramatges, as well as by the National Institutes of Health Pediatric Oncology Clinical Research Training Grant CA90433-06, the Kurt Groten Family Research Scholars' Program, and the St. Baldrick's Pediatric Cancer Foundation, who provided research support to Dr. K. Rabin.

References

1. Onciu M. Acute lymphoblastic leukemia. *Hematol Oncol Clin North Am.* 2009; 23(4):655–674. [PubMed: 19577163]
2. Nguyen K, Devidas M, Cheng SC, et al. Factors influencing survival after relapse from acute lymphoblastic leukemia: a Children's Oncology Group study. *Leukemia.* 2008; 22(12):2142–2150. [PubMed: 18818707]
3. Smith M, Arthur D, Camitta B, et al. Uniform approach to risk classification and treatment assignment for children with acute lymphoblastic leukemia. *J Clin Oncol.* 1996; 14(1):18–24. [PubMed: 8558195]
4. Panzer-Grumayer ER, Schneider M, Panzer S, et al. Rapid molecular response during early induction chemotherapy predicts a good outcome in childhood acute lymphoblastic leukemia. *Blood.* 2000; 95(3):790–794. [PubMed: 10648387]
5. Borowitz MJ, Devidas M, Hunger SP, et al. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia and its relationship to other prognostic factors: a Children's Oncology Group study. *Blood.* 2008; 111(12):5477–5485. [PubMed: 18388178]
6. Coustan-Smith E, Sancho J, Behm FG, et al. Prognostic importance of measuring early clearance of leukemic cells by flow cytometry in childhood acute lymphoblastic leukemia. *Blood.* 2002; 100(1): 52–58. [PubMed: 12070008]
7. Hann I, Vora A, Harrison G, et al. Determinants of outcome after intensified therapy of childhood lymphoblastic leukaemia: results from Medical Research Council United Kingdom acute lymphoblastic leukaemia XI protocol. *Br J Haematol.* 2001; 113(1):103–114. [PubMed: 11328289]
8. Pui CH, Robison LL, Look AT. Acute lymphoblastic leukaemia. *Lancet.* 2008; 371(9617):1030–1043. [PubMed: 18358930]
9. Nachman JB, Sather HN, Sensel MG, et al. Augmented post-induction therapy for children with high-risk acute lymphoblastic leukemia and a slow response to initial therapy. *N Engl J Med.* 1998; 338(23):1663–1671. [PubMed: 9614257]
10. Schultz KR, Pullen DJ, Sather HN, et al. Risk- and response-based classification of childhood B-precursor acute lymphoblastic leukemia: a combined analysis of prognostic markers from the Pediatric Oncology Group (POG) and Children's Cancer Group (CCG). *Blood.* 2007; 109(3):926–935. [PubMed: 17003380]

