Review Article

Testicular Involvement of Acute Lymphoblastic Leukemia in Children and Adolescents: Diagnosis, Biology, and Management

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Acute lymphoblastic leukemia (ALL) in children and adolescents can involve the testes at diagnosis or upon relapse. The testes were long considered pharmacologic sanctuary sites, presumably because of the blood-testis barrier, which prevents the entry of large-molecular-weight compounds into the seminiferous tubule. Patients with testicular involvement were historically treated with testicular irradiation or orchiectomy. With the advent of contemporary intensive chemotherapy, including high-dose methotrexate, vincristine/glucocorticoid pulses, and cyclophosphamide, testicular leukemia present at diagnosis can be eradicated, with the risk of testicular relapse being 2% or lower. However, the management of testicular leukemia is not well described in the recent literature and remains relevant in low- and middle-income countries where testicular relapse is still experienced. Chemotherapy can effectively treat late, isolated testicular B-cell ALL relapses without the need for irradiation or orchiectomy in patients with an early response and thereby preserve testicular function. For refractory or early-relapse testicular leukemia, newer treatment approaches such as chimeric antigen receptor–modified T (CAR-T) cell therapy are under investigation. The control of testicular relapse with CAR-T cells and their penetration of the blood-testis barrier have been reported. The outcome of pediatric ALL has been improved remarkably by controlling the disease in the bone marrow, central nervous system, and testes, and such success should be extended globally. ***Cancer* 2021;127:3067-3081**. © *2021 American Cancer Society*.

LAY SUMMARY:

- Acute lymphoblastic leukemia (ALL) in children and adolescents can involve the testes at diagnosis or upon relapse.
- Modern intensive chemotherapy has largely eradicated testicular relapse in high-income countries. Consequently, most current clinicians are not familiar with how to manage it if it does occur, and testicular relapse continues to be a significant problem in low- and middle-income countries that have not had access to modern intensive chemotherapy.
- The authors review the historical progress made in eradicating testicular ALL and use the lessons learned to make recommendations for treatment.

KEYWORDS: acute lymphoblastic leukemia, adolescents, biology, children, diagnosis, management, testicular leukemia.

INTRODUCTION

As a result of improved risk-directed therapy and supportive care, the 5-year survival rate for pediatric acute lymphoblastic leukemia (ALL) has been increased to more than 90% in high-income countries.[1] The testes were long regarded as pharmacologic sanctuary sites, with the blood-testis barrier reducing the efficacy of systemic chemotherapy.[2] This was considered to account, at least in part, for the worse outcomes in boys than girls with ALL, and an international meta-analysis of ALL studies showed that a longer duration of maintenance therapy was associated with lower relapse rates, including those for the testes.[3] Boys were, therefore, given maintenance therapy for longer periods.[4] The introduction of high-dose methotrexate and overall improved systemic chemotherapy have eliminated the need for local irradiation or orchiectomy for testicular leukemia at diagnosis and have reduced the risk of testicular relapse.[5,6] Consequently, overt testicular leukemia at diagnosis has therapeutic implications but little prognostic significance, with testicular relapse now being a rare event.[6,7] However, the management of testicular leukemia is not well described in the recent literature and remains relevant in low- and middle-income countries where testicular relapse is still experienced. Here, we review the incidence, clinical presentation, and biology of testicular leukemia and the sequential improvements in and recommendations for its management.

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TESTICULAR INVOLVEMENT IN PATIENTS WITH NEWLY DIAGNOSED LEUKEMIA

Overt testicular involvement is found in 1.1% to 2.4% of boys at the time of diagnosis of pediatric ALL but is very rare in adults.[4,6,7] Testicular involvement can be diagnosed on the basis of increased size, irregular swelling, and/or firm consistency of the testes on physical examination. Ultrasonography with color Doppler is useful for confirming testicular involvement and can be used to evaluate other causes of scrotal enlargement, such as hydrocele, varicocele, extratesticular mass, and torsion, and to monitor responses to therapy.[8] Grayscale sonograms typically show enlargement of one or both of the testes with diffuse or focal regions of decreased echogenicity, and Doppler examination shows increased intralesional blood flow in areas of ALL involvement (Fig. 1). Patients with testicular involvement at diagnosis are more likely than others to have higher risk clinical or biological features: an age < 1 year or an age ≥ 10 years at presentation; high initial white blood cell counts; the T-cell phenotype; and other extramedullary involvement, such as central nervous system (CNS) leukemia, mediastinal mass, and/or hepatosplenomegaly.[4,6,7] Although the information on genetic subtypes is limited, patients with high-risk genetic features such as *BCR-ABL1* and *KMT2A* rearrangement can present with testicular disease at diagnosis and/or relapse.[4,7]

Although overt testicular leukemia at diagnosis is rare, a small study of bilateral wedge testicular biopsy at the diagnosis of ALL revealed occult testicular involvement without overt changes being noted on physical examination in 1 of 4 boys with T-cell acute lymphoblastic leukemia (T-ALL) and in 4 of 20 boys with non-T-ALL.[9] No clusters of blasts were seen on repeated biopsies after remission-induction therapy in 4 of these 5 boys, and this suggests that occult testicular leukemia is sensitive to chemotherapy and that pretreatment testicular biopsy is not indicated.

At St. Jude Children's Research Hospital (St. Jude), patients with overt testicular disease who were enrolled in the Total Therapy X and XI studies (1979 to 1988) had lower event-free survival (EFS) and overall survival (OS) estimates and a higher cumulative incidence of relapse than those patients without testicular disease.[4] However, no significant differences in outcome were observed between patients with and without overt testicular disease treated in the later Total Therapy XII to XIV studies (1988-2000).[6] Similarly, in the multicenter European Organization for Research and Treatment of Cancer 58881 trial (1989-1998), testicular involvement at diagnosis was associated with the presence of worse prognostic features but had no independent adverse prognostic significance.[7] Therefore, most current frontline ALL treatment protocols consider overt testicular involvement only as a criterion for higher risk ALL, for which more intensive chemotherapy is administered.

