Outcome Differences in Patients with Precursor B Cell Acute Lymphocytic Leukemia Over Time: A Retrospective Analysis

Arie Apel MD[1], Meirav Kedmi MD[1], Etai Levi MD[2], Miriam Berkowicz MD[1], Yaron Davidovitz MD[1], Abraham Kneller MD[1], Elena Ribakovsky MD[1], Avichai Shimoni MD[1], Arnon Nagler MD[1] MSc and Abraham Avigdor MD[1]

[1]Division of Hematology and Bone Marrow Transplantation, Sheba Medical Center, Tel Hashomer, affiliated with Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel
[2]Depatment of Hematology, Soroka Medical Center and Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel

ABSTRACT: **Background:** Acute lymphocytic leukemia (ALL) is a rare disease with a poor outcome in adults. Over the years different protocols have been developed with the aim of improving the outcome. The German study group protocols (GMALL), which are the most frequently used in our institutions, changed significantly between the periods 1989–93 and 1999–2003.
Objectives: To investigate whether the change in protocols over the years resulted in an outcome difference at two hospitals in Israel.
Methods: We thoroughly reviewed the records of 153 patients from Sheba Medical Center and Soroka Medical Center, of whom 106 comprised the study group. The patients were divided into two groups according to the treatment protocol used: 40 patients with the 1989/93 protocol and 66 with the 1999/2003 protocol. Outcome was analyzed for the two groups.
Results: We found a significant difference in disease-free survival (DFS) between the two groups for B cell-ALL (B-ALL) patients who achieved complete remission after induction. There was no difference in overall survival. We did not find any difference in outcome for T cell-ALL patients or for CD20-positive patients.
Conclusions: In our retrospective analysis, GMALL 99/2003 led to a better DFS for B-ALL patients who were in complete remission after induction. This is possibly related to the differences in medications between the protocols but may also be due to better supportive care. Despite the proven advantage of the newer protocols regarding overall survival, in our experience there was no other significant difference between the two regimens.

IMAJ 2014; 16: 224–228

KEY WORDS: acute lymphoblastic lymphoma (ALL), German multi-center trial for adult ALL (GMALL) protocol, progression-free survival, overall survival

Acute lymphoblastic leukemia is a heterogeneous group of diseases characterized by malignant proliferation of precursor B or T lymphocytes in the bone marrow. In our institutions the most commonly used protocol for treatment of ALL is the GMALL [1]. According to the French-American-British Group Classification System, ALL is defined by the presence of 30% or more lymphoblasts in the bone marrow, as compared to only 20% required by the World Health Organization definition [2-5]. Seventy-five percent of adult ALL cases originate from B cell lineage and 25% from T cell lineage [2-5]. B-ALL is divided into four subgroups according to the level of blast maturation: pro-B, common-B, pre-B, and mature-B. This classification is based on cell-differentiating antigens using flow cytometry. T-ALL is classified as early T, thymic T, and mature T [4]. The classic prognostic factors in ALL, such as age, white blood cell count at diagnosis, immune phenotype, and response to steroid therapy, have limited ability to predict the outcome [1,6-9].

The German multi-center trial for adult ALL protocol for the treatment of ALL was developed by Hoelzer et al. [1] and is a leading protocol for the treatment of adult ALL.

Until the year 2000 we used GMALL 1989/93; we then switched to GMALL 1999 and later to GMALL 2003 [10-12]. The GMALL includes two induction phases and two consolidation phases, after which patients are assigned to either continuation of chemotherapy for up to 52 weeks or allogeneic stem cell transplantation according to risk stratification and subject to the availability of an HLA-matched donor. After completion of 52 weeks of chemotherapy the patients continue with maintenance therapy for 2 years [10-12].