11. Moricke A, Zimmermann M, Reiter A, et al. Long-term results of five consecutive trials in childhood acute lymphoblastic leukemia performed by the ALL-BFM study group from 1981 to 2000. *Leukemia*. 2010; 24(2):265–284. [PubMed: 20010625]
12. Rivera GK, Pinkel D, Simone JV, et al. Treatment of acute lymphoblastic leukemia. 30 years' experience at St. Jude Children's Research Hospital. *N Engl J Med*. 1993; 329(18):1289–1295. [PubMed: 8413409]
13. Cave H, van der Werff ten Bosch J, Suciu S, et al. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia. European Organization for Research and Treatment of Cancer--Childhood Leukemia Cooperative Group. *N Engl J Med*. 1998; 339(9):591–598. [PubMed: 9718378]
14. Campana D. Status of minimal residual disease testing in childhood haematological malignancies. *Br J Haematol*. 2008; 143(4):481–489. [PubMed: 18710378]
15. Dworzak MN, Froschl G, Printz D, et al. Prognostic significance and modalities of flow cytometric minimal residual disease detection in childhood acute lymphoblastic leukemia. *Blood*. 2002; 99(6):1952–1958. [PubMed: 11877265]
16. Metzger ML, Howard SC, Fu LC, et al. Outcome of childhood acute lymphoblastic leukaemia in resource-poor countries. *Lancet*. 2003; 362(9385):706–708. [PubMed: 12957095]
17. Kumar S, Chen MG, Gastineau DA, et al. Lymphocyte recovery after allogeneic bone marrow transplantation predicts risk of relapse in acute lymphoblastic leukemia. *Leukemia*. 2003; 17(9):1865–1870. [PubMed: 12970788]
18. Le Blanc K, Barrett AJ, Schaffer M, et al. Lymphocyte recovery is a major determinant of outcome after matched unrelated myeloablative transplantation for myelogenous malignancies. *Biol Blood Marrow Transplant*. 2009; 15(9):1108–1115. [PubMed: 19660724]
19. Kim DH, Kim JG, Sohn SK, et al. Clinical impact of early absolute lymphocyte count after allogeneic stem cell transplantation. *Br J Haematol*. 2004; 125(2):217–224. [PubMed: 15059145]
20. Ray-Coquard I, Cropet C, Van Glabbeke M, et al. Lymphopenia as a prognostic factor for overall survival in advanced carcinomas, sarcomas, and lymphomas. *Cancer Res*. 2009; 69(13):5383–5391. [PubMed: 19549917]
21. Behl D, Porrata LF, Markovic SN, et al. Absolute lymphocyte count recovery after induction chemotherapy predicts superior survival in acute myelogenous leukemia. *Leukemia*. 2006; 20(1):29–34. [PubMed: 16281063]
22. Cox MC, Nofroni I, Ruco L, et al. Low absolute lymphocyte count is a poor prognostic factor in diffuse-large-B-cell-lymphoma. *Leuk Lymphoma*. 2008; 49(9):1745–1751. [PubMed: 18798109]
23. Ege H, Gertz MA, Markovic SN, et al. Prediction of survival using absolute lymphocyte count for newly diagnosed patients with multiple myeloma: a retrospective study. *Br J Haematol*. 2008; 141(6):792–798. [PubMed: 18410454]
24. Oki Y, Yamamoto K, Kato H, et al. Low absolute lymphocyte count is a poor prognostic marker in patients with diffuse large B-cell lymphoma and suggests patients' survival benefit from rituximab. *Eur J Haematol*. 2008; 81(6):448–453. [PubMed: 18691256]
25. Siddiqui M, Ristow K, Markovic SN, et al. Absolute lymphocyte count predicts overall survival in follicular lymphomas. *Br J Haematol*. 2006; 134(6):596–601. [PubMed: 16889618]
26. Yanada M, Borthakur G, Garcia-Manero G, et al. Blood counts at time of complete remission provide additional independent prognostic information in acute myeloid leukemia. *Leuk Res*. 2008; 32(10):1505–1509. [PubMed: 18405972]
27. De Angulo G, Hernandez M, Morales-Arias J, et al. Early lymphocyte recovery as a prognostic indicator for high-risk Ewing sarcoma. *J Pediatr Hematol Oncol*. 2007; 29(1):48–52. [PubMed: 17230066]
28. DuBois SG, Elterman K, Grier HE. Early lymphocyte recovery in Ewing sarcoma. *J Pediatr Hematol Oncol*. 2007; 29(5):351–352. [PubMed: 17483722]
29. Moore C, Eslin D, Levy A, et al. Prognostic significance of early lymphocyte recovery in pediatric osteosarcoma. *Pediatr Blood Cancer*. 2010
30. De Angulo G, Yuen C, Palla SL, et al. Absolute lymphocyte count is a novel prognostic indicator in ALL and AML: implications for risk stratification and future studies. *Cancer*. 2008; 112(2):407–415. [PubMed: 18058809]