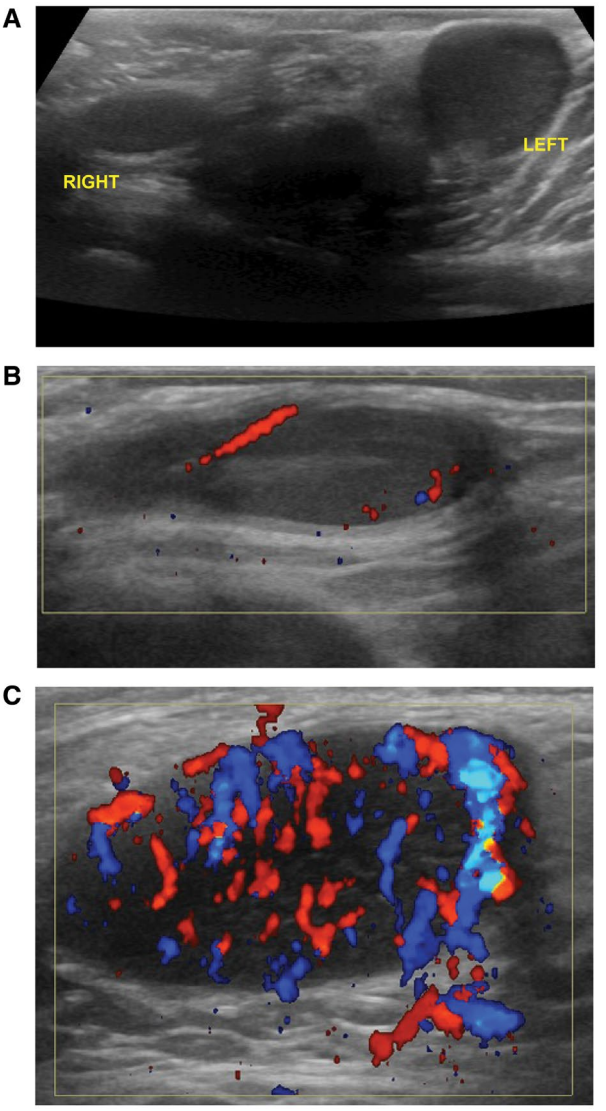

Figure 1. Ultrasound imaging of testicular leukemia. (A) Asymmetric testes (left > right). (B) Doppler sagittal imaging of the right testis shows normal vascularity at the epididymis. (C) Doppler sagittal imaging of the left testis shows hypervascularity.

TESTICULAR RELAPSE IN THE 1970s AND THE USE OF TESTICULAR BIOPSY TO DEMONSTRATE RESIDUAL DISEASE

In the 1970s, isolated overt testicular relapses occurred in 1% to 4% of boys during chemotherapy and in

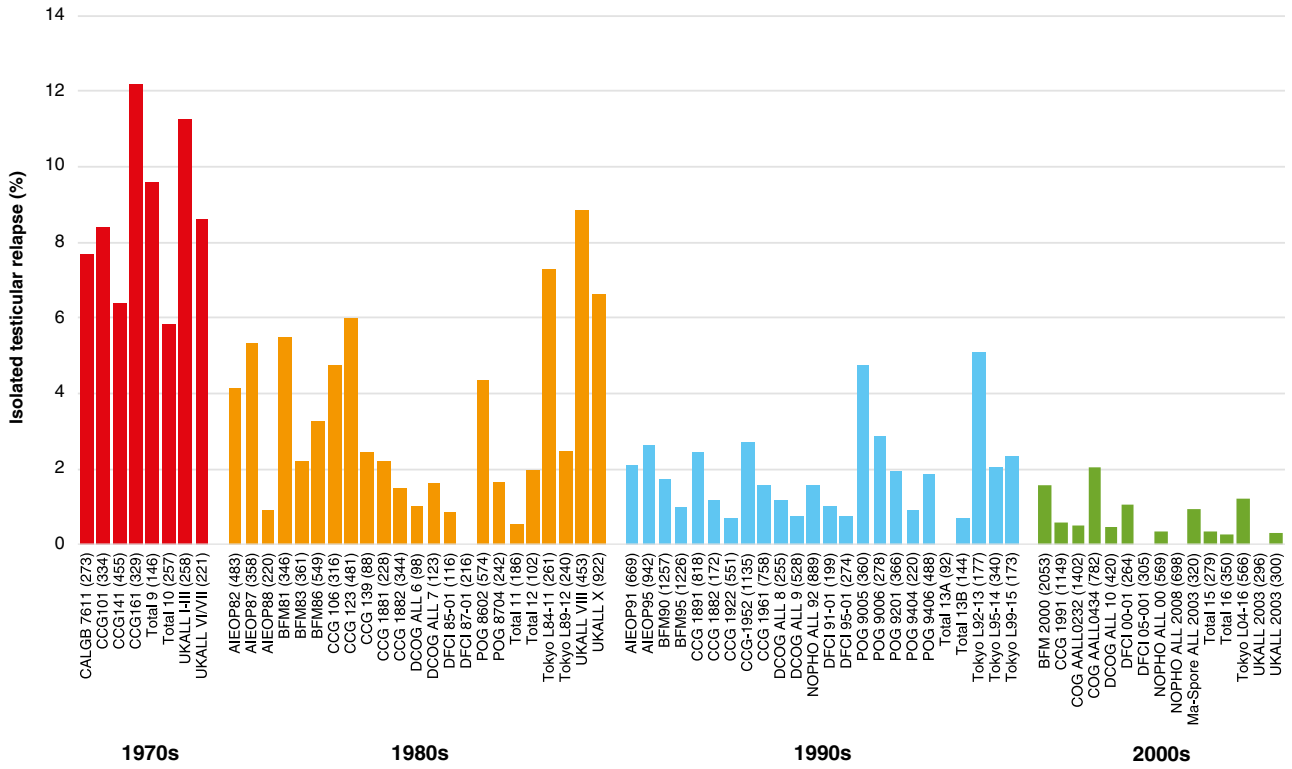

Figure 2. Decreasing incidences of isolated testicular relapses. The percentages of isolated testicular relapse are shown according to the decade in which the treatment protocols were initiated. Because testicular relapses were rarely presented as survival curves, the percentages of relapses represent proportions (total isolated testicular relapses/total male enrollments) during the median follow-up period of each treatment protocol. The numbers in parentheses show the total male enrollments in the studies. References for the treatment studies are provided in the supporting information. AIEOP indicates Associazione Italiana di Ematologia e Oncologia Pediatrica; ALL, acute lymphoblastic leukemia; BFM, Berlin-Frankfurt-Münster; CALGB, Cancer and Leukemia Group B; CCG, Children's Cancer Group; COG, Children's Oncology Group; DCOG, Dutch Childhood Oncology Group; DFCI, Dana-Farber Cancer Institute; Ma-Spore, Malaysia-Singapore; NOPHO, Nordic Society of Pediatric Hematology and Oncology; POG, Pediatric Oncology Group; UKALL, United Kingdom Acute Lymphoblastic Leukemia.

approximately 10% of those patients who completed 24 to 36 months of therapy (Fig. 2 and Supporting Table 1).[5,10-16] Therefore, testicular biopsy was often performed during and at the completion of chemotherapy and revealed occult testicular leukemia in 8% to 33% of the patients in hematologic remission.[16-20] However, a search for other occult extramedullary disease in 29 children with ALL in continuous complete remission at the end of therapy via kidney and liver biopsies, pelvic ultrasonography, intravenous pyelograms, skeletal surveys, cranial computed axial tomography scans, electroencephalography, and ophthalmologic examinations failed to find occult disease.[18] Patients with occult testicular leukemia in otherwise continuous remission in the bone marrow had significantly worse outcomes than patients with negative biopsies.[16,17] Testicular irradiation, orchiectomy, or both were commonly used for local control in patients with occult testicular leukemia. However, those patients often experienced subsequent relapse in their bone marrow and CNS without further relapse in the testes, a finding that led to additional intensified systemic chemotherapy in the salvage therapy.