The protocols that we used until 2000 differ from the newer protocols in a few major aspects. In the newer protocols the patients are stratified into three risk groups: standard risk, high risk, and very high risk (presence of Philadelphia chromosome), in contrast to only SR and HR in the old protocols. Risk stratification is based on white blood cell count at presentation,

ALL = acute lymphoblastic lymphoma
B-All = B cell ALL
T-ALL = T cell ALL
GMALL = German multi-center trial for adult ALL
SR = standard risk
HR = high risk

blast maturation, presence of translocations (4:11 and 9:22), and day of achieving complete remission. In the older protocols the HR group included patients with (9:22) translocation, while in the newer ones they are defined as VHR [10,11]. In addition, according to the newer protocols, HR and VHR patients who achieve CR after induction therapy are referred to allo-SCT following the first consolidation. The older protocols do not recommend that T-ALL patients undergo allo-SCT in the first CR, whereas in the newer protocols the treatment is the same as for B-ALL patients.

The backbone of chemotherapy in ALL is: anthracyclins, alkylating agents, methotrexate and asparaginase. However, there are some differences between the newer and older protocols with regard to medications: the introduction of pegylated asparaginase instead of asparaginase, dexamethasone instead of prednisone, and the application of tyrosine kinase inhibitors in the treatment of Philadelphia-positive ALL. In this work we aimed to retrospectively evaluate the difference in outcome of ALL patients in the different eras (pre- and post-2000) at the Sheba and Soroka medical centers.

PATIENTS AND METHODS

We reviewed the records of 153 ALL patients, aged 15–65 years, at the Sheba and Soroka medical centers. The study protocol was approved by the Helsinki Ethics Committee of both centers. Exclusion criteria were severe comorbidity that would influence the physician's ability to administer full treatment (severe heart failure, severe renal impairment not related to leukemia, human immunodeficiency virus and severe psychiatric illness), pregnancy, participation in other clinical trials, ALL as secondary malignancy, or initiation of therapy in a different hospital or department.

The final study population comprised 106 patients: 93 from Sheba and 13 from Soroka. The patients were divided into two groups according to protocol: group 1 – GMALL 89/93 and group 2 – GMALL 99/2003.

DEFINITIONS AND STATISTICS

Overall survival for the entire group was defined as the time from diagnosis to death from any cause or to last follow-up. Disease-free survival was defined as the time from diagnosis to relapse, death (from any cause), or last follow-up. Analysis for OS and DFS for the subset of patients who achieved CR was also performed. For this subset analysis, OS was defined as the time from CR to death of any cause or to last follow-up, and DFS as the time from CR to relapse, death or last follow-up. The Kaplan-Meier method was used to calculate OS and DFS.

VHR = very high risk
CR = complete remission
allo-SCT = allogeneic stem cell transplantation
OS = overall survival
DFS = disease-free survival

Differences between the groups were analyzed with chi-square and Fisher's exact test. *P* values < 0.05 were considered statistically significant.

RESULTS

PATIENT AND DISEASE CHARACTERISTICS

Between 1984 and 2009, 106 eligible ALL patients were treated at the Sheba and Soroka medical centers. Demographics and disease characteristics are presented in Table 1. Forty patients (group 1) were treated with the GMALL 89/93 protocol and 66 patients (group 2) with the GMALL 99/2003 protocol. Female-to-male ratio was 1:1.5 and was not different between the groups. Median age was 35.1 years in group 1 and 37.4 in group 2 (not significant). Group 1 included 30 B-ALL and 10 T-ALL patients (75% and 25% respectively), while group 2 included 47 B-ALL and 19 T-ALL patients (71.2% and 28.8% respectively) (NS). Among the 30 B-ALL patients in group 1, 17 (56.6%) were SR, 6 (20%) were HR and 7 (23.3%) were VHR. Among the 47 B-ALL patients in group 2, 24 (51%) were SR, 11 (23.4%) HR, and 12 (25.6%) were VHR (NS). Among the 10 T-ALL patients in group 1, 50% were SR and 50% HR. In group 2, 6 T-ALL patients (31.6%) were SR and 13 (68.4%) HR. Thirty-seven patients (92.5%) from group 1 and 53 (80%) from group 2 achieved CR after induction ($P = 0.35$).