31. Kantarjian H, Thomas D, O'Brien S, et al. Long-term follow-up results of hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone (Hyper-CVAD), a dose-intensive regimen, in adult acute lymphocytic leukemia. *Cancer*. 2004; 101(12):2788–2801. [PubMed: 15481055]
32. Weir EG, Cowan K, LeBeau P, et al. A limited antibody panel can distinguish B-precursor acute lymphoblastic leukemia from normal B precursors with four color flow cytometry: implications for residual disease detection. *Leukemia*. 1999; 13(4):558–567. [PubMed: 10214862]
33. Kaplan ELMP. Nonparametric estimation from incomplete observations. *Journal of the American Statistical Association*. 1958; 53:457–481.
34. Wei WH, Su JS. Model choice and influential cases for survival studies. *Biometrics*. 1999; 55(4): 1295–1299. [PubMed: 11315087]
35. Klein, JP.; Moeschberger, ML. *Survival Analysis: Techniques for Censored and Truncated Data*. New York: Springer-Verlag; 1997.
36. Cox D. Regression models and life tables. *Journal of the Royal Statistical Society*. 1972; 34:187–220.
37. Faderl S, Thall PF, Kantarjian HM, et al. Time to platelet recovery predicts outcome of patients with de novo acute lymphoblastic leukaemia who have achieved a complete remission. *Br J Haematol*. 2002; 117(4):869–874. [PubMed: 12060122]
38. Disis ML. Immune regulation of cancer. *J Clin Oncol*. 2010; 28(29):4531–4538. [PubMed: 20516428]
39. Lowdell MW, Craston R, Samuel D, et al. Evidence that continued remission in patients treated for acute leukaemia is dependent upon autologous natural killer cells. *Br J Haematol*. 2002; 117(4): 821–827. [PubMed: 12060116]
40. Kosmidis S, Baka M, Bouhoutsou D, et al. Longitudinal assessment of immunological status and rate of immune recovery following treatment in children with ALL. *Pediatr Blood Cancer*. 2008; 50(3):528–532. [PubMed: 17853465]
41. Komada Y, Zhang SL, Zhou YW, et al. Cellular immunosuppression in children with acute lymphoblastic leukemia: effect of consolidation chemotherapy. *Cancer Immunol Immunother*. 1992; 35(4):271–276. [PubMed: 1511462]
42. Ohnishi K, Yamanishi H, Naito K, et al. Reconstitution of peripheral blood lymphocyte subsets in the long-term disease-free survivors of patients with acute myeloblastic leukemia. *Leukemia*. 1998; 12(1):52–58. [PubMed: 9436920]
43. Szczepanski MJ, Szajnik M, Czystowska M, et al. Increased frequency and suppression by regulatory T cells in patients with acute myelogenous leukemia. *Clin Cancer Res*. 2009; 15(10): 3325–3332. [PubMed: 19417016]
44. Kanakry CG, Hess AD, Gocke CD, et al. Early lymphocyte recovery after intensive timed sequential chemotherapy for acute myelogenous leukemia: peripheral oligoclonal expansion of regulatory T cells. *Blood*. 2011; 117(2):608–617. [PubMed: 20935254]
45. Li B, Cai Y, Qi C, et al. Orally administered particulate beta-glucan modulates tumor-capturing dendritic cells and improves antitumor T-cell responses in cancer. *Clin Cancer Res*. 2010; 16(21): 5153–5164. [PubMed: 20855461]
46. Sugimoto H, Ito T, Torii Y, et al. Thymic stromal lymphopoietin plays an adjuvant role in BCG-mediated CD8(+) cytotoxic T cell responses through dendritic cell activation. *Clin Immunol*. 2010; 136(2):205–216. [PubMed: 20471323]

Figure 1. The distribution of ALC values approximates a log-normal distribution with sigma of 1/2

Using a Martingale residual analysis, the cut off point for high and low ALC with the most predictive power was determined to be 1,500 cells/ μ L (approximately the 30th percentile). The median ALC in this population was 2,280 cells/ μ L, which was also predictive for OS, with a p -value of 0.011.

Figure 2. High ALC at Induction day 29 predicts improved survival

Kaplan-Meier estimates of (A) relapse-free survival (RFS) and (B) overall survival (OS) in patients with ALC-29 $\geq 1,500$ cells/ μ L (solid lines) or ALC-29 $<1,500$ cells/ μ L (dashed lines). p -values based on log rank. ALC indicates absolute lymphocyte count.

Figure 3. ALC refines MRD stratification

Kaplan-Meier estimates of (A) relapse-free survival (RFS) and (B) overall survival (OS) in patients with MRD^{neg} (<0.01%) or MRD^{pos} (0.01%) and ALC^{hi} (ALC-29 ≥ 1,500 cells/μL) or ALC^{lo} (ALC-29 <1,500 cells/μL). *p*-values based on log rank. ALC indicates absolute lymphocyte count; MRD indicates minimal residual disease.