In the St. Jude Total Therapy X study, which featured intensive chemotherapy, only 1 of 120 biopsies (0.8%) revealed occult testicular disease during continuation therapy or at the end of therapy.[13] In this study, 13 boys, including the one with occult disease, developed testicular relapses; 6 of the boys had had a negative biopsy finding 12 to 28 months before their testicular relapse, and 6 did not have a biopsy. The false-negative biopsy results were probably due to an overall improvement in systemic chemotherapy and/or to focal or scanty leukemic testicular infiltration that escaped microscopic detection. Consequently, elective testicular biopsy was considered not clinically beneficial and has now been discontinued; this decision is supported by

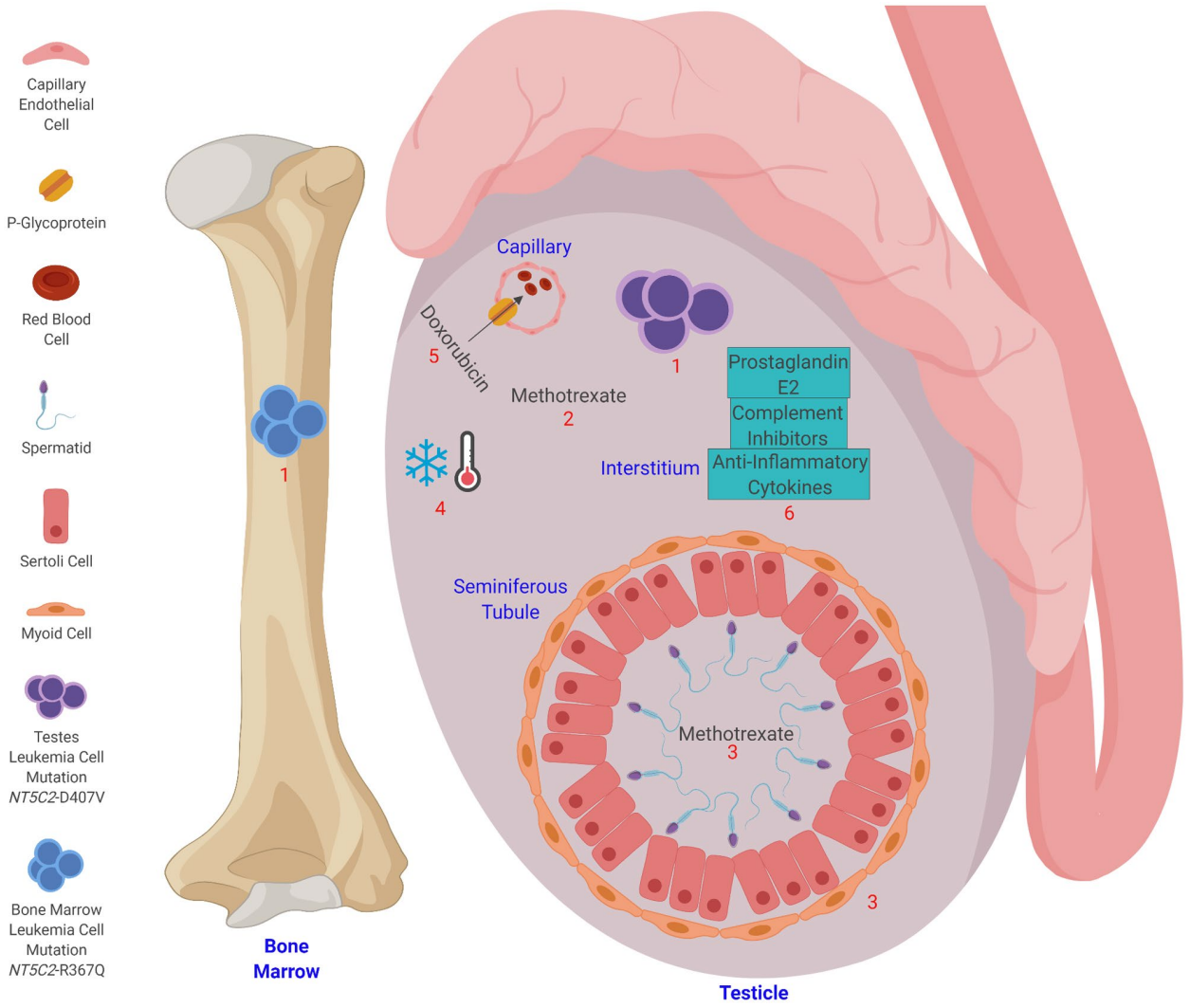

Figure 3. Possible mechanisms of chemotherapy resistance in the testes. Testicular leukemia relapse is considered to occur as a result of insufficient exposure to chemotherapy. (1) Leukemia relapse in the testes can develop from a clone that is independent of the one seen in the bone marrow relapse; in a patient with relapsed acute lymphoblastic leukemia, the testicular leukemia cells were found to have an *NT5C2*-D407V mutation, whereas the bone marrow cells had an *NT5C2*-R367Q mutation. (2) The chemotherapy concentration can be reduced in the interstitium; for example, methotrexate levels in the interstitium are lower than those in the serum by a factor of 2 to 4. (3) Tight junctions between the myoid cells and the Sertoli cells prevent the entry of large molecules into the seminiferous tubule. Methotrexate levels in the seminiferous tubule are lower than those in the serum by a factor of 18 to 50. (4) The testes are 2.5 °C colder than the body temperature, and this could decrease the efficacy of chemotherapy. (5) P-glycoprotein can export chemotherapy agents (eg, doxorubicin) from the testes into capillaries. (6) Prostaglandin E2, anti-inflammatory cytokines, and complement inhibitors in the testes help to make the testes an immune-privileged site.

the low frequency of testicular relapse observed in subsequent clinical trials.

BIOLOGY OF TESTICULAR INVOLVEMENT/RELAPSE

A mutational profiling study in a patient with combined bone marrow and testicular ALL relapse suggested that the leukemia in the testes represented a different subclone from that in the bone marrow, although both subclones were derived from the same ancestral clone.[21] Specifically, the leukemia cells in the testes of this patient were found to have an *NT5C2* mutation (*NT5C2*-D407V) that was different from the *NT5C2* mutation in the bone marrow leukemia cells (*NT5C2*-R367Q). The *NT5C2* mutation confers 6-mercaptopurine resistance on leukemia cells.[22] These findings suggest that

the chemotherapy exposure can differ between the bone marrow and the testes, and this in turn suggests that the testes are indeed pharmacologic sanctuary sites. Patients with isolated testicular relapses or combined testicular/bone marrow relapses (bone marrow disease in this setting is presumed to be due to seeding from testicular disease) have better survival than patients with isolated bone marrow relapses, and this also supports the concept of sanctuary sites.[23,24] Isolated bone marrow relapses are probably caused by selected drug-resistant clones, whereas testicular and combined relapses are caused by clones that are protected from selection pressure and remain susceptible to salvage therapy.

