T–CELL ACUTE LYMPHOBLASTIC LEUKEMIA PATHOGENESIS AND THERAPY

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1. Introduction

1.1- Scope -

T-cell acute lymphoblastic leukemia (T-ALL) is an uncommon form of acute leukemia, accounting for about 10% of cases in children and 3% in adults. Nonetheless, T-ALL is important beyond its numbers for several reasons. First, it strikes at a young age. Second, although it is often lethal, it is potentially curable. Finally, T-ALL has been a fertile ground for investigations of mutations of growth-regulating genes which contribute to the pathogenesis of leukemia. Today, I will review characteristic features of the biology of T-ALL, recent advances in its treatment and new understanding of its molecular pathogenesis. I thank my collaborators, Richard Baer (Dept. of Microbiology) and George Buchanan and Dr. Julie Katz (Dept. of Pediatrics) for allowing me to present some of their data.

1.2- Leukemias and Lymphomas originating in the T-Lymphocyte Lineage

T-ALL is just one of several hematopoietic malignancies which originate in the T-cell lineage (1,2). To avoid confusion, I have listed these malignancies in Table 1. These tumors can be divided into phenotypically immature malignancies, including T-ALL and lymphoblastic lymphoma, and phenotypically mature tumors, including diffuse aggressive lymphomas, adult T-cell lymphoma leukemia, cutaneous T-cell lymphoma/Sezary's syndrome and various other leukemias of mature T-cell phenotype (Table 1). My focus today is upon the leukemias of immature lymphocyte phenotype, namely, T-ALL. In general, these are high grade, high growth fraction tumors which express markers of T lymphocyte precursors such as terminal deoxynucleotidyl transferase (TdT). I will not discuss the other T lineage malignancies today; references to their characteristics (Table 1) are included at this end of this protocol.

Table 1. Characteristics of Leukemias/Lymphomas Derived from the T-Lymphocyte Lineage

Immature Phenotype	Pathology	Immunophenotype	Clinical Features	References
T-ALL	Acute Leukemia, mass disease	TdT^+ $CD5^+$ $CD7^+$ $CD3^-$	Acute leukemia +/- mass disease	3
Lymphoblastic lymphoma	Mediastinal mass, nodes	TdT^+ $CD5^+$ $CD7^+$ $CD3^-$	Mass disease→acute leukemia	4
Mature Phenotype				
Diffuse mixe and large cell lymphomas	Nodes, spleen, liver	TdT^- $CD3^+$ $CD4^+$	Diffuse aggressive lymphoma (25% of cases)	5
Adult T cell lymphoma/ leukemia	Skin, nodes, any organ, leukemia	TdT^- $CD3^+$ $CD4^+$ $CD8^-$ CD25+	Fulminant course, $\uparrow Ca^{++}$, HTLV-1+	6
Cutaneous T cell lymphoma/ Sezary's syndrome	Skin, nodes	TdT^- $CD3^+$ $CD4^+$ $CD8^-$	Indolent skin neoplasia	7
T cell CLL	Marrow, nodes, spleen, CNS	TdT^- $CD3^+$ $CD4^+$ $CD8^-$ or $CD4^-$ $CD8^+$	More aggressive than B-CLL	8
T cell prolymphocyte leukemia	Spleen, liver, leukemia	TdT^- ,$CD3^+$ $CD4^+$ $CD8^-$ or $CD4^-$ $CD8^+$	Splenomegaly, high WBC	9
T-Cell hairy cell leukemia	Spleen	TdT^- $CD3^+$ $CD4^+$ $CD8^-$	Rare - like B- lineage HCL. HTLV-2+	10
Large granular lymphocytosis	Blood, spleen, ± marrow	TdT^- $CD3^+$ $CD8^+$ or TdT^- $CD3^-$ $CD8^-$ $CD57^+$	Splenomegaly, neutropenia	11,12

1.3- Acute lymphoblastic leukemia; defining characteristics.

Pathologists and hematologists have long divided acute leukemia into lymphoblastic and myelogenous types. This distinction, based upon morphologic and cytochemical criteria, has been verified by modern phenotypic analysis (13). Currently, three morphologic subtypes of ALL are recognized: L1, L2 and L3 (14). Subtype L2, by far the most common in adults, signifies lymphoblasts with an intermediate pattern of nuclear chromatin condensation, occasional nucleoli; small to moderate amounts of cytoplasm, and variability in size and nucleocytoplasmic ratio. Immunophenotypic analysis has shown that about 85% of ALL is of the B-cell precursor type (BCP-ALL), while about 15% is of T-cell precursor type (T-ALL) (Table 2).

Table 2. Immunophenotype Classification of ALL

	CD19	CD10	Cμ	SmIg	CD7	CD5	TdT
B-Lineage ALL							
Early B	+	-	-	-	-	-	+
Common	+	+	-	-	-	-	+
Pre-B	+	+	+	-	-	-	+
B	+	+/-	+/-	+	-	-	-
T-ALL	-	-/+	-	-	+	+	+

Adapted from Ref. 13

The significance of immunophenotype is now increasing, with the discovery that immunophenotype and specific gene derangements are tightly correlated.

1.4- Adult Versus Childhood ALL: General Comparisons.

While the development of therapy for childhood ALL has been a model of success in medical oncology, unfortunately the same cannot be said of adult ALL. Compared to children, adults with ALL gain fewer complete remissions, relapse earlier and have substantially lower rates of cure (15). Although the reasons for these differences are not entirely clear, some of the key factors are listed in Table 3.

Table 3. Adults Fare Poorly - Why?

1. Increased drug resistance
2. More Ph translocations
3. Less hyperdiploid karyotypes
4. Poor tolerance for therapy

Clearly, adult ALLs acquire resistance to the standard therapeutic drugs more commonly than do pediatric ALLs. Acquisition of the multidrug resistance (mdr) phenotype may account in part for this difference (16). Cytogeneticists have recognized that certain karyotypes predict an adverse therapeutic outcome in pediatric ALL, and these karyotypes are more common in adult ALL. These cytogenetic differences include a decreased percentage of the favorable hyperdiploid karyotype, and an increased percentage of the Philadelphia translocation (or its molecular equivalent) in adult ALL. Bartram and associates have reported that 55% of adults with common ALL have molecular evidence of the Philadelphia translocation, in comparison to 5% of pediatric ALLs (17). Cytogenetic analysis has shown a smaller but still highly significant excess of Philadelphia translocations in adult ALL (~25%) in comparison to pediatric ALL (~5%). This difference cannot account for an adverse outcome in T-ALL, since the Philadelphia chromosome (or its molecular equivalent) is found almost exclusively in BCP-ALL rather than in T-ALL. Finally, host factors are important in therapeutic outcome in ALL. Tolerance for aggressive multiagent chemotherapy decreases continuously with age in adults, but this does not entirely explain the adverse effect of age on prognosis (18,19). The age distribution of patients must be taken into account when comparing results of treatment in different published reports.