Table I

Patient characteristics

	Subject Cohort	P9900 Trials
Number of patients enrolled, n	171	2153
Mean age at diagnosis (years)	5.4 (1–17)	5.7 (1–20)
Age 1–4 years	94	
Age 5–10 years	48	
Age 10–18 years	29	
Median initial WBC ($\times 10^3$ cells/ml)	8.4 (0.3–321)	7.8 (0.3–958.8)
Male gender, n (%)	87 (51%)	1152 (53.5%)
Treatment protocol		
P9904 (low risk), n (%)	60 (35.1%)	828 (38.4%)
P9905 (standard risk), n (%)	100 (58.5%)	1054 (48.9%)
P9906 (high risk), n (%)	11 (6%)	272 (12.6%)
Mean follow-up (months)	59 (5.6–98)	81.2 (1.3–126.0)

Table II

Univariate analyses in all patients predicting relapse-free and overall survival

Relapse-free Survival	n	Relapse/deaths (%)	HR (95% CI)	p-value
ALC day 29 < 1500 (cells/ μ L)	49	33% vs. 18%	2.2 (1.1–4.2)	0.018
Age at diagnosis < 10 (years)	142	17% vs. 45%	0.28 (0.14–0.56)	0.001
Mean initial WBC < 50 ($\times 10^3$ cells/ml)	158	21% vs. 31%	0.66 (0.23–1.9)	0.439
Favorable cytogenetics	65	11% vs. 29%	0.32 (0.14–0.74)	0.008
MRD day 29 0.01%	26	46% vs. 17%	3.3 (1.6–6.7)	0.001
Platelets day 29 100 ($\times 10^3$ cells/ μ l)	17	35% vs. 20%	2.4 (0.99–5.70)	0.053
Overall Survival	n	Deaths	HR (95% CI)	p-value
ALC day 29 < 1500 (cells/ μ L)	49	20% vs. 3%	7.0 (2.2–22.3)	0.001
Age at diagnosis < 10 (years)	142	4% vs. 28%	0.13 (0.04–0.38)	0.001
Mean initial WBC < 50 ($\times 10^3$ cells/ml)	158	8% vs. 15%	0.50 (0.11–2.2)	0.367
Favorable cytogenetics	65	0% vs. 13%	---	---
MRD day 29 0.01%	26	27% vs. 4%	6.6 (2.1–20.9)	0.001
Platelets day 29 100 ($\times 10^3$ cells/ μ l)	17	35% vs. 5%	9.1 (3.1–26.3)	<0.001

Table III

Multivariate analyses of multiple risk factors affecting relapse-free and overall survival

Relapse-free Survival	HR (95% CI)	p-value
ALC day 29 < 1500 (cells/ μ L)	1.0 (0.40, 2.7)	0.92
Age at diagnosis < 10 (years)	0.37 (0.14, 0.95)	0.04
Mean initial WBC < 50 ($\times 10^3$ cells/ μ L)	0.64 (0.15, 2.8)	0.57
Favorable cytogenetics	0.33 (0.12, 0.91)	0.03
MRD day 29 0.01%	3.1 (1.3, 7.2)	0.01
Platelets day 29 100 ($\times 10^3$ cells/ μ L)	0.95 (0.27, 3.4)	0.94
Overall Survival	HR (95% CI)	p-value
ALC day 29 < 1500 (cells/ μ L)	5.8 (1.04, 31.9)	0.04
Age at diagnosis < 10 (years)	0.31 (0.08, 1.1)	0.08
Mean initial WBC < 50 ($\times 10^3$ cells/ μ L)	0.59 (0.12, 3.0)	0.52
Favorable cytogenetics	NA *	---
MRD day 29 0.01%	3.5 (0.98, 12.8)	0.05
Platelets day 29 100 ($\times 10^3$ cells/ μ L)	1.86 (0.44, 7.8)	0.39

* NA indicates not applicable, due to no deaths occurring among cases with favorable cytogenetics.