One possible explanation for the testes being sanctuary sites is the existence of a physiologic blood-testis barrier that prevents the entry of large-molecular-weight compounds into the seminiferous tubule (Fig. 3).[2] Anatomically, this barrier is believed to comprise the tight junctions of the myoid cells and Sertoli cells that line the seminiferous tubule. Whether a second physiologic barrier exists between the blood and the interstitial space has not been well defined. One or both barriers could modify the therapeutic efficacy and/or toxicity of the commonly used chemotherapeutic agents. The decreased ability of methotrexate to pass from the blood into the interstitial space (where the levels are lower than those in the serum by a factor of 2 to 4) and the seminiferous tubule (where the levels are lower than those in the serum by a factor of 18 to 50) has been demonstrated in a rat model.[2] ALL cells were usually found in the interstitial space, and infiltration of seminiferous tubules occurred only in the advanced stages of disease.[25] Although the difference in methotrexate penetration between the blood and the interstitium was relatively small, these findings suggested that ALL cells in the testes might be exposed to lower concentrations of chemotherapeutic agents. However, higher doses of chemotherapy could conceivably overcome the blood-testis barrier,[5,26,27] and cyclophosphamide and vincristine have been found to cross the barrier.[28]

Another possible cause of testicular relapse is the lower temperature of the testes compared with that of the rest of the body (Fig. 3), which might decrease the cytotoxic effects of chemotherapy.[29] This could explain why ovarian relapse is rarer than testicular relapse: the ovaries are located inside the abdomen, where the temperature is higher in comparison with the scrotum. Furthermore, it has been reported that treatment with estradiol significantly decreases the occurrence of leukemic infiltrates in rat testes.[30] Estrogen may prevent Leydig cells from binding to leukemia cells and may directly inhibit lymphoblast infiltration. P-glycoprotein present in the capillary endothelium of the testes could also lead to decreased chemotherapy exposure for leukemia cells by serving as an energy-dependent pump for the efflux of various chemotherapeutic agents (Fig. 3).[31]

The immune-privileged status of the testes could contribute to leukemia cell survival. The testes contain macrophages producing prostaglandin E2, which inhibits T-cell proliferation and natural killer cell function; testicular cells also produce anti-inflammatory cytokines and complement inhibitors (Fig. 3).[32] Leukemia cells themselves have mechanisms of immune tolerance. It has been postulated that a relatively low mutational burden results in fewer neoantigens being available for recognition by host T cells.[33] Similarly, danger-associated molecular patterns may not be present in concentrations sufficient to mediate dendritic cell maturation, and leukemia antigen presentation by immature dendritic cells results in T-cell tolerance.[34]

There are several possible explanations for the incidence of testicular involvement of ALL at diagnosis and relapse being lower in adults than pediatric patients. Intraperitoneal injection of T-ALL cells induced testicular infiltrates in 100% of sexually immature (25-day-old) rats but in only 42% of sexually mature (50-day-old) rats.[35] Infiltrates were located in the perivascular interstitial tissue. The testis extracts from the sexually matured rats, but not those from the immature rats, inhibited the proliferation of concanavalin A–stimulated normal lymphoblasts and leukemia cells. This suggests that the permeability of vascular endothelium and the immunosuppressive effect of the testes change with physiologic pubertal development. Also, the tight junctions of the blood-testis barrier diminish with age through reduced expression of genes and proteins involved in cell adhesion of the seminiferous epithelium.[36] This allows better chemotherapy penetration into the testes of adults, who typically receive intensive regimens with high-dose methotrexate and cytarabine.

FRONTLINE THERAPY TO CONTROL TESTICULAR LEUKEMIA

In the 1970s, presymptomatic testicular irradiation (12-18 Gy) after remission-induction therapy was used to reduce testicular relapse and prevent possible reseeding of the testicular leukemia to the bone marrow.[10,15] United Kingdom Acute Lymphoblastic Leukemia (UKALL) studies VI and VII found that testicular irradiation completely

TABLE 1. Randomized or Historical-Comparison Studies That Benefited for Testicular Disease Control

Protocol[a]	Risk	Steroid, mg/m^2			Methotrexate, g/m^2	Cranial Irradiation	Patient, No.	Male, No.	Relapse, %			
		Induction	DI	Maintenance					Isolated Testicular	Isolated CNS	BM	Any
Testicular radiation												
UKALL VI/VII	VI: HR VII: SR	Pred 40	No	Pred 40	No	Yes	NA	221	8.6	NA	NA	NA
(Testicular irradiation)	VI: HR VII: SR	Pred 40	No	Pred 40	No	Yes	NA	83	0.0[b]	NA	NA	NS
(No testicular irradiation)	VI: HR VII: SR	Pred 40	No	Pred 40	No	Yes	NA	138	13.8[b]	NA	NA	NS
MTX dosing												
CALGB 7611	SR/HR	Pred 40	No	Pred 40	0.5/no [R]	R	525	273	NA	NA	NA	NA
(IDMTX/ITMTX)	SR/HR	Pred 40	No	Pred 40	0.5	No	266	131	2.0[b]	28.0[b]	27.0[b]	NS
(CRI/ITMTX)	SR/HR	Pred 40	No	Pred 40	No	Yes	259	142	13.0[b]	8.0[b]	43.0[b]	NS
BFM 81, no MTX	SR/MR/HR	Pred 60	Dex 10	No	0	Yes	NA	241	6.7[b]	NA	NA	30.3
BFM 83, MTX 0.5	SR/MR/HR	Pred 60	Dex 10	No	0.5	SR-H/MR/HR	NA	313	2.5[b]	NA	NA	35.8
BFM 86, MTX 5	SR/Ri/E	Pred 60	Dex 10	No	5	Ri/E	NA	485	2.3[b]	NA	NA	25.8
Total 10	SR/HR	Pred 40	No	No	1 [R for SR]	Yes [R for SR]	427	257	5.8	8.2	22.2	38.4
(SR, HD-MTX)	SR	Pred 40	No	No	1	No	154	89	2.2[b]	11.0[b]	17.5[b]	36.4[b]
(SR, CRI)	SR	Pred 40	No	No	No	Yes	155	90	11.1[b]	3.9[b]	32.3[b]	45.2[b]
(HR)	HR	Pred 40	No	No	No	Yes	101	68	4.4	11.9	17.8	37.6
CCG 1882	HR/SER	Pred 60	Dex 10	Pred 40	Escalating [R]	Yes	311	172	1.2	2.6	23.5	28.6
(No MTX)	HR/SER	Pred 60	Dex 10	Pred 40	No	Yes	156	89	2.2	5.1	27.6	37.2[b]
(Escalating MTX)	HR/SER	Pred 60	Dex 10	Pred 60	Escalating	Yes	155	83	0.0	0.0	19.4	20.0[b]
CCG 1961	HR	Pred 60	Dex 10	Pred 40	Capizzi [R]	CNS+	1299	758	1.6	4.7	10.2	3.2
(No MTX)	HR	Pred 60	Dex 10	Pred 40	No	CNS+	649	392	2.6	4.9	12.9	4.0[b]
(Capizzi MTX)	HR	Pred 60	Dex 10	Pred 40	Capizzi	CNS+	650	366	0.5	4.5	7.5	2.3[b]
CCG 1991	B-SR	Dex 6	Dex 10	Dex 6	Escalating [R]	CNS+	2078	1149	0.6	1.8	4.5	8.1
(No MTX)	B-SR	Dex 6	Dex 10	Dex 6	No	CNS+	1036	561	1.2[b]	2.5[b]	4.2	9.3[b]
(Escalating MTX)	B-SR	Dex 6	Dex 10	Dex 6	Escalating	CNS+	1042	588	0.0[b]	1.1[b]	4.9	6.9[b]
Intrathecal therapy												
CCG 1952	SR	Pred 40	Dex 10	Pred 40	No	CNS+	2027	1135	2.7	4.4	7.0	16.0
(ITMTX)	SR	Pred 40	Dex 10	Pred 40	No	CNS+	1018	570	1.9[b]	5.7[b]	5.3[b]	14.8
(ITTriple)	SR	Pred 40	Dex 10	Pred 40	No	CNS+	1009	565	3.5[b]	3.1[b]	8.7[b]	17.2
Glucocorticoids												
CCG 161[c]	SR	Pred 40	No	Pred/No [R]	No	R	605	329	12.2	5.3	17.7	25.6
(VCR/Pred)	SR	Pred 40	No	Pred 40	No	R	302	163	6.1[b]	6.3	12.6[b]	18.9[b]
(No VCR/Pred)	SR	Pred 40	No	No	No	R	303	166	18.1[b]	4.3	22.8[b]	32.3[b]
CCG 1922	SR	Pred/Dex [R]	Dex 10	Pred/Dex [R]	No	CNS+	1060	551	0.7	5.4	10.6	16.4
(Pred)	SR	Pred 40	Dex 10	Pred 40	No	CNS+	530	281	1.1	7.0	12.1	19.8[b]
(Dex)	SR	Dex 6	Dex 10	Dex 6	No	CNS+	530	270	0.4	3.8	9.1	13.0[b]
BFM 2000	SR/MR/HR	Pred/Dex [R]	Dex 10	No	5	HR/T/CNS+	3720	2053	1.6	1.5	9.7	14.8
(Pred)	SR/MR/HR	Pred 60	Dex 10	No	5	HR/T/CNS+	1867	864	2.7[b]	2.0[b]	10.9[b]	17.3[b]
(Dex)	SR/MR/HR	Dex 10	Dex 10	No	5	HR/T/CNS+	1853	803	1.1[b]	1.0[b]	8.5[b]	12.4[b]
Asparaginase												
POG 8704	T	Pred 40	Pred 40	Pred 120	No	WBC > 50,000	317	242	1.7	3.5	26.5	41.6