2.- T-ALL: Characteristic Clinical Features (Table 4)

Table 4. Clinical Features of T-ALL

Age range: preadolescent to young adult
3:1 male predominance
Mass disease
High lymphoblast count in peripheral blood
Increased incidence CNS leukemia
Tumor lysis syndrome

Ref 3

The incidence of T-ALL peaks in the preadolescent through early adult years (3). This pattern differs sharply from the age incidence of BCP-ALL, which peaks between ages two and five. There is a three to one male predominance. T-ALL often presents with extramedullary mass disease, including anterior mediastinal masses, lymphadenopathy and hepatosplenomegaly. T-ALL is closely related to another neoplasm, lymphoblastic lymphoma, which presents in the same extramedullary sites but without marrow involvement. The immunophenotypes of T-ALL and lymphoblastic lymphoma are quite similar, and if inadequately controlled lymphoblastic lymphoma regularly progresses to a leukemic stage. The presenting white blood cell count in T-ALL is higher on average than in BCP-ALL. Central nervous system (CNS) involvement (meningeal leukemia) is more common at presentation in T-ALL than in BCP-ALL. At diagnosis, asymptomatic CNS involvement may be detected by the presence of lymphoblasts in the cerebrospinal fluid (CSF). On the other hand, full-blown symptomatic presentations either at diagnosis or at relapse feature lethargy, headache, nausea, vomiting, and cranial nerve palsies. CNS leukemia is easier to prevent than to treat, especially once cranial nerve deficits appear. This fact mandates surveillance for asymptomatic disease, which can then be treated to prevent progression. The high burden of leukemia frequently seen at presentation in T-ALL, together with the large growth fraction and increased cell turnover, explains the risk of the tumor lysis syndrome (20). Since this complication may be aggravated by chemotherapy, adequate preventive measure prior to beginning therapy are essential, including volume diuresis, allopurinol and alkalinization of the urine.

In summary, the unusual epidemiology and presentation with a high burden of disease suggest common pathogenetic events which differ from those behind BCP-ALL. Molecular genetic analysis is beginning to clarify some of these events.

2.1- Illustrative Case Histories

2.1.1- RD is a 30 year old man who presented with lethargy and cervical lymphadenopathy. His white blood count was 260,000, all CD2+ lymphoblasts. Under Dr. Barry Cooper's care, he began on multiagent remission induction chemotherapy according to a modification of the "Linker" regimen (21). Within 5 weeks he was in complete remission, received prophylactic intrathecal chemotherapy and radiotherapy, and began cycles of intensive multiagent consolidation chemotherapy. Seventeen months after diagnosis, while receiving oral maintenance chemotherapy, he developed a rapidly expanding subcutaneous mass over the lower sternum. Biopsy showed sheets of lymphoblasts. His peripheral blood, bone marrow and CSF were normal. He was referred to Dr. Robert Collins at Baylor University Medical Center, where he received high dose Cytoxan and total body radiation, followed by a bone marrow transplant from a sibling donor. Except for mild manifestations of chronic graft versus host disease, he is free of disease one year post-transplant.

2.1.2- CM, an 11 year old boy, presented to Childrens Medical Center with generalized adenopathy, a white blood count of 350,000 and a mediastinal mass. Circulating cells were CD5+, CD7+, CD19-. He was begun on multiagent remission induction chemotherapy (Pediatric Oncology Group (POG) Protocol T-3 (#8704)). Within five weeks he was in complete remission and began cycles of intensive reinduction and continuation chemotherapy. In month 22, while still receiving intensive therapy, he developed bone marrow, testicular and CNS relapse. He was begun on salvage chemotherapy and achieved a remission which lasted only ten weeks.

2.1.3- *Comment.* These cases illustrate the tendency of T-ALL to recur in spite of intensive chemotherapy. These patients also illustrate the nasty habit of T-ALL to recur with explosive rapidity, which is a reflection of the high growth fraction of the disease. Such recurrence usually implies selection for drug resistant subclones and generally portends a fatal outcome. In the case of RD, the option of high dose therapy with two modalities not previously used, together with a possible graft versus leukemia effect, offers some hope for salvage.

3. Treatment.

3.1 - Basic Outline of Therapy for ALL

Over the past 30 years, principals of therapy of childhood and adult ALL have been established in sequential clinical trials (15,22,23) (Table 5).

Table 5. Treatment of ALL - General Approach

Supportive care -	infections, bleeding, tumor lysis
Remission induction -	vincristine, prednisone, L-asparaginase, anthracyclines
CNS prophylaxis/therapy -	cranio (spinal) radiation, intrathecal methotrexate or cytosine arabinoside
Post-induction therapy -	intensive rotating polychemotherapy
Maintenance therapy -	Low-intensity chemotherapy.

First, supportive care is given prior to remission induction chemotherapy. This includes the treatment of infection, bleeding, anemia, and measures to prevent or treat the tumor lysis syndrome. Remission induction is designed to reduce the burden of leukemic blast cells at least 1,000 fold; and consists of multiagent chemotherapy, including the lympholytic drugs vincristine, prednisone, and especially in adults anthracyclines and/or L-asparaginase. Remission is

accomplished in more than 95% of children and in 70-80% of adults. Next, therapy is given to treat microscopic or overt central nervous system leukemia. For prophylactic therapy, methotrexate and/or cytosine arabinoside is given intrathecally, and cranial irradiation is routinely given to adults. High-dose methotrexate or cytosine arabinoside is also effective prophylaxis because these drugs penetrate the CNS when given in large dosage. Depending upon the risk of recurrence, which is comparatively high in childhood T-ALL and in all forms of adult ALL, intensive cycles of multiagent chemotherapy are given for 12-18 months after remission, with the aim of eradicating minimal residual disease. For lower risk ALL, which includes the majority of childhood BCP-ALL, low intensity post-remission therapy has been shown to increase cure rates (23). This phase of therapy is termed "maintenance" and typically includes daily oral 6-mercaptopurine and weekly methotrexate, with or without intermittent vincristine/prednisone. This therapy is routinely continued for 12-18 months. "Maintenance" therapy has not been proven beneficial in high risk disease when given following intensive post-remission therapy.

The results of this general approach to therapy have been gratifying, particularly in childhood BCP-ALL, with cure rates now approximating 70%. However, in all forms of adult ALL and in pediatric T-ALL the outcome has not been as favorable. Even with intensive chemotherapy, only 30-40% of adults (15) and 50-60% of children (3) with T-ALL will be cured.

3.2 - Treatment of Pediatric T-ALL

The dividing line between pediatric and adult T-ALL is blurred, because a large fraction of patients are in the adolescent age range. Moreover, biologic features of T-ALL in both age groups are similar. No separate studies on the treatment of adult T-ALL exist, as large therapeutic trials have included adults with both T-ALL and BCP-ALL. In the pediatric arena, T-ALL is recognized as a high-risk subset of disease which has prompted studies specifically designed to treat this phenotype. Therefore, most of the advances in the treatment of T-ALL have come from the pediatric cooperative trials.

In the mid 1970's, immunophenotypic studies were developed which led to the recognition of the high-risk nature of T-ALL in children. There followed a series of empiric sequential trials to improve outcome. One early trial originated at the Memorial Sloan-Kettering

Cancer Center in an effort to improve the treatment of lymphoblastic lymphoma. An innovative regimen called LSA2-L2 was developed which incorporated cyclic therapy with pairs of drugs for 2 years after remission induction (24). This approach was designed to overcome drug resistance but the selection of drugs was totally empiric. Nonetheless, the LSA2-L2 program achieved a 30-40% long-term disease-free survival in T-ALL (25).