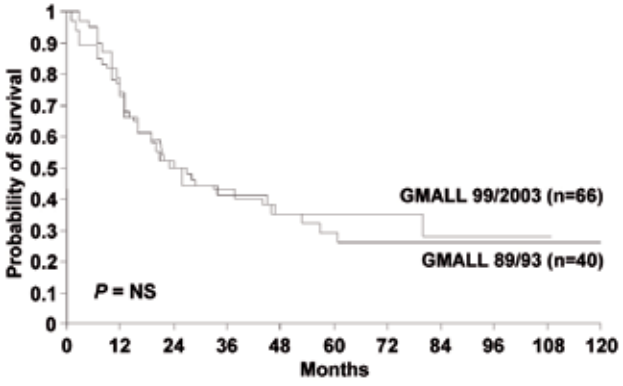
OVERALL SURVIVAL

Five year OS for the entire cohort was 29% and 35% (NS) for group 1 and 2, respectively [Figure 1]. Analysis of the B-ALL cases only did not reveal any difference in OS between the groups. Analysis of OS by lineage for the entire cohort showed borderline advantage in OS for T-ALL in group 1 but no differ-

Table 1. Patients and disease characteristics

	GMALL 89/93 (group 1) (%)	GMALL 99/2003 (group 2) (%)	
	n=40	n=66	*P* value
B-ALL	30 (75%)	47 (71.2%)	NS
T-ALL	10 (25%)	19 (28.8%)	NS
Female	15 (37.5%)	24 (36.4%)	NS
Male	25 (62.5%)	24 (63.6)	NS
Age (yr)	35.1	37.4	NS
B-ALL	GMALL 89/93	GMALL 99/2003	
SR	17 (56.6%)	24 (51%)	NS
HR	6 (20%)	11 (23.4%)	NS
VHR	(23.3%)7	12 (25.6%)	NS
T-ALL	GMALL 89/93	GMALL 99/2003	
SR	5 (50%)	6 (31.6%)	NS
HR	5 (50%)	13 (68.4%)	NS

Figure 1. Overall survival for the entire cohort by group (group 1: GMALL 89/93, group 2: GMALL 99/2003)



ence in group 2. Five year OS in group 1 was 23% versus 51% for B-All and T-ALL, respectively ($P = 0.07$). In group 2, 5 year OS was 34% for B-ALL and 35% for T-ALL (NS).

Six patients aged 35–55 died during induction and all were from group 2; they comprised five B-ALL patients, two SR, one HR and two VHR. One patient had T-ALL. Five of the six died of sepsis and one from colon perforation.

OS ACCORDING TO RISK STRATIFICATION

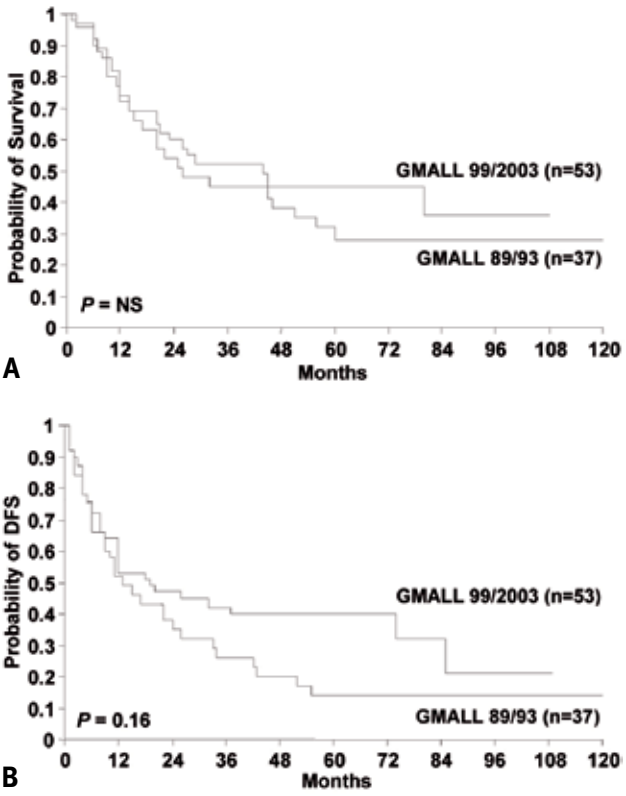
Five year OS was significantly higher for SR as compared to VHR patients in group 2 ($P = 0.04$). In group 1 the difference did not reach significance ($P = 0.09$), probably due to the smaller number of patients.