TABLE 1. *Continued*

Protocol[a]	Risk	Steroid, mg/m^2			Methotrexate, g/m^2	Cranial Irradiation	Patient, No.	Male, No.	Relapse, %			
		Induction	DI	Maintenance					Isolated Testicular	Isolated CNS	BM	Any
(No asparaginase)	T	Pred 40	Pred 40	Pred 120	No	WBC > 50,000	157	124	3.2	4.5	34.4	55.4[b]
(Asparaginase)	T	Pred 40	Pred 40	Pred 120	No	WBC > 50,000	160	118	0.0	2.5	18.8	28.1[b]
Chemotherapy intensity												
CCG 1881	LR	Pred 40	Dex 10 [R]	Pred 40	No	CNS+	700	228	2.2	5.3	10.3	18.9
(No DI)	LR	Pred 40	No	Pred 40	No	CNS+	351	113	3.5	6.0	11.7	22.2
(DI)	LR	Pred 40	Dex 10	Pred 40	No	CNS+	349	115	0.9	4.6	8.9	15.5
CCG 1891	IR	Pred 40	Dex 10	Pred 40	No	CNS+	1204	818	2.4	6.5	8.1	18.4
(DI)	IR	Pred 40	Dex 10	Pred 40	No	CNS+	405	279	2.5	7.9	9.9	21.7[b]
(DDI)	IR	Pred 40	Dex 10	Pred 40	No	CNS+	402	264	1.9	4.7	5.7	13.7[b]
(DIVPI)	IR	Pred 40	Dex 10	Pred 40	No	CNS+	397	275	2.9	6.8	8.8	19.9[b]

Abbreviations: BFM; Berlin-Frankfurt-Münster; BM, bone marrow; B-SR, B-cell acute lymphoblastic leukemia, standard risk; CALGB; Cancer and Leukemia Group B; CCG, Children's Cancer Group; CNS, central nervous system; CRI, cranial irradiation; DDI, double delayed intensification; Dex, dexamethasone; DI, delayed intensification; DIVPI, delayed intensification with an increased number of vincristine and prednisone pulses; E, experimental group; HD-MTX, high-dose methotrexate; HR, high risk; IDMTX, intermediate-dose methotrexate; IR, intermediate risk; ITMTX; intrathecal methotrexate; ITTriple, triple intrathecal therapy; LR, low risk; MR, medium risk; MTX, methotrexate; NA, not available; NS, not significant; POG, Pediatric Oncology Group; Pred, prednisone; R, randomization; Ri, risk group; SER, slow early response; SR, standard risk; SR-H, standard risk–high; T, T-cell acute lymphoblastic leukemia; UKALL, United Kingdom Acute Lymphoblastic Leukemia; VCR, vincristine; WBC, white blood cell count.

Because the testicular relapses were rarely presented as survival curves, the relapse percentages represent the proportions (total of the specified event/total protocol enrollments [total male enrollments for testicular relapse]) during the median follow-up period of each treatment protocol. References for the treatment studies are provided in the supporting information.

[a]Parentheses indicate randomized groups.

[b]Significant difference between randomized groups.

[c]No data for isolated relapses are available; data for combined relapses are shown.

prevented testicular relapse, whereas the incidence in patients who received no irradiation was 13.8% (Table 1).[15] However, adding testicular irradiation to the treatment regimen did not improve the rates of bone marrow remission or survival and was associated with infertility and gonadal dysfunction.[10,15] Therefore, prophylactic testicular irradiation was discontinued.

Improvements in systemic therapy have been most efficacious in overcoming the pharmacologic sanctuary status of the testes. Methotrexate is given at a wide range of doses (eg, 20-5000 mg/m^2) in ALL therapy. The Cancer and Leukemia Group B 7611 protocol for children and adolescents with ALL randomized patients to receive either 3 courses of intermediate-dose methotrexate (500 mg/m^2 over 24 hours) plus intrathecal methotrexate or cranial irradiation plus intrathecal methotrexate (Table 1).[5] Patients who received the intermediate-dose methotrexate had a significantly lower incidence of testicular relapse (2% ± 1% vs 13% ± 3% at 12 years after diagnosis) and a lower incidence of bone marrow relapse (27% ± 3% vs 43% ± 3%) in comparison with those treated with cranial irradiation. The higher methotrexate plasma concentration and the increased penetration of methotrexate into the interstitium of the testes probably eradicated sequestered residual leukemia cells and prevented the emergence of drug-resistant clones. However, the CNS relapse rate was significantly higher in the intermediate-dose methotrexate group than the cranial irradiation group as a result of insufficient CNS-directed therapy (28% ± 3% vs 8% ± 2%).

Intermediate-dose methotrexate (500 mg/m^2) and high-dose methotrexate (5000 mg/m^2) were introduced in the Berlin-Frankfurt-Münster (BFM) 83 and 86 studies, respectively (Table 1).[37] Patients treated in those studies had equally low rates of testicular relapse (2.5% and 2.3%, respectively) in comparison with patients treated in the BFM 81 study without the increased dose of methotrexate (for whom the testicular relapse rate was 6.7%). In the Children's Cancer Group (CCG) 1882 (high risk and slow early response), 1961 (high risk and rapid early response), and 1991 (standard risk) studies, patients who received augmented postinduction therapy, including escalated intermediate doses of methotrexate without leucovorin rescue, had fewer relapses, including isolated testicular relapses, in comparison with those who did not receive it (Table 1).[38,39] Therefore, most current frontline regimens/protocols for patients with National Cancer Institute high-risk ALL include high-dose methotrexate or escalating doses of methotrexate and asparaginase without leucovorin rescue (the Capizzi regimen).