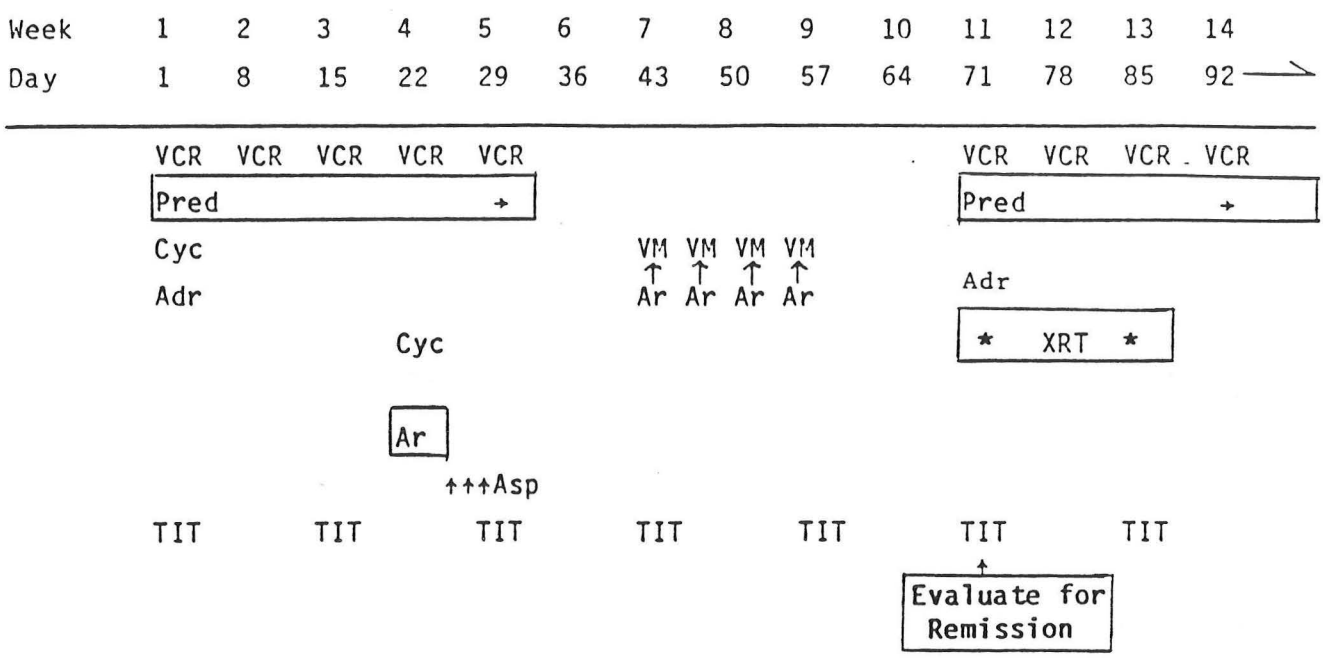
A more disease-specific approach began with the observation that T-ALL cell lines were up to 100 times more sensitive to cytosine arabinoside than were B lineage cell lines. Many of the subsequent innovations in the therapy of T-ALL have centered around increases in the dose-intensity of this drug. Cytosine arabinoside is commonly given with Cytoxan or teniposide, and the contribution of each drug to cell kill is sometimes difficult to unravel.

To understand why cytosine arabinoside might be selectively toxic for T-ALL blasts, its biochemical pharmacology must be reviewed. In order to kill cells, cytosine arabinoside must be transported across the cell membrane by a process of facilitated diffusion, phosphorylated to cytosine arabinoside triphosphate, and incorporated into growing DNA chains. Intracellular cytosine arabinoside triphosphate must avoid dephosphorylation and deamination by degradative enzymes. Wiley and co-workers have carefully studied the comparative handling of cytosine arabinoside by fresh BCP- and T-ALL blast cells (26). The rates of phosphorylation, dephosphorylation and deamination were approximately equal in the two types of cells. On the other hand, the density of nucleoside transport sites was far greater in T-ALL than in BCP-ALL cells. Although this difference may be partially explained by the higher growth fraction of T-ALL blasts, kinetic behavior alone is unlikely to account for the large excess of transport sites in T-ALL cells.

These *in vitro* differences led to a trial which compared the efficacy of cytosine arabinoside/Cytoxan pulses in BCP- and T-ALL. In a small collaborative study conducted at the Midwest Cancer Center of Milwaukee and UT Southwestern Pediatric Hematology/Oncology Division, pulses of cytosine arabinoside and Cytoxan during maintenance were found to improve the outcome of children with T-ALL but not BCP-ALL (27). This small study lead to a large trial in the Pediatric Oncology Group (POG). An outline of this protocol (POG #8704; T-3 Study) is shown in Figure 1 (28). Following a period of three months of induction and reinduction therapy, nine week cycles of these alternating groups of drugs are given for a total of 10 cycles.

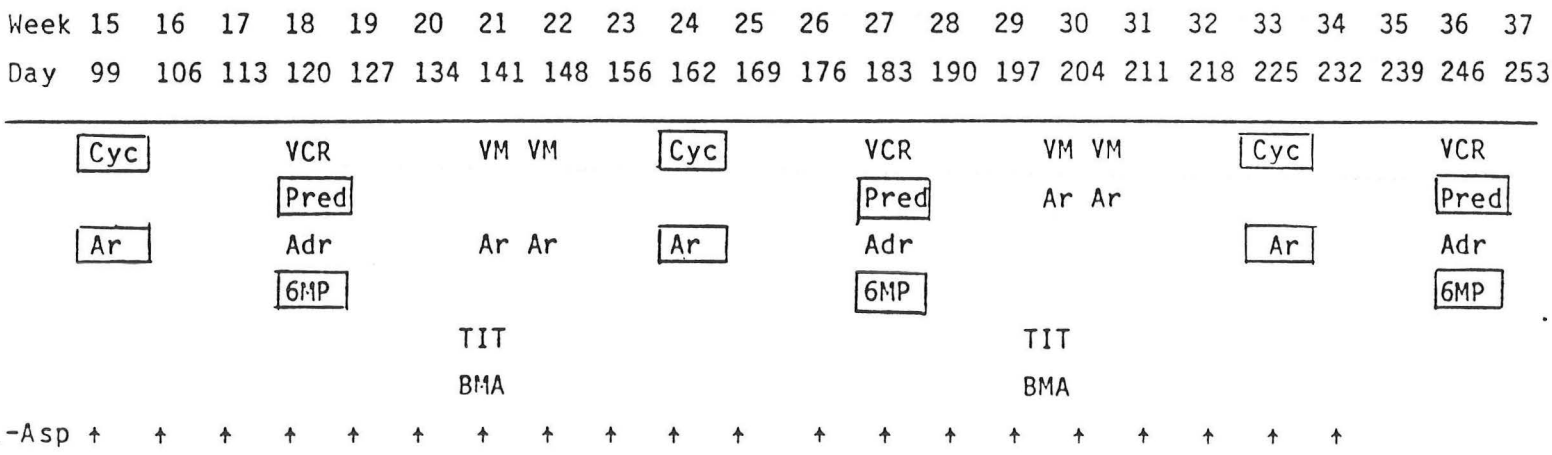
APPENDIX III - POG #8704 - TREATMENT II
CHEMOTHERAPY SCHEMA: T-3 PROTOCOL - INDUCTION AND CONSOLIDATION

Patients without CNS Disease



* XRT for patients with WBC >50,000 at diagnosis

CHEMOTHERAPY SCHEMA: T-3 PROTOCOL CONTINUATION



TO CONTINUE FOR 10 NINE-WEEK CYCLES

VCR = Vincristine
Pred = Prednisone
Cyc = Cyclophosphamide
Adr = Adriamycin
VM = VM-26
Ar = Cytosine arabinoside (Ara-C)

ASP = Asparaginase
TIT = Triple intrathecal therapy: Methotrexate, hydrocortisone, and cytosine arabinoside
6-MP = 6-mercaptopurine

(From Drs. Julie Katz and George Buchanan)

The three drug groups include cytosine arabinoside and cyclophosphamide; vincristine, prednisone, Adriamycin and 6-mercaptopurine; and finally, VM26 and cytosine arabinoside. Thus, drugs known to be particularly active in T-ALL were emphasized during the prolonged post-remission therapy period, as opposed to the strictly empiric use of many different agents in the earlier LSA2-L2 program. The results of the POG T-3 study demonstrated a modest increase in relapse-free survival when compared with the historical results with the LSA2-L2 regimen. Thus, four-year event free survival on the T-3 study was 50%, compared to 42% on the LSA2-L2 program (29). When compared to the older regimen, the current data suggest that the T-3 program may be delaying recurrences rather than increasing cure rates.