Among the VHR (Philadelphia +) patients who were treated with the newer protocols (n=12), 10 patients received imatinib mesylate as part of their protocol and 2 did not. Seven patients (70%) of the 10 who were treated with imatinib died. The OS at 5 years was 30%. Both patients (100%) who were not treated with imatinib died. For the B-ALL patients treated with the newer protocols (n=45, 11 CD20 + and 34 CD20-), the OS was 53% at 5 years for CD20-positive patients and 44% for CD20-negative patients, DFS was 50% in CD20-positive cases compared to 42% for CD20-negative cases.

ANALYSIS OF PATIENTS WHO ACHIEVED CR

A subset analysis of the patients who achieved CR in both groups (n=90) showed that 37 of the 40 patients in group 1 and 53 of the 66 in group 2 achieved CR (NS). Overall survival and disease-free survival did not significantly differ between B-ALL and T-ALL patients in either of the groups. Comparison of the patients who achieved CR in the two groups revealed no difference in OS or DFS between the groups [Figure 2]. Analysis of the B-ALL patients who achieved CR revealed significantly better DFS for group 2 ($P = 0.05$) but no difference in OS (P = 0.15) [Figure 3]. The same analysis for T-ALL did not show any difference. The 5 year OS for standard risk B-ALL patients who achieved CR was 19% in group 1 (n=16, 95% confidence interval 5–40) and 53% in group 2 (n=21, 95%CI 29–73) (P = 0.22). Patients who did not achieve CR were generally referred to salvage protocols and allogeneic stem cell transplantation. This group of patients was too small for analysis.

Figure 2. [A] Probability of overall survival for patients in group 1 who achieved complete remission (GMALL 89/93) vs. group 2 (GMALL 99/2003). **[B]** Probability of disease-free survival for patients in group 1 who achieved complete remission (GMALL 89/93) vs. group 2 (GMALL 99/2003)