In the CCG 1952 study, which compared triple intrathecal therapy (methotrexate, hydrocortisone, and cytarabine) and single intrathecal therapy (methotrexate alone) in patients with standard-risk ALL who did not receive systemic high-dose methotrexate, triple intrathecal therapy was associated with better CNS control but also with significantly higher incidences of bone marrow and testicular relapse (Table 1).[38] These results, especially those of the Cancer and Leukemia Group B 7611, UKALL VI and VII, and CCG 1952 studies,[5,15,38] suggest that bone marrow, CNS, and testicular relapses are competing events. Therefore, treatment regimens should provide effective systemic (and testicular) therapy and CNS-directed therapy.

Vincristine and glucocorticoids have been used in remission induction and as pulses during continuation therapy. The CCG 161 study showed that monthly administrations of vincristine and prednisone pulses in patients with low-risk ALL significantly reduced relapses in the marrow and testes (both overt and occult) in comparison with patients not receiving the pulses (Table 1).[11] However, the vincristine/prednisone pulses did not improve OS; the patients receiving the vincristine/prednisone pulses had increased mortality in remission due to viral or *Pneumocystis jirovecii* infections. Vincristine is probably useful for preventing testicular relapse because of its ability to cross the blood-testis barrier.[28] Currently, dexamethasone is often used during delayed intensification and continuation therapy because the extramedullary tissue penetration of dexamethasone is superior to that of prednisone, especially in the CNS. The CCG 1922 study demonstrated that dexamethasone was more efficacious than prednisone for controlling systemic leukemia (including testicular relapse) in patients with standard-risk ALL.[38] The Associazione Italiana di Ematologia e Oncologia Pediatrica (AIEOP)–BFM ALL 2000 study also compared dexamethasone and prednisone during induction therapy and found that the incidences of overall relapse and isolated testicular relapse were significantly reduced in the dexamethasone arm.[40] Nevertheless, dexamethasone needs to be used judiciously because it was associated with an increased risk of severe infection and death.[40]

Asparaginase is routinely used to treat ALL. The Pediatric Oncology Group (POG) 8704 study for T-ALL demonstrated that intensive *Escherichia coli* L-asparaginase administration during consolidation resulted in outcomes superior to those obtained with the control regimen without asparaginase (Table 1).[41] No testicular relapse was seen in the 118 male patients who received

asparaginase, whereas 4 of 124 patients (3.2%) in the control arm experienced testicular relapse. CNS relapses were also less frequent in the asparaginase arm (2.5% vs 4.5%). Systemic administration of native or polyethylene glycol–conjugated *E. coli* asparaginase reduced asparagine levels in the cerebrospinal fluid, albeit completely in only 28% of the patients who received the latter agent.[42] Asparaginase would also be effective against ALL cells by depleting asparagine in the testes.

Cyclophosphamide is an important component of ALL therapy and can penetrate and exert antileukemia effects in the testes.[28] However, cumulative doses of 4000 mg/m^2 or greater are associated with Leydig cell dysfunction/failure and infertility (Table 2).[43,44]

TABLE 2. Impact of Various Treatments for Testicular Leukemia on Fertility

Therapy	Impact on Fertility
Chemotherapy	Alkylating agents: cumulative cyclophosphamide-equivalent doses of ≥4000 mg/m^2 are associated with Leydig cell dysfunction/failure and infertility.
Testicular irradiation	24 Gy: Severe gonadal dysfunction and azoospermia are expected. 15 Gy: Leydig cell function can be preserved to allow spontaneous pubertal development. Testicular irradiation with ≥4 Gy is associated with infertility.
Orchiectomy	Even unilateral orchiectomy is associated with infertility.
Hematopoietic cell transplantation	Total body irradiation is a significant risk factor for infertility.
Chimeric antigen receptor T cells	Unknown but expected to be minimal.

THE INCIDENCE OF TESTICULAR RELAPSE HAS SIGNIFICANTLY DECREASED WITH RECENT PROTOCOLS

In the 1980s, the reported incidence of isolated testicular relapses ranged from 0.0% to 7.3%; this was an improvement over the incidence of 5.8% to 12.2% in the 1970s (Fig. 2 and Supporting Table 1).[38,41,45-51] Higher incidences of testicular relapse were seen with treatment protocols such as BFM 81 (5.5%), CCG 123 (6.0%), Tokyo L84-11 (7.3%), and UKALL VIII and X (8.8% and 6.6%, respectively).[38,46,50,51] In those studies, routine use of intermediate- or high-dose methotrexate was not incorporated. Other protocols had very low incidences of testicular relapse during this period: 0.9% in AIEOP 88, 0.9% in Dana-Farber Cancer Institute (DFCI) 85-01, 0.0% in DFCI 87-01, and 0.5% in St. Jude Total Therapy XI.[45,48,49] All of these protocols included high-dose methotrexate (2-5 g/m^2), and the DFCI protocols used high-dose prednisone (120 mg/m^2) for high-risk patients during the intensification and maintenance therapy phases. During this period, the incidences of isolated CNS relapse and bone marrow relapse were still generally high: they ranged from 0.2% to 14.6% and from 10.1% to 26.5%, respectively. The addition of delayed intensification to lower risk ALL therapy in the CCG 1881 study decreased all bone marrow, CNS, and testicular relapses (Table 1).[38,41]

In the 1990s, the incidence of isolated testicular relapse was decreased to 0.0% to 2.9% (Fig. 2 and Supporting Table 1) in most studies,[38,41,45-50,52-55] although the incidence remained high in the POG 9005 and Tokyo L92-13 studies (4.7% and 5.1%, respectively)[41,50]; POG 9005 used prednisone only during induction without delayed intensification or routine use of high-dose methotrexate, and Tokyo L92-13 was characterized by a short duration of therapy (a total of 1 year) and was also associated with a high incidence of any relapse (32.3%). Most of the groups, other than the CCG, used high-dose methotrexate (1-8 g/m^2), and most protocols incorporated delayed intensification. Furthermore, improved risk stratification based on age, white blood cell count at diagnosis, immunophenotype, and cytogenetics was applied. In addition to isolated testicular relapse, the incidences of isolated CNS relapse and bone marrow relapses improved in the 1990s: they ranged from 0.6% to 6.7% for CNS relapses and from 4.0% to 24.3% for bone marrow relapses. Two delayed intensification phases, as opposed to a single phase, in patients with intermediate-risk ALL (in CCG 1891) and more intensive postinduction therapy in higher risk patients with a slow early response (in CCG 1882) or a rapid early response (in CCG 1961), in comparison with the control arms, resulted in improved systemic control, including better control of testicular relapse (Table 1).[38]

Since 2000, testicular relapses have been substantially reduced, and the incidences have ranged from 0.0% to 2.0% (Fig. 2 and Supporting Table 1).[39,40,56-67] As a result of the improvements in systemic and intrathecal chemotherapy, the use of cranial irradiation therapy for CNS-directed treatment has been markedly reduced. In most protocols, dexamethasone during either induction or postinduction therapy, high-dose methotrexate with close pharmacodynamics monitoring, and intensive asparaginase have been used for high-risk disease. The Children's Oncology Group

studies showed improved systemic control with high-dose methotrexate in high-risk B-cell acute lymphoblastic leukemia (B-ALL) and with Capizzi methotrexate in T-ALL.[56,57] Furthermore, many protocols improved risk stratification by incorporating genetic/cytogenetic classification and minimal residual disease (MRD). In addition to testicular relapses being better controlled, the rates of relapse in both CNS and bone marrow decreased during this period: they ranged from 1.2% to 3.2% and from 1.0% to 11.0%, respectively.