3.3 - Relapse with Acute Myelogenous Leukemia.

Recently at the St. Jude Hospital and in POG trials, a number of children with ALL have relapsed with acute myelogenous leukemia (AML). In the St. Jude series, children with T-ALL were at greatest risk (30) However, current experience also indicates that patients with BCP-ALL are also at risk. It now appears that the major risk factor for these myeloid relapses is intensive treatment with the drug VM-26. This drug, which produces single-and-double-stranded breaks in DNA, was introduced into front-line therapy after its combined use with cytosine arabinoside in relapsed ALL demonstrated noncross-resistance with older combinations of drugs. Given its actions on DNA, it is reasonable to expect that VM-26 and its commercially available relative VP-16 may be leukemogenic.

Interestingly, a large fraction of these myeloid relapses have had different cytogenetic abnormalities from those present in the earlier ALLs. The new karyotypes commonly include abnormalities involving chromosome 11 band q23. This locus is of interest because abnormalities here have previously been noticed in infants with acute leukemias of very primitive immunophenotypes, and in both children and adults with ALL with mixed lymphoid-myeloid features (31). In several well studied cases, however, a clonal relationship between the original T-ALL and the relapse AML was clearly demonstrated (32,33). In the absence of more extensive studies on the clonal relationships between these two leukemias, it is not yet clear whether these cases represent the induction of a new leukemia or a selection of subclones of the original leukemia with a different developmental potential.

3.4 - Therapy of Adult T-ALL

As mentioned earlier, adults with T-ALL have been lumped together with the more prevalent BCP-ALL patients in treatment trials. Recall that adults with ALL as a group do much less well than do children. Nonetheless, the overall results in adult ALL have improved with careful increases in dose intensity in sequential trials (15,18,19,21,34) (Table 6).

Interestingly, in three trials of intensive and prolonged post-remission therapy, which included cytosine arabinoside, adults with T-ALL fared at least as well as did patients with BCP-ALL. In the German trial, the T immunophenotype was a favorable marker, with 45% of patients continuously free of disease at five years (19). Similar results were achieved in the trial at the Memorial Sloan-Kettering Cancer Center (18). In a smaller community study carried out in the San Francisco Bay area, adults with T-ALL did about as well as did patients with BCP-ALL (21). This Bay Area protocol, devised by Charles Linker, is currently our choice for treatment of adults with both T and BCP-ALL. In summary, given the high risk of treatment failure in adult ALL, T-ALL is not an especially bad form of the disease when treated with prolonged intensive chemotherapy. Moreover, perhaps one-third of adults with T-ALL appear to have been cured with these regimens.

Table 6. Adult ALL -- Recent Therapeutic Results

Trial (Ref)	N	Median Age	Induction	CNS Rx	Intensification	Maintenance	CR, %, (Median Months)	Median Survival Months	Probability CCR
Germany (19)	368	25	V,P,A,D,C Ara-C,MTX, 6MP	IT MTX, RT	V,Dex,ADM Ara-C,C,TG	6MP, MTX	74 (24)	25	35% at 10yr
Memorial (18)	199	<25=47% 25-50=37% >50=16%	V,P (D,ADM,C)	MTX (via Ommaya reservoir)	Ara-C,TG,A,V (D,P,MTX,C, int.. Ara-C,BCNU)	MTX,V,C,BCNU (TG,6MP,HU, D,P,ADM,ActD)	82 (31)	28	38% at 15yr
Italy (34)	358	31	V,P,A,D	IT MTX,	V,P,int. Ara-C, VM,Ara-C,D	MTX, 6MP,VP (int. Ara-C, VM,Ara-C,D) (Randomized Study)	79 (22)	22	21% at 4yr
Bay Area ("Linker") (21)	109	24 (range16-49)	V,P,D,A	IT MTX, RT	V,P,D,A VM, Ara-C, int. MTX	6MP, MTX	87	?	39% at 5yr

V, vincristine; P, prednisone; A, L- asparaginase; D, daunorubicin; C, Cytoxan; Ara-C ,cytosine arabinoside; MTX, metrotrexate; 6MP, mercaptopurine, ADM, adriamycin; TG, throguanine; VM, VM-26 (teniposide); Dex, dexamethasone; BCNU, carmustine; HU hydroxyurea; Act D, actinomycin D; IT, intrathecal; RT, cranial radiotherapy, int., intermediate dose.

3.5 - Role of Bone Marrow Transplantation.

In children and young adults with acute myelogenous leukemia (AML), allogeneic bone marrow transplantation clearly reduces the risk of disease relapse (35). Therefore, it is appropriate to ask whether similar treatment for high risk acute lymphoblastic leukemia might increase cure rates. Unfortunately, in a recent retrospective comparison, allogeneic bone marrow transplantation in first remission ALL (including T-ALL) was not superior to intensive prolonged post-remission chemotherapy when measured by leukemia free survival (36). Moreover, bone marrow transplantation did not seem to offer any advantage within any particular high-risk subset, such as patients with a high white blood count at diagnosis, any specific immunophenotype, or prolonged time to achievement of first remission. The reason for this lack of difference is the balancing liabilities of each form of treatment (Figure 2). Specifically, with intensive chemotherapy the risk of disease relapse is higher than with bone marrow transplantation. On the other hand, the complications of bone marrow transplantation lead to a higher treatment-related mortality which cancels the benefits of decreased risk of disease recurrence.

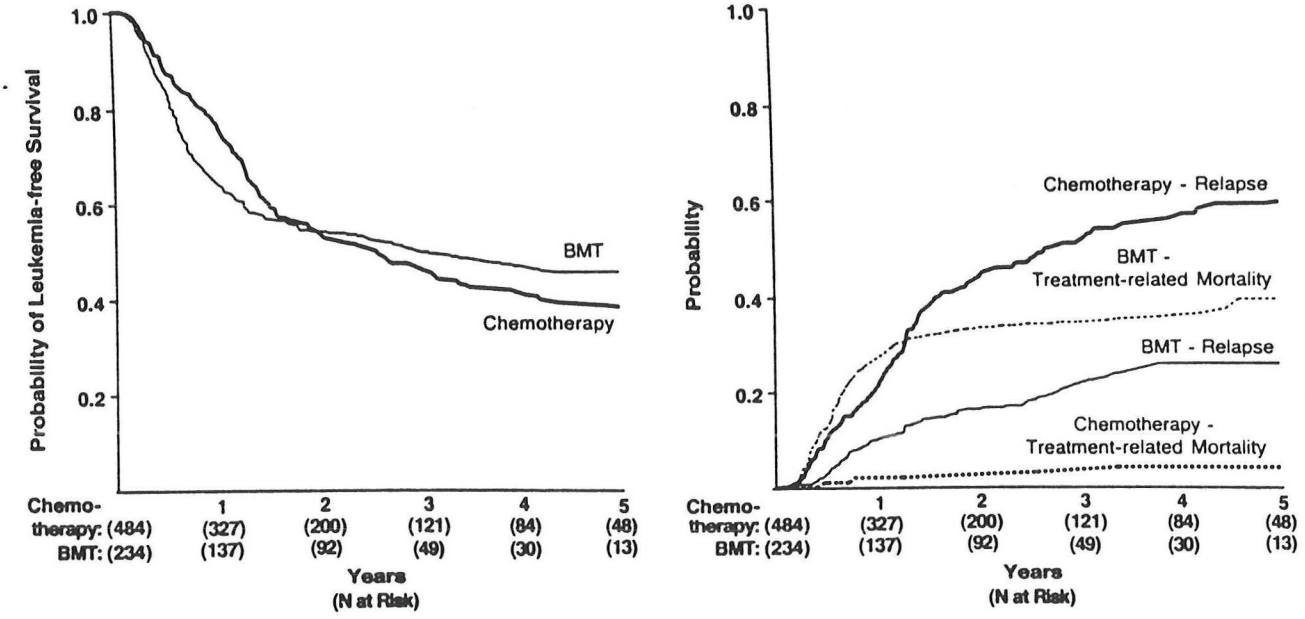


Figure 2. Adjusted probabilities for adults with acute lymphoblastic leukemia in first remission according to type of postremission therapy. Left. Leukemia-free survival. **Right.** Relapse and treatment-related mortality. Numbers in parentheses indicate number (*N*) of patients remaining at risk (alive in remission). BMT = bone marrow transplant.