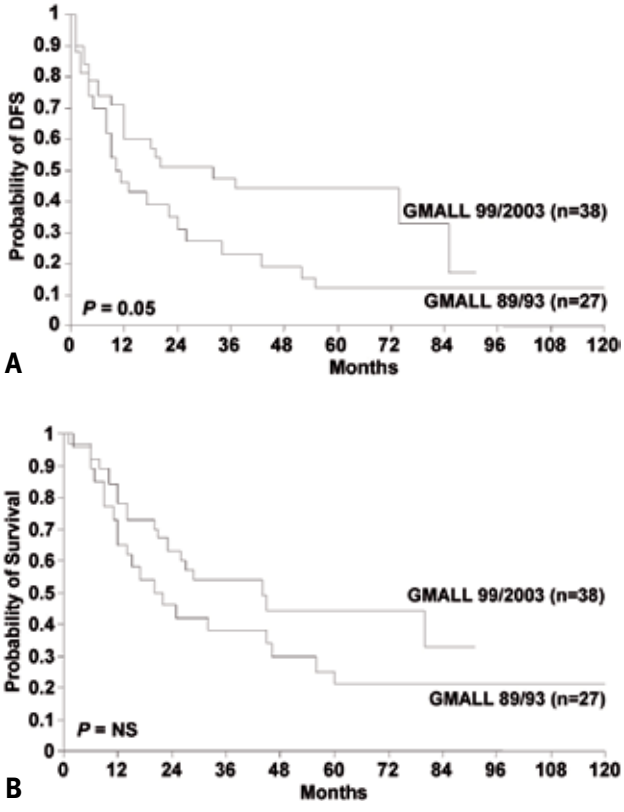
DISCUSSION

Acute lymphoblastic leukemia is one of the most common hematological malignancies in pediatric oncology, with a cure rate of about 80%. In adults it is a rare disease with an estimated annual incidence of 1/100,000 and a poor prognosis [13]. Despite the improvement in CR rates, supportive care and allo-SCT, most adults with ALL will relapse and eventually die of their disease. Although advanced therapy for ALL did improve the OS rate, unfortunately it is still very poor with only 30–40% OS at 5 years [14-17].

CD = cell differentiation

CI = confidence interval

Figure 3. [A] Probability of disease-free survival for B-ALL patients who achieved complete remission in group 1 (GMALL 89/93) vs. group 2 (GMALL 99/2003). **[B]** Probability of overall survival for B-ALL patients who achieved complete remission in group 1 (GMALL 89/93) vs. group 2 (GMALL 99/2003)



This retrospective study evaluated 106 ALL patients treated at the Sheba Medical Center in Tel Hashomer and Soroka Medical Center in Beer Sheva during the last 25 years. We compared the treatment results between the two different GMALL protocols – 89/93 and 99/2003 – in order to examine whether there was an improvement in survival with time.

Complete remission rates in both groups were similar to what is reported in the literature [10]. Our results demonstrate that DFS significantly improved for B-ALL patients who achieved CR from 12% to 44%, while the OS was not significantly different. A possible explanation is that some of the relapsing patients are salvageable with allo-SCT. Some reports in the literature indicate that patients younger than 55 years with SR ALL will benefit from allo-SCT after remission induction if they have a matched sibling donor [18]. In our cohort, for SR B-ALL patients who achieved CR, the 5 year OS improved from 19% in group 1 to 53% in group 2. This difference was not statistically significant, probably due to the very small number of patients in our cohort. Nevertheless, we still maintain our local policy not to perform allo-SCT in patients with SR ALL achieving first CR, based on the data provided by the larger scale GMALL studies that demonstrated relatively good survival outcomes in this group of patients [10-12]. Furthermore, we have also incorporated a risk-adapted strategy using frequent polymerase chain reaction analyses of minimal residual disease during the first year of therapy in patients with SR ALL. This policy, which is based on the recent GMALL data, provides new guidance for performing allo-SCT in this subgroup of patients. Patients with a high level of MRD ($> 10^{-4}$) after consolidation are defined now as MRD high risk and allocated to SCT in the first CR, whereas patients with a low level of MRD ($< 10^{-4}$) continue to receive conventional chemotherapy according to the protocol [19]. In contrast to the literature [8,13,20], we did not find any difference in outcome for T-ALL patients between the two eras. However, these data are very limited due to the small sample size. We believe that in a larger group of patients the differences would have been shown. The Philadelphia-positive group of patients was also too small to be analyzed.

The improved outcome may be related to the use of pegylated asparaginase, repeated cycles of consolidation which includes high dose methotrexate and cytarabine to HR and VHR patients, increased use of allo-SCT for HR and VHR patients after one cycle of consolidation, and the use of tyrosine kinase inhibitors [13, 14, 20, 21].

The fact that our study is retrospective makes it difficult to measure one of the most important parameters – improvement in supportive care. It is clear, however, that the difference between the two eras was major. Therefore, we cannot attribute the improved outcome for B-ALL patients solely to the change of protocol. The fact that we did not see a difference in outcome in any other group but B-ALL, which achieved CR, may indicate that the difference is related to the change in protocol and not only to improved supportive care.