Currently, for patients with overt testicular disease at diagnosis, most groups monitor the response to induction therapy by physical examination and/or ultrasound and administer testicular irradiation only if persistent testicular involvement is confirmed by biopsy unless the involvement is considered unequivocal (Table 3). Because of the efficacy of multi-agent induction therapy, such cases are rare. The practice of administering longer maintenance therapy to boys than girls has been discontinued in most studies.[68]

MANAGEMENT OF TESTICULAR RELAPSE

Clinically isolated testicular leukemia should be considered a systemic disease because treatment by testicular irradiation or orchiectomy alone is usually followed by a bone marrow relapse within a few months.[16,17] Similarly, if no CNS treatment is given after a testicular relapse, 10% to 20% of the patients will subsequently develop CNS relapse. Indeed, an MRD assay using polymerase chain reaction or flow cytometry analysis can detect bone marrow disease in more than 50% of patients in whom a so-called isolated testicular relapse has been diagnosed by morphologic examination.[69] Therefore, systemic chemotherapy with effective CNS prophylaxis is required for boys with isolated testicular relapse, and MRD should be monitored (Table 3).

As shown in patients with bone marrow and isolated CNS relapses, the prognosis for patients with isolated testicular relapse depends on the time of relapse.[23] The 5-year OS for patients who had an early testicular relapse (<18 months after diagnosis; 13.6%) was significantly poorer than that for patients who had an intermediate relapse (18-36 months after diagnosis; 52.2%) or a later relapse (≥36 months after diagnosis; 60.0%). Furthermore, postrelapse survival is significantly worse in patients with T-ALL than those with B-ALL.[23] Therefore, for patients with B-ALL and early testicular relapse and for those with T-ALL and relapse at any time, very aggressive treatment, including hematopoietic cell transplantation (HCT), has been recommended (Table 3).[70-72] When total body irradiation (~12 Gy) is included in the conditioning regimen, a testicular boost (an additional ~12 Gy) is typically administered. Patients with combined bone marrow and testicular relapse are treated similarly to those with isolated bone marrow relapse.

Patients with B-ALL and an intermediate or late isolated testicular relapse can generally be cured by salvage chemotherapy (Table 3).[27,71] In addition to systemic chemotherapy, many salvage regimens have used testicular irradiation or orchiectomy of the affected testis, and it has been suggested that at least 24 Gy of scrotal irradiation (typically given bilaterally) is needed unless orchiectomy is performed.[27,71] Although there is insufficient evidence to favor one treatment modality over the other, orchiectomy can be justified if the patient has bulky testicular disease, if unilateral disease is probable, or if radiation is refused by the patient/family. The AIEOP-BFM group suggests orchiectomy for patients with unilateral testicular relapse along with biopsy of the contralateral testis to confirm that there is no involvement before the administration of prophylactic radiotherapy (15 Gy).[71] For patients with bilateral testicular involvement, either radiotherapy (24 Gy) or orchiectomy can be considered. Severe gonadal dysfunction and azoospermia are expected after 24 Gy of testicular radiation, but Leydig cell function can be preserved at 15 Gy to allow spontaneous pubertal development (Table 2).[73] Alkylating agents with cumulative cyclophosphamide-equivalent doses of ≥4000 mg/m^2, testicular irradiation with ≥4 Gy, unilateral orchiectomy, and total body irradiation in HCT recipients are also associated with infertility (Table 2).[43,44,74,75] Patients should be offered the option of fertility preservation through semen banking before testicular irradiation or orchiectomy. If patients are prepubertal, testicular tissue harvesting can be considered, although this is experimental and should be performed under a protocol approved by the institutional review board or research ethics committee.

To avoid the late effects on fertility and hormonal function resulting from testicular irradiation or orchiectomy, the Dutch Late Effects Study Group successfully treated 5 boys with late isolated testicular relapse with only systemic chemotherapy, including high-dose methotrexate (5-12 g/m^2).[26] Encouraged by this study, the Children's Oncology Group developed a study for patients with first isolated testicular relapse of B-ALL and an initial remission of at least 18 months.[27] Patients were treated with intensive multi-agent chemotherapy without testicular irradiation if their testicular leukemia resolved (as indicated by the resolution of enlargement or negative testicular biopsies) after induction therapy. Multi-agent chemotherapy included drugs known to penetrate

TABLE 3. Recommended Management of Testicular Involvement of Acute Lymphoblastic Leukemia

Evaluation at diagnosis and relapse
Physical examination of testes
Ultrasound examination of suspected cases
Biopsy to confirm testicular disease for patients with isolated testicular involvement at diagnosis or relapse or for those with equivocal testicular involvement at the time of concurrent bone marrow or central nervous system relapse
Treatment
Patients with newly diagnosed ALL
Induction chemotherapy
Biopsy for patients with persistent enlargement or abnormal ultrasound imaging after induction therapy (if involvement is unequivocal, biopsy may not be necessary)
Testicular irradiation (24 Gy, given bilaterally) for persistent leukemia involvement
Isolated testicular relapse
—Early relapse (<18 mo after diagnosis) in B-ALL and relapse at any time in T-ALL
Intensive chemotherapy[a] with hematopoietic cell transplantation
Testicular boost (12 Gy) with total body irradiation (12 Gy , given bilaterally)[b]
(Chimeric antigen receptor T cells for patients with B-ALL)[c]
—Intermediate relapse (18-36 mo after diagnosis) and late relapse (≥36 mo after diagnosis) in B-ALL
Systemic chemotherapy[a,d,e]
Bilateral: testicular irradiation (24 Gy) or orchiectomy
Unilateral: orchiectomy of the affected testis and prophylactic irradiation (15 Gy) of the biopsy-negative contralateral testis or testicular irradiation (24 Gy, given bilaterally)
(Chimeric antigen receptor T cells for patients with B-ALL)[c]
Combined testicular and bone marrow relapse
—Early relapse (<18 mo after diagnosis) and intermediate relapse (18-36 mo after diagnosis) in B-ALL and relapse at any time in T-ALL
Intensive chemotherapy[a] with hematopoietic cell transplantation
Testicular boost (12 Gy) with total body irradiation (12 Gy)[b]
(Chimeric antigen receptor T cells for patients with B-ALL)[c]
—Late relapse (≥36 mo after diagnosis) in B-ALL
Systemic chemotherapy[a,d,e]
Bilateral: testicular irradiation (24 Gy) or orchiectomy
Unilateral: orchiectomy of the affected testis and prophylactic irradiation (15 Gy) of the biopsy-negative contralateral testis or testicular irradiation (24 Gy, given bilaterally)
(Chimeric antigen receptor T cells for patients with B-ALL)[c]