(From Reference 36)

4. Pathogenesis of T-ALL

4.1 - Disorders of Cell Regulation in T-ALL

Certain disorders of cellular regulation, common to all forms of acute leukemia, are particularly well illustrated in T-ALL (Table 7).

Table 7. Regulatory Defects in Acute Leukemia

1. Excessive proliferation (self-renewal).
2. Failure to complete differentiation program.
3. Aberrant differentiation.
4. Proliferation outside normal microenvironments.

First, there is excessive self-renewal in the leukemic population, demonstrating a clearly impaired regulation of proliferation. Second, there is massive failure of the leukemic population to complete a normal program of differentiation. Furthermore, leukemia cells often express combinations of genes not seen in normal development, for example "mixed" lymphoid-myeloid phenotypes. Finally, these malignant T-cell precursors proliferate outside their normal microenvironments. Increasing evidence suggests that somatic alterations or mutations in genes which regulate growth and differentiation ("oncogenes") are responsible for these disorders of the normally tightly coupled processes of cell proliferation and differentiation.

4.2 - The Key Clue: Recurrent Chromosomal Translocations in T-ALL

A number of cytogeneticists, mainly those who serve large cooperative pediatric leukemia/lymphoma groups, have found recurring chromosomal translocations in T-ALL blast cells collected at the time of diagnosis (37-39). These translocations frequently disrupt chromosome 14 band q11 or somewhat less commonly, chromosome 7 band q35. The second chromosome involved in these balanced translocations is variable, but the most common breakpoints are

chromosome 11 band p13, chromosome 10 band q24, chromosome 1 band p34, chromosome 9 band q35 and chromosome 8 band q24 .

The paradigm for understanding the significance of these translocations emerged from earlier studies of the consistent chromosome translocation found in Burkitt's lymphoma, namely t(8:14) (q24;q32) (40). In these Burkitt lymphomas, the breakpoint in chromosome 14 band q32 in every case involved the immunoglobulin heavy chain gene locus. The other breakpoint, on chromosome 8 band q24, was either in or close to the c-*myc* locus. c-*myc* is known to be a growth-regulated gene whose expression rises quickly after cells receive a signal to proliferate. Moreover, c-*myc* is known to be a powerful oncogene when captured by chicken retroviruses. Current hypothesis argues that in the Burkitt's translocations, the c-*myc* gene is brought under the influence of active immunoglobulin heavy chain gene enhancer regions on chromosome 14. The true situation is probably more complicated, but it is clear that the regulation of c-*myc* expression is dramatically altered by these translocations (40).

In T-ALL, a similar situation began to emerge from analysis of the recurring chromosomal translocations (41). As noted above, in virtually every case, one of the two translocation breakpoints involved either chromosome 14 band q11 or chromosome 7 and q35. It turns out that these breakpoints cleave either the T-cell antigen receptor $\alpha-\delta$ (chromosome 14 band q11) or β (chromosome 7 band q35) locus. As in Burkitt's lymphoma, in the T lineage cells the antigen receptor loci contain active enhancer sequences which could stimulate the expression of growth regulatory genes brought into their vicinity by the translocations. Therefore, the next step was to identify what growth-regulatory genes ("oncogenes" analogous to c-*myc* in Burkitt's lymphoma) might be involved in these T-ALL translocations..

In studying these translocations, a systematic approach to the characterization of the involved genes has evolved (Table 8).

Table 8. Workup of Translocations in Leukemia-Lymphoma

1. Identify immune receptor breakpoint using immune receptor sequences as probes.
2. Clone breakpoint fragments.
3. Locate open reading frames (ORFs) near breakpoints.
4. Sequence ORFs.
5. Compare sequence to known sequences (homology to genes regulating growth, development, signal transduction?).
6. Study expression in normal and tumor tissue.
7. Isolate full-length cDNA clones (structural alterations of gene product ?).
8. Characterize genomic organization and surrounding regulatory sequences.
9. Test whether translocated gene is oncogenic.

The hypothesis argues that the immune receptor gene is joined to the candidate oncogene, thus activating its expression. Therefore, the immune receptor gene can be used as a tag to isolate cloned pieces of the genome within which to search for the oncogene. It is possible to "walk" or "jump" away from the immune receptor gene sequences, looking for open reading frames (ORFs). The ORF sequences can be determined and compared systematically to other sequences in the databases for clues regarding gene function. The anatomy of the rearrangement is studied and compared to the normal counterpart gene and its adjacent regulatory sequences. The expression of this gene in normal versus tumor tissue is studied in order to detect increased or inappropriately timed expression. One possibility (not yet observed in T-ALL) is that the structure of the gene is altered by the translocation to produce a new, fusion gene composed of sequences normally present on separate chromosomes (42).

With all this information in hand, if the newly identified gene adjacent to the translocation breakpoint is plausibly related to a growth regulatory gene, experiments can be designed to ask whether the translocation is an oncogenic event. For example, the gene as it is altered by translocation can be transferred into suitable test cells to

determine whether it is a transforming gene; that is, whether the altered gene changes the pattern of growth and differentiation in the test cells (43). A more powerful test of the oncogenic nature of the disrupted gene is to study its effect in transgenic mice. Thus, a c-*myc* gene, when joined to immunoglobulin gene enhancer sequences and overexpressed in B lineage cells, yields B-cell lymphomas and leukemias in transgenic mice (44).

4.3 - Candidate Oncogenes Adjacent to T-ALL Translocaton Breakpoints

Following this approach (Table 8), a number of investigators have characterized candidate growth regulatory genes immediately adjacent to the recurrent translocation breakpoints found in T-ALLs. These genes are summarized in Table 9.

Several important points concerning these genes are worthy of discussion. First, with the exception of the *c-myc* oncogene, cleaved by the t(8;14)(q24;q11) translocation, these gene disruptions are specific for T-ALL. To date, translocations involving these genes have not been reported in other hematopoietic neoplasms. Second, in all, these translocations are found in about 20-25% of T-ALLs. Third, as mentioned earlier, all of these translocations involve either the T-cell receptor α-δ or the T-cell receptor β locus.

Two implications of T-cell receptor gene involvement deserve mention. First, these translocations are likely to have been generated by mistaken immune receptor recombinase action. The normal role of this recombinase is to rearrange gene segments to create a functional immune receptor variable region gene during lymphocyte ontogeny. This recombinase is active only at the precursor stage of lymphocyte development, and T-ALL blasts are arrested at this stage of maturation. In support of this hypothesis, certain molecular details of these chromosomal translocations strongly suggest that the immune receptor recombinase participates in their generation (41). Second, the juxtaposition of growth-activating genes and T-cell receptor loci enables enhancer sequences to activate the adjacent oncogenes. The analogy to c-*myc* activation by the immunoglobulin heavy chain enhancers in Burkitts lymphoma (t(8;14)(q24;q32) translocation) is obvious.

