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**Presentation**

# The roles of adoptive and active forms of immunotherapy in the cure of children suffering from acute lymphoid leukemia: a) underestimation of active immunotherapy benefit, b) its immunogenetic indications to select sensitive patients, hence prevent chemotherapy's late effects

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**Summary –** Children's acute lymphoid leukemia (ALL) chemotherapy started in 1948 with antimetabolites combined with steroids. It was enriched in 1959 with vincristine and cyclophosphamide and, in 1970, with daunomycin. It induced more and more apparently what were called complete remissions, and prolonged more and more the survivals without reducing however, until 1973, the (100%) mortality. It started to reduce it at the fifth year, to 20 and even 40% between 1973 and 1976, due to progressive and maximal intensification and duration of chemotherapy.

It is in the same period that we proposed to apply in ALL remissions after relapses and in the first remissions of the most malignant type, allogeneic bone marrow grafts; we published the first success in human ALL in 1963, and clinically observed the same actions as those described experimentally: cytoablation of both leukemia and hematopoiesis, the latter being restored by the graft, whose reaction versus the residual neoplastic cells (called graft versus leukemia or GvL) appeared to be able to often eradicate them, at the cost however of a graft-versus-host reaction (both reactions sharing the same mechanism). One of us became a member of the Committee of the International Bone Marrow Transplant Registry, whose results showed the improvement in the prognosis of the aggressive form of ALL.

The intensity and length established for chemotherapy for the most severe form of children's ALL have often been applied to the intermediary and to the least aggressive ones. The global 5-year survival increased to 60% between 1976 and 1984, and is around 80% today.

But the registration of late debilitating or malignant effects of chemotherapy toxicities makes us wonder if some patients have not received an excessively intense and long application of cytostatics (often combined with ionizing radiations on CNS).

In fact, the patients belonging to some HLA phenotypes (A33 and B17) have appeared to be especially often cured with active immunotherapy (killed leukemic cells and/or BCG), whose action was shown by specific cytotoxicity amplification, which was applied after short adjuvant chemotherapy, and hence is able to reduce the long chemotherapy incidence of debilitating or malignant late effects. Sakurai's group confirmed our absence of late relapses after ALL active immunotherapy, which contrasts with their risk after maintenance chemotherapy, whose minimal residual disease is a worrisome stumbling block to the cure. © 2001 Éditions scientifiques et médicales Elsevier SAS

**active immunotherapy / adoptive immunotherapy / children's acute lymphoid leukemia / intensive chemotherapy late effects / result underestimation**

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## CHILDREN'S ALL DEFINITION AND CATEGORIZATION

Children's acute lymphoid leukemia constitutes the group of the lymphocyte precursor neoplasias in children aged ≤ 15 years [1]. Not all of the adolescents and young adults [2] are included in this group, which comprises more neoplasias of the B-cell precursors than of the T-cell one [1].

The definition of this group is far from being respected, and we take the opportunity of this paper to underline the fact that it cannot rationally include the lymphoid neoplasias constituted of activated, hence mature lymphocytes, which are often called blastoid*, (characterized by cytoplasmic pyroninophilia or hyperbasophilia visible on Giemsa smears [3] and by cytoplasmic numerous ribosomes visible at E.M. [4]).

The simplistic, so-called 'French-American-British' (FAB) classification [5] should not have included, as one of its three entities '$L_3$', the leukemic evolution of Burkitt's lymphoma, whose cells are sIg+, hence mature (and not precursors of) B-lymphocytes, and are pyroninophilic blastoids [6]. The International Lymphoma Groups Study [7] should not have included in ALL another lymphoma composed of proplasmocytic, middle-sized, sIg+, blastoid cells [8], which Galton [9] had described in 1974 as "prolymphocytic leukemia."

The Ph1 blastic crises [10] are more difficult to eliminate morphologically from ALL. Their detection in the cases of chronic myeloid leukemia and myeloid metaplasia reveals the evolution of the terminal phase.