Abbreviations: ALL, acute lymphoblastic leukemia; B-ALL, B-cell acute lymphoblastic leukemia; T-ALL, T-cell acute lymphoblastic leukemia.
The radiation dose, schedule, and timing may vary between treatment regimens.
[a]Immunotherapy (eg, blinatumomab) can be used in B-ALL, although the efficacy of blinatumomab for the treatment of testicular relapse is not established.
[b]For patients who do not receive total body irradiation, testicular radiation (24 Gy) or orchiectomy can be considered.
[c]The number of patients with testicular relapse who have been treated with chimeric antigen receptor T cells is limited.
[d]When intensified chemotherapy, including high-dose methotrexate, is given, some treatment regimens omit testicular irradiation or orchiectomy if no residual disease is observed after the induction regimen.
[e]Patients with minimal residual disease of ≥0.1% at the end of induction therapy should be treated in the same way as those with early relapse. Even in cases of isolated testicular relapse, minimal residual disease in the bone marrow can be positive at relapse.

the blood-testis barrier, such as high-dose methotrexate (5 g/m^2, 9 courses), cyclophosphamide (a maximum cumulative dose of 6.4 g/m^2, including prerelapse therapy), dexamethasone (10 mg/m^2/d during induction, reinduction, and maintenance), vincristine, and high-dose cytarabine (3 g/m^2, 8 doses). At the end of induction, 26 of 40 patients had persistent testicular enlargement, and 12 (30.0%) had confirmed residual leukemia by biopsy and were offered 24 Gy of bilateral testicular irradiation. The 5-year OS was 73.1%, and it did not differ between those patients who received bilateral testicular irradiation and those who did not. However, this study could not address the necessity of testicular irradiation for patients with T-ALL or for those with B-ALL and early relapse.

Rarely, an isolated testicular relapse can occur after HCT for refractory or relapsed ALL. Although treatment options are limited in these cases, because of the previous high cumulative exposure of the patients to chemotherapy and radiation, some patients with very late relapses after HCT have been successfully treated with orchiectomy.[76]

Recently, chimeric antigen receptor–modified T (CAR-T) cells targeting B-cell antigens (eg, CD19, CD22, or both) have shown efficacy in patients with relapsed/refractory B-ALL.[77] Although CAR-T cells have mostly been used to treat bone marrow relapse, CAR-T therapy has been effective for CNS leukemia, and CAR-T cells have been observed beyond the blood-brain barrier. Similarly, the control of testicular relapse with CAR-T cells and their penetration of the blood-testis barrier have been reported.[78-80] If other studies confirm CAR-T therapy to be effective for treating testicular relapse, this approach will be quite attractive because CAR-T cells can control both medullary and extramedullary disease and are expected to have less gonadal toxicity. It has not yet been shown whether other immunotherapeutic approaches, such as blinatumomab and inotuzumab ozogamicin, or molecularly targeted agents are effective against testicular leukemia.

TESTICULAR RELAPSE IN LOW- AND MIDDLE-INCOME COUNTRIES

Although the treatment and survival rates for ALL have improved significantly in high-income countries, clinicians and patients in low- and middle-income countries face many obstacles to successful treatment, including a late presentation/delayed diagnosis, coexisting debilitating conditions such as malnutrition and infections, suboptimal treatment adherence, abandonment of treatment, shortages of chemotherapeutic agents, inadequate supportive care, and a lack of specialists.[81] Therefore, patients in low- and middle-income countries still experience testicular relapse. Salvage therapy is not always available, and when it is, salvage rates are dismal. A single-institution study in India showed that the rates for isolated relapse and any (isolated and combined) testicular relapse were

similar for 93 boys treated from 1984 to 1986 (3.2% and 6.5%, respectively) and for 361 boys treated from 1986 to 1993 (3.3% and 6.1%, respectively).[82,83] Another single-institution study in India reported 17 patients (4.2%) with isolated testicular relapses and 30 patients (7.4%) with any testicular relapse among 407 boys treated with a regimen based on the UKALL X study from 1990 to 2006.[84] A salvage regimen (reinduction, testicular irradiation, and maintenance therapy) was given to 12 of the 17 boys with isolated testicular relapses but to only 1 of the 13 boys with combined relapses. At the Hue Central Hospital in Viet Nam, 156 children with ALL (including 93 boys) were treated with the CCG 1881 or CCG 1882 regimen from 2012 to 2018. Four patients (4.3%) developed any testicular relapses, including 2 patients (2.2%) with isolated testicular relapses.[85] Neither study used intermediate- or high-dose methotrexate.

It has been shown that the administration of high-dose methotrexate is feasible with extended hydration and leucovorin rescue along with monitoring of serum creatinine and urine pH without measuring methotrexate levels.[86] High-dose methotrexate (3 g/m^2 for low-risk disease and 5 g/m^2 for intermediate- and high-risk disease) was incorporated into the Shanghai Children's Medical Center (SCMC) ALL-2005 protocol.[87] At the SCMC, the 1085 patients (including 667 boys) treated between 2005 and 2014 had 5-year EFS and OS rates of 68.3% and 80.0%, respectively. The cumulative incidence of relapse was 24.5% at 10 years, and isolated and any testicular relapses were seen in 30 (4.5%) and 38 (5.7%) of the 667 boys, respectively. In addition, 165 of the 1085 patients (15.2%) experienced isolated bone marrow relapses, and 29 (2.7%) had any CNS relapse. This suggests that merely incorporating high-dose methotrexate is insufficient.

It is important to develop centers of excellence and study groups with an adequate number of trained professionals who can adapt well-designed protocols to the local conditions.[81,88] Twinning or global collaboration between centers or groups in low/middle-income countries and those in high-income countries will facilitate the advancement of treatment. To overcome socioeconomic hardships, charitable organizations, philanthropists, and government agencies should be involved to enable better access to medical resources and patient/family support.[88] In this regard, 20 major hospitals in the China Children's Cancer Group, including the SCMC, collaborated with St. Jude to launch the ALL-2015 protocol.[89] Between 2015 and 2019, 7640 patients (including 4521 boys) were enrolled, and the 5-year EFS and OS rates were 80.3% and 91.1%, respectively. Of the 7640 patients, 658 (8.6%) experienced relapse, with 470 experiencing (6.2%) isolated bone marrow relapses and 136 (1.8%) experiencing any CNS relapse. Remarkably, isolated and any testicular relapses were limited to 27 (0.6%) and 46 (1.0%) of the 4521 boys, respectively. This excellent result validates the effectiveness of a comprehensive collaborative approach between low/middle-income countries and high-income countries, which can lead to improved survival and a reduction in both medullary and extramedullary relapses.[81]

In conclusion, as a result of the introduction of improved systemic combination chemotherapy regimens that eradicate testicular leukemia by penetrating the blood-testis barrier, the incidence of testicular relapse has significantly decreased. Recent advances in immunotherapy and molecularly targeted therapy are expected to result in further improvements in the survival of patients with ALL and to decrease the intensity and toxicity of treatment with conventional chemotherapeutic agents. ALL is cured by controlling disease in the 3 major body compartments: the bone marrow, the CNS, and the testes. It is important to confirm that newer treatment strategies do not compromise the leukemia control in any of these compartments. Furthermore, successful treatment strategies used in high-income countries need to be adapted for use in low- and middle-income countries through comprehensive collaborations so that all patients have a chance for a cure, regardless of their geographic location.

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