In contrast to reports in the literature [20,22,23], we did not find differences in outcome between CD20-positive and CD-negative cases.

Achievement of CR is a key factor for good outcome in ALL. In our cohort as well as others there was no significant change in CR rate between the 89/93 and the 99/2003 protocols [13,20,21,24,25].

Six patients died early during induction mostly from sepsis; as mentioned, all were treated with the newer protocols. It is possible that the early deaths resulted in comparable OS rates between the protocols. Since the groups are rather small, it is difficult to prove or refute the hypothesis that the newer induction regimen is more toxic. The GMALL literature does not support the finding of higher toxicity of the newer protocols compared to the older ones. The major differences in induction between the older and newer protocol is the change

MRD = minimal residual disease

from *Escherichia coli* asparaginase to pegylated asparaginase and prednisone to dexamethasone. A possible explanation for the higher early mortality with the newer protocol could relate to the replacement of prednisone by dexamethasone, which may be associated with a higher rate of infection.

To conclude, this retrospective study showed a significant improvement in disease-free survival but no difference in overall survival for B-ALL patients treated with the newer GMALL protocols. The CR rates for the groups were equal, possibly indicating that post-remission therapy differences contributed to the outcome. Our study was too small to demonstrate differences for T-ALL patients as well as other subgroups of patients. The most difficult parameter to compare is the impact of supportive care on the outcome. The results are far from satisfactory and we need to further optimize the treatment in order to achieve better outcome in adult ALL.

Corresponding author:
Dr. A. Avigdor
Division of Hematology and Bone Marrow Transplantation, Sheba Medical Center, Tel Hashomer 52621, Israel
Phone: (972-3) 530-2588
Fax: (972-3) 530-5343
email: Abraham.Avigdor@Sheba.health.gov.il