Today's progress in the knowledge of B and T lineage differentiation [1] has not changed the rationale of the categorization of children's ALL that was proposed in 1976 by the WHO [11]. The ALL categorization terminology considered in this paper is given in *table I*, which compares treatment actions.

If we have, for rationalization, eliminated the above-cited three entities from the ALL frame, we must include, for the same reason, the mediastinal thymocytic pre-T leukemia (the thymocytes being T-cell precursors). Excluding it and calling it 'lymphoblastic lymphoma' improves the general result of an ALL population, as its prognosis is poor.

The prognosis of children's ALL is determined by the immunologic [12] and cytologic types, and the total tumoral volume [13], accessorily by cytogenetic abnormalities [10]. Serum lactic dehydrogenase level [14] is also a less well known prognosis marker.

## CHEMOTHERAPY HAS BEEN SINCE 1988, AND STILL IS, THE FIRST AND BASIC TREATMENT FOR ALL CHILDREN'S ALL

One of us was lucky to be a fellow of David Karnovsky and Joseph Burchenal at Memorial Hospital, when the first antimetabolites were applied to children's ALL. Joseph and John Burchenal [15] illustrate in *figure 1* the survival of children having suffered from ALL before 1948, and its progressive extension with the availability of oncostatics: children treated between 1948 and 1952 by the first antimetabolites combined with corticosteroids presented a survival median of 10 months, and an end at 30 months; children treated between 1959 and 1965, when vincristine and cyclophosphamide were added, presented a median at 15 months and its end at the thirtieth. The overall survival and event-free survival curves were exponential.

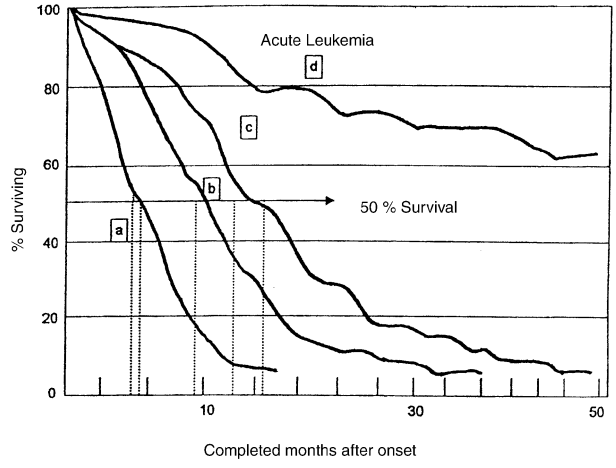


**Figure 1**. (a) Forty years ago, the median survival time for untreated children with acute lymphoblastic leukemia was between 3 and 4 months. (b) A report of 160 patients treated (Jan. 1948–Jan. 1952) with antimetabolites and steroids showed some increase in survival time. (c) Further improvement was demonstrated with treatment of 205 cases (Sept. 1959–April 1965) using steroids, cyclophosphamide, vincristine, and antimetabolites. (d) A later study involving 43 children (Nov. 1969–March 1972) used steroids, daunomycin, and vincristine, as well as two antimetabolites (methotrexate and 6-mercaptopurine) and one alkylating agent (cyclophosphamide). (From [15]).

* The term 'blast' mean root. The proposed term 'blastoid' designates activated lymphocytes.

**Table I.** Main types of children's ALL and their respective prognoses.

| *Markers* | *CD10* | *CD20* | *cIg* | *sIg* | *Ig gene rearranged* | *CD1* | *CD3* | *TCR gene rearranged* | *Pyronino-philia* |
|---|---|---|---|---|---|---|---|---|---|
| *Types* | | | | | | | | | |
| Late pre-B (20%) | – | + | + | – | + | – | – | – | – |
| Early pre-B (20%) | + | – | + | – | + | – | – | – | – |
| Lymphoblastic pro-B (5%) | – | – | – | – | – | – | – | – | – |
| Late, medullary thymocytic, pre-T | – | – | – | – | – | – | + | + | – |
| Early, cortical thymocytic, pre-T | – | – | – | – | – | + | – | | – |
| Lymphoblastic, prothymocytic, pro-T | – | – | – | – | – | – | – | | – |
| | *Prognosis* | | | | *The best* | | *Median* | *The most reserved* | |
| | *Total tumor* | | | | | | | | |
| | *Small* | | *Large* | | | | | | |
| Late pre-B | + | | | | + | | | | |
| Early pre-B | + | or | + | | + | or | + | + | |
| Lymphoblastic pro-B | + | or | + | | | | | + | |
| Late, medullary thymocytic, pre-T | | | mediastinal mass | | | | | + | |
| Early, cortical thymocytic, pre-T | | | mediastinal mass | | | | | + | |
| Lymphoblastic, prothymocytic, pro-T | + | or | + | | | | | + | |