Table 9. Genes Disrupted by Translocation in T-ALL: Functions of their Homologues in Normal Growth/Development

Investigator (Ref.)	Translocation	Gene	Fr	Regulatory Family	Example of Normal Function (Ref.)
Rabbitts (45)	t(11;14)(p13;q11) t(7;11)(q35;p13)	*rhom-2*	11%	LIM family (cysteine-rich domains)	cell fate decision (*lin-11, c. elegans*) (60)
Dube (46)	t(10;14)(q24;q11)	*hox-t*	4%	homeodomain	regional morphogenesis (embryogenesis; *Hox-1.6*, mouse) (61)
Baer/Kirsch/Croce (47-51)	t(1;14)(p34;q11)	*tal-1*	3%	basic-helix-loop-helix (bHLH); homology to *lyl-1*	myogenesis (*myoD*, mammals etc.) (62) sensory organ formation (*achaete-scute, drosophila*) (63)
Sklar (52)	t(7;9)(q35;q34.3)	*tan-1*	2%	cell membrane protein	sensory organ formation (*notch, drosophila*) (64)
Rabbitts/Korsmeyer (53, 54)	t(11;14)(p15;q11)	*rhombotin-1* (*ttg-1*)	1%	(see *rhom-2*)	(see *rhom-2*)
Several (55)	t(8;14)(q24;q11)	c-*myc*	1%	bHLH/leucine zipper	cell proliferation (widespread) (65)
Baer (56)	t(7;9)(q35;q34)	*tal-2*	<1%	(see *tal-1*)	(see *tal-1*)
Cleary (57)	t(7;19)(q35;p13)	*lyl-1*	<1%	bHLH; homology to *tal-1*	(see *tal-1*)
Rabbitts,Croce (58, 59)	t(14;14)(q11;q32.1)	unknown	<1%	unknown	unknown

Fr, frequency of translocation in T-ALL blasts

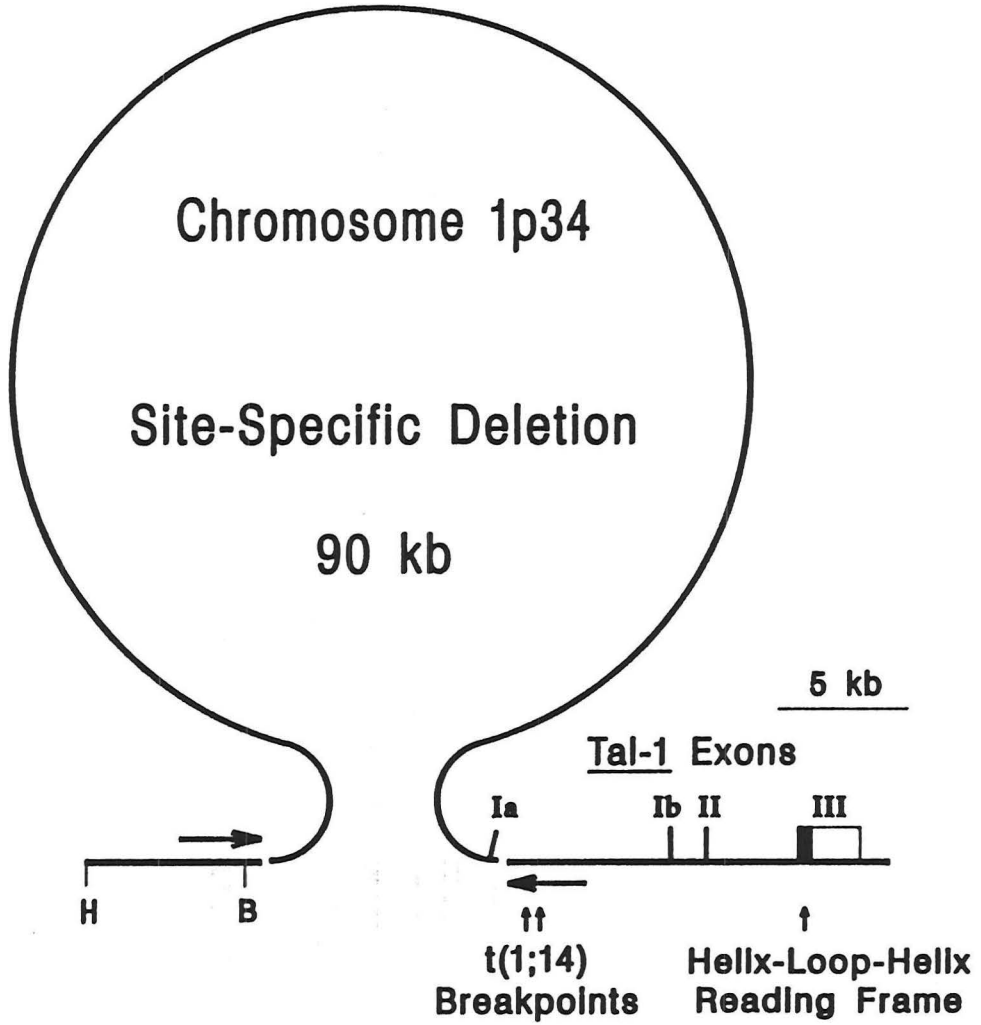
Analysis of ORFs near these T-ALL translocation breakpoints has shown that all but one of the candidate genes are related to genes known to encode transcription control factors. Many of these transcription factors are known to control growth and development in a variety of biological systems (Table 9). The one exception, the TAN-1 gene, is related to a gene in *drosophila* which encodes an integral membrane protein. However, like the transcription factors, the function of this protein, called notch, is to regulate development, in this case of cutaneous sensory organs in *drosophila* (64). The implication of the homology between genes disrupted in T-ALL and growth regulatory genes in other biologic systems is clear: namely, these disrupted genes very likely mediate the imbalance between proliferation and differentiation characteristic of T-ALL.

4.4 - The *tal-1* gene: A Transcriptional Regulator Disrupted in 28% of T-ALLs.

Several of the genes listed in Table 9 encode so-called basic-helix-loop-helix (bHLH) proteins. These proteins are members of a growing class of transcriptional regulators which dimerize by hydrophobic interactions along one face of the α-helices. Dimerization is required for binding to DNA along the basic domains which are adjacent to the helix-loop-helix domains. Analyzing the t(1;14)(p34;q11) translocations, Richard Baer of our Department of Microbiology and independently groups led by Ilan Kirsch and Carlo Croce, discovered the *tal-1* gene immediately adjacent to the breakpoint on chromosome 1 band p34 (47,50,51).This gene encodes a bHLH protein and has close homology to the *lyl-1* gene disrupted in another case of T-ALL (57) (Table 9).

The t(1;14) translocation is found in 3% of T-ALLs. Probing with the *tal*-1 gene in T-ALLs which lacked the t(1;14) translocation, Richard Baer found a common rearrangement of this locus in 25% of these leukemias submitted from the cell bank of the Pediatric Oncology Group (48). It turned out that this rearrangement was a precise site specific deletion on chromosome-1, one end of which was within an the intron of this gene and the other end 90kb upstream. The distal end of this deletion (within a *tal-1* intron) was within about 1kb of the clustered translocations breakpoints seen in the t(1;14)(p34;q11) cases (Figure 3). Thus, the functional effects of the translocation and

Site-Specific Deletions Truncate the tal-1 Gene in T-ALL



tal^{d} Allele

deletions may be similar. The remarkable site specificity of the deletions together with certain molecular details of these rearrangements, suggest that aberrant recombinase activity may create the deletions as well as the translocations. Taken together, the translocations and deletions are therefore seen in 28% of T-ALLs. This observation illustrates an important point in molecular oncology: there are numerous ways that a growth-regulatory gene may be activated, and only some of these ways are visible by cytogenetic analysis. Thus, these genes may play a role in a larger fraction of tumors than would be guessed from cytogenetic analysis alone. When the *tal-1* site-specific deletions and all the translocations listed in Table 9 are both taken into account, nearly 50% of all T-ALLs have already defined rearrangements of candidate oncogenes. Additional types of mutations and rearrangements of these and other oncogenes will surely further increase this figure.