References

1. Hoelzer D, Thiel E, Loffler H, et al. Prognostic factors in a multicenter study for treatment of acute lymphoblastic leukemia in adults. *Blood* 1988; 71 (1): 123-31.
2. Bennett JM, Catovsky D, Daniel MT, et al. Proposals for the classification of the acute leukaemias. French-American-British (FAB) co-operative group. *Br J Haematol* 1976; 33 (4): 451-8.
3. Vardiman JW, Thiele J, Arber DA, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. *Blood* 2009; 114 (5): 937-51.
4. Harris NL, Jaffe ES, Diebold J, et al. The World Health Organization classification of neoplastic diseases of the haematopoietic and lymphoid tissues: Report of the Clinical Advisory Committee Meeting, Airlie House, Virginia, November 1997. *Histopathology* 2000; 36 (1): 69-86.
5. Foa R, Vitale A. Towards an integrated classification of adult acute lymphoblastic leukemia. *Rev Clin Exp Hematol* 2002; 6 (2): 181-99, discussion 200-2.
6. Rowe JM. Prognostic factors in adult acute lymphoblastic leukaemia. *Br J Haematol* 2010; 150 (4): 389-405.
7. Le QH, Thomas X, Ecochard R, et al. Initial and late prognostic factors to predict survival in adult acute lymphoblastic leukaemia. *Eur J Haematol* 2006; 77 (6): 471-9.
8. Gokbuget N, Hoelzer D. Recent approaches in acute lymphoblastic leukemia in adults. *Rev Clin Exp Hematol* 2002; 6 (2): 114-41, discussion 200-2.
9. Lazarus HM, Richards SM, Chopra R, et al. Central nervous system involvement in adult acute lymphoblastic leukemia at diagnosis: results from the international ALL trial MRC UKALL XII/ECOG E2993. *Blood* 2006; 108 (2): 465-72.
10. Gokbuget N, Baur KH, Beck J, et al. Dexamethasone dose and schedule significantly influences remission rate and toxicity of induction therapy in adult acute lymphoblastic leukemia (ALL): results of the GMALL pilot trial 06/99. *Blood* 2005; 106: 1832a.
11. Gokbuget N, Hoelzer D, Arnold R, et al. Treatment of adult ALL according to protocols of the German Multicenter Study Group for Adult ALL (GMALL). *Hematol Oncol Clin North Am* 2000; 14 (6): 1307-25, ix.
12. Gokbuget N, Arnold R, Buechner TH, et al. Intensification of induction and consolidation improves only subgroups of adult ALL: analysis 0f 1200 patients in GMALL study 05/93. *Blood* 2001; 98: 802a
13. Rowe JM. Buck G, Burnett AK, et al. Induction therapy for adults with acute lymphoblastic leukemia: results of more than 1500 patients from the international ALL trial: MRC UKALL XII/ECOG E2993. *Blood* 2005; 106 (12): 3760-7.
14. Pulte D, Gondos A, Brenner H. Improvement in survival in younger patients with acute lymphoblastic leukemia from the 1980s to the early 21st century. *Blood* 2009; 113 (7): 1408-11.
15. Pui CH, Robison LL, Look AT. Acute lymphoblastic leukaemia. *Lancet* 2008; 371 (9617): 1030-43.
16. Vitale A, Guarini A, Chiaretti S, Foa R. The changing scene of adult acute lymphoblastic leukemia. *Curr Opin Oncol* 2006; 18 (6): 652-9.
17. Pui CH, Evans WE. Treatment of acute lymphoblastic leukemia. *N Engl J Med* 2006; 354 (2): 166-78.
18. Goldstone AH, Richards SM, Lazarus HM, et al. In adults with standard-risk acute lymphoblastic leukemia, the greatest benefit is achieved from a matched sibling allogeneic transplantation in first complete remission, and an autologous transplantation is less effective than conventional consolidation/maintenance chemotherapy in all patients: final results of the International ALL Trial (MRC UKALL XII/ECOG E2993). *Blood* 2008; 111 (4): 1827-33.
19. Bruggemann M, Raff T, Flohr T, et al. Clinical significance of minimal residual disease quantification in adult patients with standard-risk acute lymphoblastic leukemia. *Blood* 2006; 107 (3): 1116-23.
20. Bassan R, Hoelzer D. Modern therapy of acute lymphoblastic leukemia. *J Clin Oncol* 2011; 29 (5): 532-43.
21. Bachanova V, Sandhu K, Yohe S, et al. Allogeneic hematopoietic stem cell transplantation overcomes the adverse prognostic impact of CD20 expression in acute lymphoblastic leukemia. *Blood* 2011; 117 (19): 5261-3.
22. de Labarthe A, Rousselot P, Huguet-Rigal F, et al. Imatinib combined with induction or consolidation chemotherapy in patients with de novo Philadelphia chromosome-positive acute lymphoblastic leukemia: results of the GRAAPH-2003 study. *Blood* 2007; 109 (4): 1408-13.
23. Narayanan S, Shami PJ. Treatment of acute lymphoblastic leukemia in adults. *Crit Rev Oncol Hematol* 2012; 81 (1): 94-102.
24. Rabin KR, Poplack DG. Management strategies in acute lymphoblastic leukemia. *Oncology* 2011; 25 (4): 328-35.
25. Faderl S, O'Brien S, Pui CH. Adult acute lymphoblastic leukemia: concepts and strategies. *Cancer* 2010; 116 (5): 1165-76.

Capsule

Serial mutation

Mutations that affect gene function and, ultimately, the phenotype of an organism are grist to the mill of evolution. While examining the genetic basis for a stable polymorphism observed in bacteria during a long-term mutation experiment, Plucain et al. identified three specific, successive mutational events exhibiting synergistic epistatic and frequency-dependent interactions that enabled one lineage to invade the other and to be maintained. Thus, a series of specific mutations conferred the invasion phenotype and allowed the use of novel resources only when all mutations were present.

Science 2104; 343: 1366

Eitan Israeli