Such curves only started to present asymptotic tendencies after 1970, when daunomycin was added and systemic chemotherapy was intensified and combined with CNS radio- and/or local chemotherapy, as *figure 2* from Steinhorn and Ries [16] illustrates: 1) the event-free survival curves of the patients treated between 1973 and 1976 were the first to consist of exponentials with asymptotes. The latter started between the fourth and the seventh years, representing about 40% of patients; it did not present a plateau; 2) the curves of the children of two groups treated between 1977 and 1980, and between 1981 and 1984, were also exponential, counting 80% of the patients alive at the third year, and the one with the longest follow-up, 60% at 7 years, without conversion into asymptotes; and 3) the later trials have been the objects of more intensive chemotherapy. Some have applied the same to all types of ALL; others have tried to adapt the intensity to the predicted prognosis [17-20].

## CHEMOTHERAPY, EVEN MAXIMALLY INTENSIFIED, CURES NEITHER RELAPSING CASES, NOR THOSE OF THE SEVERE PROGNOSIS TYPES. AS IT OBEYS FIRST-ORDER KINETICS, ITS INTENSIFICATION DOES NOT PREVENT THE PERSISTANCE OF MINIMAL RESIDUAL DISEASE

Oncostatics [21, 22] as virostatics when facing HIV-1 [23], do not kill 100% of the cell (or of the virus) population they attack. This fact that chemotherapy obeys first-order kinetics [21-23] explains that it leaves a so-called minimum residual disease (MRD), which we detected in the blood under the form of leukemic cell rebounds *(figure 3)* [24]. It explains late relapses after short- and even long-maintenance chemotherapy [25] and any kind of intensification [26]. The detection of an MRD after remission induction is thus an important prognosis factor [27]. MRD

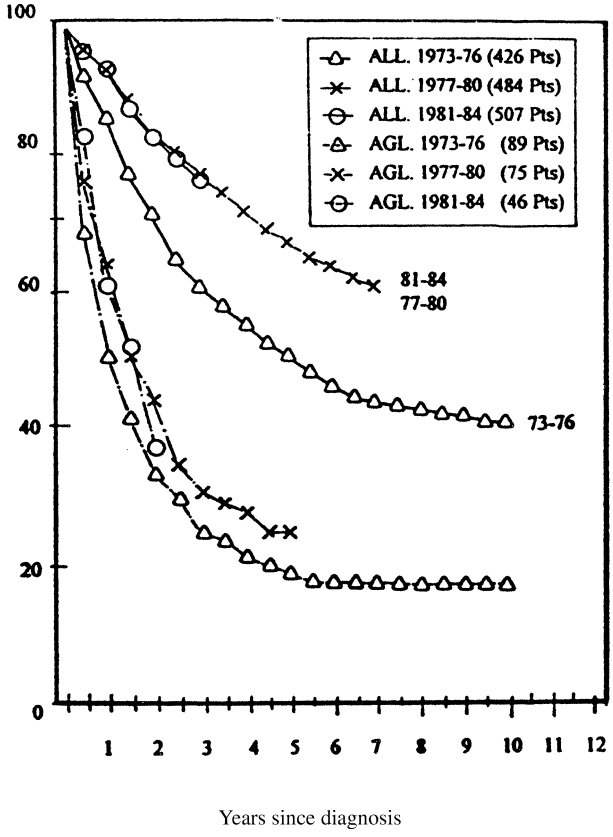


**Figure 2**. Survival among children with acute lymphoid leukemia submitted to chemotherapy protocols of different periods. (From [16]).