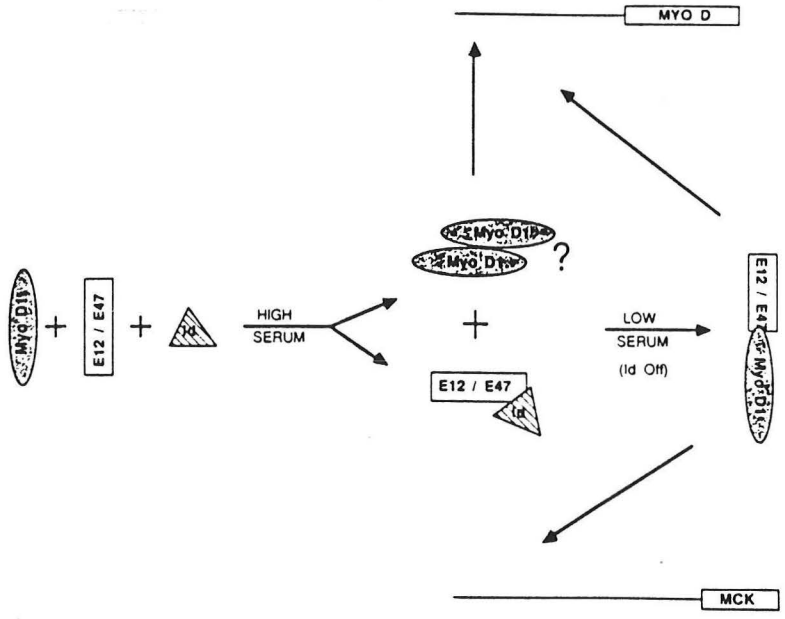
4.5 - Basic-Helix-Loop-Helix Genes: Functions and Possible Consequences of Rearrangements.

In order to understand possible roles of these rearranged genes in leukemogenesis, a brief digression on their structure and function is in order. These bHLH proteins are "master genes" which regulate complex programs of proliferation, cell type specification and cell fate determination in a variety of biologic systems (41). They accomplish this regulation by binding to specific core sequences in the enhancers adjacent to many target genes. Binding does not occur until these proteins dimerize, which is accomplished by interactions between the helix-loop-helix domains. These helixes are amphipathic, with one face being substantially hydrophobic with the other being hydrophilic (66). Protein-protein interactions are thought to occur between hydrophobic faces of the α helical regions. Interaction can occur between a large variety of proteins containing these helix-loop-helix domains, creating a flexible system of transcriptional control of a large group of genes.

The *myo-D* family of genes is illustrative of these functions in mammalian development (62, 67). This family of genes encodes several closely related bHLH proteins which are specifically induced in mesenchymal cells destined to differentiate towards a skeletal muscle phenotype. To accomplish this, the *myo-D* protein interacts with a tissue-nonspecific bHLH protein called E12. This heterodimer then binds to the enhancers which regulate a large number of muscle

specific genes, for example, creatine kinase. This binding activates expression of these muscle-specific genes, thus regulating skeletal muscle differentiation. Remarkably, in several experimental systems the *myo-D* gene singlehandedly induces conversion of morphologically and biochemically undifferentiated mesenchymal cells into myotubes and myocytes (62, 67); hence the term "master gene (41)."

This system is subject to negative regulation by the expression of a protein called Id (68). The Id protein is a helix-loop-helix molecule which lacks a basic DNA binding domain. When this Id protein binds to *myo-D*'s ardent partner E12, *myo-D* cannot dimerize with E12 protein, cannot bind to any enhancers and therefore cannot complete its differentiation-inducing function. Thus, through dimerization, an intricate positive and negative regulation of enhancer binding is possible (Figure 4A). Combinatorial variety provides flexible control of development while utilizing a relatively limited number of transcription factors.



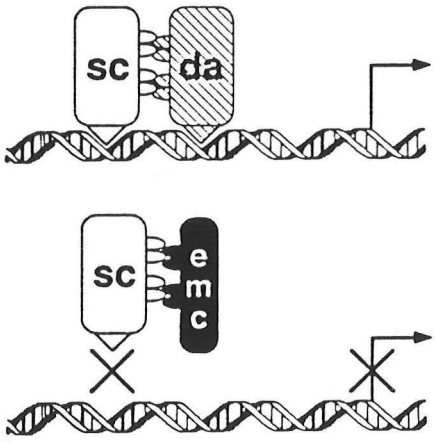
4A
Figure X A Schematic Representation of the Myogenic Regulatory Scheme Proposed in the Text

Myoblasts proliferating in high serum are imagined to have high enough levels of Id protein to titrate the available pool of E12/E47 protein and prevent the formation of E12/E47–MyoD heterodimers. The MyoD gene, under these conditions, could be activated by either a MyoD homodimer or a heterodimer of MyoD with an unknown protein (indicted with the question mark). When the cells are deprived of serum factors, Id protein levels are imagined to decrease and some E12/E47 protein is now available to heterodimerize with MyoD. This heterodimer would activate the MCK gene, the MyoD gene, and perhaps other muscle structural genes required for mature myotube formation.

(From Reference 68)

This same kind of regulation is also utilized in *drosophila* to regulate neurocutaneous organ development (63, 69). Here, the regulated steps involve choice of developmental pathway for neurectodermal cells. Development of this organ requires precise regulation of commitment to neural versus epidermal cell type. This choice is regulated by bHLH transcription factors termed achaete-scute. A variety of mutants genes for these factors have been characterized which yield specific abnormal neurocutaneous (bristle) phenotypes. Here again, dimerization is critical; both positive and negative interactions with respect to transcriptional activation are well characterized (Figure 4B).

extramacrochaetae Encodes a Novel HLH Protein
35



4B
Figure 4 Negative Regulation of *AS-C* or *da* Function by the *emc* Protein

By analogy to other members of the HLH class of proteins, the *AS-C* and *da* proteins may act as dimeric transcriptional regulators in the control of sensory organ determination. They might bind to DNA as homodimers, heterodimers between various *AS-C* proteins, or as shown here, *AS-C–da* heterodimers. Dimerization in the HLH class of proteins depends on interaction between the amphipathic helices, indicated here by the interdigitation of the proteins. DNA binding activity of these proteins requires a basic region N-terminal to the helices, which is symbolized here by the triangles on the *sc* and *da* proteins. The *emc* protein is a novel member of the HLH family of proteins; sequence analysis suggests that *emc* lacks the DNA binding activity demonstrated for several of these proteins but shares their ability to form dimers. We propose that heterodimers between *AS-C* and *emc* proteins, as illustrated here, or between the *da* and *emc* proteins, would lack or have altered DNA binding or transcriptional regulatory activity. In this illustration of our model we show the *sc–emc* heterodimer as lacking DNA binding activity and consequently failing to activate a gene that is activated by the *sc–da* heterodimer.

(From Reference 69)

To return to the *tal -1* gene, what is its normal function? Richard Baer has shown that it shares several features expected of a transcriptional control gene. First, it is a bHLH protein whose expression is limited to certain hematopoietic tissues, including early lymphoid and erythroid cells. Second, TAL-1, a tissue specific bHLH protein, dimerizes with a ubiquitous bHLH protein named E47 (70). This complex can then bind the core sequences ("E boxes") found in a variety of gene enhancers, including those found adjacent to immune receptor genes. Dr. Baer's current working hypothesis is that the TAL-1 protein is a lineage-specific transcriptional regulator which specifies development in the hematopoietic system just as the *myo-D* family of proteins directs myogenesis in the mesenchymal cells . Although the evidence is circumstantial at the moment, it seems plausible that the disruption of the *tal-1* gene by translocation and deletion could contribute to the excess of self renewal and blocked differentiation characteristic of T-ALL. Consistent with this hypothesis is the finding that *tal-1* expression is high in T-ALL cell lines which have disrupted this gene by translocation or deletion (48,49).

4.6 - Summary of Pathogenesis

The consistent finding in T-ALL of rearrangements of genes that are homologous to genes known to regulate development is strong evidence that we already have in hand the key oncogenes in T-ALL. However, I do not want to leave the impression that a single molecular defect causes leukemia. A great deal of evidence in experimental and clinical oncology indicates that multiple steps are necessary to create a fully malignant phenotype. The steps are thought to include several dominant acting genetic changes which promote growth, alter patterns of differentiation, change cell-cell interaction etc. To date, most well studied human tumors contain many mutations. Even in T-ALL, two of the genes listed in Table 9 are often rearranged, and their effects may be complementary. A good example is the patient CM, described above, whose T-ALL blasts contained both the *tal-1* deletion and the t(11;14) (p13;q11) translocation which deregulates the *rhom-2* gene. A second class of mutations is now known to be at least as important in the pathogenesis of cancer. These are the recessive loss-of-function mutations of genes whose normal role is apparently to restrain growth (71). In a number of other human tumors, these antioncogenes have been well characterized and include such genes as p53 and *rb*. Interestingly, these particular genes, so commonly involved in a host of

other cancers, do not appear to be damaged in T-ALL, although further data are necessary to be sure. Perhaps additional, yet to be described tumor suppressor genes are important in the regulation of growth of T-lineage precursors.

5. - T-ALL: Prospects for Improved Therapy

In closing, I would like to touch on several possible avenues to improved therapy for T-ALL. Obviously, we would like to target the dysregulated growth controlling gene products which I have summarized in Table 9. Unfortunately, since we lack detailed information on the molecular interactions of these proteins, rational design of drugs to inhibit their function is not yet feasible. For now, trials are ongoing which attempt to maximize the selectivity of cytosine arabinoside for T-ALL. Specifically, various regimens of high-dose cytosine arabinoside are currently being studied in an attempt to increase the cure rate. Pilot studies of this sort are now underway within the Pediatric Oncology Group. Another newer cytotoxic agent with selectivity for T-lineage cells is 2-deoxyadenosine. or pentostatin (72,73). This drug is an analog of adenosine and is a specific inhibitor of adenosine deaminase (ADA). This enzyme is necessary for the catabolic deamination of adenosine and deoxyoadenine. Pentostatin is selectively toxic for T-lineage cells. One possible basis for this selectivity is the unusually large accumulation of dATP in T-lineage cells consequent to ADA inhibition. Such accumulation appears lethal. Early phase I trials of pentostatin yielded high grade renal, neurologic, hepatic, gastrointestinal and ocular toxicity at doses greater than 10mg per square meter (73). Currently, trials are ongoing to define a safer dose range of this agent in order to capitalize on its selective toxicity for T-lineage malignancies. However, cytotoxicity is not limited to T-cells. In fact, pentostatin has already become the treatment of choice for advanced hairy cell leukemia, an uncommon indolent leukemia of B-lineage phenotype (74).

We are taking a different approach to the improvement of therapy for patients with T-ALL. This approach is based on the hypothesis that therapy for T-ALL needs to be individualized. Thus, leukemia stemlines vary in their sensitivity to the wide variety of available antileukemic drugs, such an vincristine, cytosine arabinoside etc. Since most patients achieve complete remission, yet nearly 50% will ultimately relapse, the critical phase of treatment is likely to be the intensive post-remission chemotherapy period. It is likely that the early success

of this therapy in reducing tumor cell burden below some critical threshold will determine the ultimate success or failure of treatment. In order to permit selection of the most active drugs for individual patients during this phase, we have sought to develop quantitative assays for minimal residual disease present during the remission phase of T-ALL. Our current approach is to focus upon the specific molecular rearrangements at the *tal-1* locus found in a substantial fraction of these leukemias (75). Since the translocations and deletions in the *tal-1* locus are usually exquisitely site-specific, it is possible to develop specific assays to detect these rearrangements with great sensitivity and specificity. These assays are based on the polymerase chain reaction (PCR) and can detect one cell containing the specific rearrangement in a background of 10^5 normal cells. Moreover, since several alleles of this deletion are available to serve as internal standards, the assay can be quantitated so that the number of leukemic cells per 10^6 normal cells can be estimated. This quantitative assay allows us to determine when the leukemic clone is expanding at a level well below the threshold required for detection by ordinary laboratory methods such as microscopic analysis of blood, bone marrow or spinal fluid. An example of detection of minimal residual disease in patient CM is shown in Figure 5.

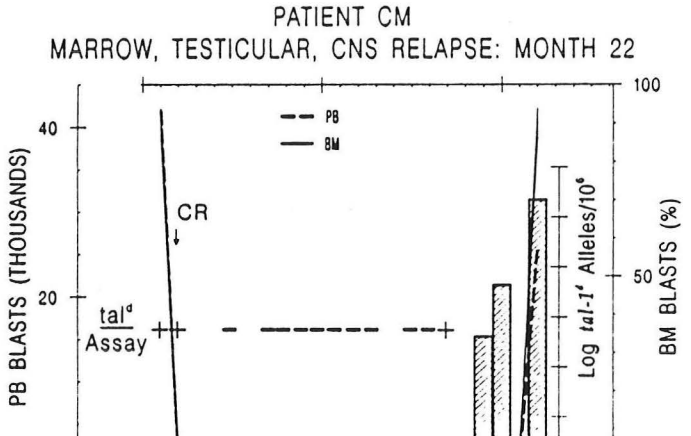


Figure 5. Course of Patient CM. Detection assays (+/-) for deleted *tal-1* (*tal-1*^{d}) alleles shown as + or -; quantitative assays shown as bars on a log scale extending from 10 to 10^6 *tal-1*^{d} alleles per 10^6 normal genomes. PB, peripheral blood (---); BM, bone marrow ( ); CR, complete remission.

In this patient, we were able to detect the reappearance of the neoplastic clone 5 months prior to overt relapse. Moreover, quantitative analysis demonstrated the expansion of the clone 2 months prior to relapse. We are now organizing a trial within multiple institutions of the Pediatric Oncology Group to test whether such detection predicts relapse and, if so, with what lead time. Should such analysis provide a high positive predictive value for disease relapse, trials to adjust therapy on the basis of these assays would seem reasonable. In essence, the aim is to develop *in vivo* drug sensitivity assays. Ideally, these assays would dovetail with future drug development to provide more precise and effective individual therapy for patients with T-ALL.

6. Summary and Conclusions

T-ALL is an uncommon subtype of acute lymphoblastic leukemia with characteristic clinical and laboratory features, including peak evidence in late childhood through early adulthood; predilection for males; presentation with a high burden of disease including mediastinal mass, lymphadenopathy and a high white blood count; and high cell turnover predisposing to the tumor lysis syndrome. Therapeutic outcome in T-ALL has improved with sequential increases in intensity of chemotherapy, including recently an emphasis on cytosine arabinoside. The molecular pathogenesis of T-ALL is currently unfolding. Apparently due to mistakes in the immune receptor recombinase enzyme system, rearrangements of growth regulatory genes, including primarily those encoding transcription factors, appear consistently to contribute to leukemogenesis. Further advances in controlling this often lethal disease will depend on more selective utilization of existing cytotoxic agents, development of new agents, further understanding of the molecular interactions of dysregulated growth regulatory gene products, design of antagonists of these growth regulatory proteins and molecular monitoring of minimal residual disease in order to individualize therapy.

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