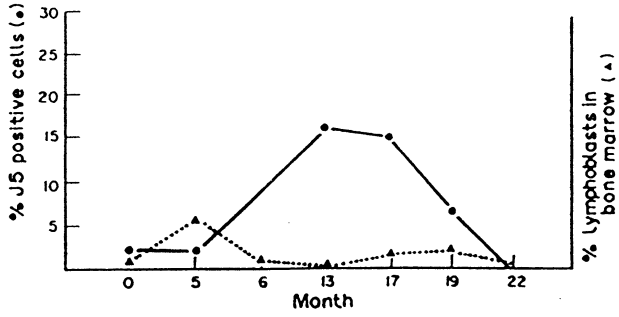


**Figure 3**. Percentage of CALLA-positive (•) cells and of lymphoblasts (Δ) in the bone marrow of a girl in complete remission 5 years after the treatment of acute lymphatic leukemia. Despite abstention from treatment, there was spontaneous regression and no relapse [24].

appeared to be more frequent than what we had described on smears [24], to those who searched for it systematically with marker combinations [28], or with combinations of monoclonal antibodies (CD10 and CD19) with TdT*, which allows one to distinguish the respective expression of the B-lineages's precursors of ALL and of those of normal bone marrow [29], or with PCR immunoglobulin gene [30] or TCR rearrangements [31].

* The DNA polymerase enzyme, TdT or terminal deoxynucleotidyl transferase, is an enzyme that catalyzes the polymerization of deoxynucleoside triphosphates without requiring a template formation: its reaction is positive in more than 90% of cases of ALL.

## DOUBTS ON THE POWER AND NECESSITY OF CHEMOTHERAPY INTENSIFICATION, AND CERTITUDE OF ITS RISK OF LATE TOXIC, DEBILITATING OR MALIGNANT COMPLICATIONS

Finally, when authors eliminate all bias, they doubt the necessity and importance in several conditions of intensification. It has been proven ineffective in adults [32], and infants [33] whose ALL prognosis is poor. It is not necessary to underline the excellent results in non-high-risk patients, which are obtained with moderately intensive treatments [34].

On the other hand, beyond the immediate chemotherapy toxicities whose risk is correlated with their intensity and length [35], a long list of late, debilitating or malignant complications has been established *(table II)*.

They could and should have been dreaded by experts and public agencies more than they have sometimes been. The mutagenic effect of most cytostatics [35], especially the alkylating agents, the induction of metabolic, hence genomic dysmethylation [36], the cardiac lesions induced by anthracyclines [37], were known. Only the acute myeloid leukemia and myelodysplasia induction by epipodophyllotoxins was unpredictable [38]. *Table II* gives the list of the most frequent late effects, among which the second neoplasias are the most impressive [39-42].

One understands that irradiation included in the first protocols has been replaced by intrathecal injection of cytostatics. Rotational combinations seem to

**Table II.** Late, toxic, debilitating or malignant complications of ALL chemotherapy.

| *Authors* | *Riveras* | *Van der Does-Van den Berg* | *Belpomme* | *Silverman (infants)* |
|---|---|---|---|---|
| Manifestations | 43 | 41 | 42 | 40 |
| Learning disability | | | | 82% |
| Psychological delay | 12% | 50% | | |
| Pituitary and other endocrine gland and gonadic defects | | | | |
| Short stature | | + | | 18% |
| Growth delay | 58% | | | |
| Obesity | + | + | | 27% |
| Delayed puberty and infertility | | + | | |
| Ovarian failures | 9% | | | |
| Male gonadal development insufficiency | 31% | | | |
| Asymptomatic cataract | | | | 67% |
| Heart echographic abnormality | | | | 30% |
| Bone necrosis | 4% | | | |
| MLC gene rearranged | | | | 13/23 |
| Secondary malignancy | + | | + | 8% |
| Myeloid leukemia | 1/33 | | 13/28 | |

increase tolerance [42], as we have offered for virostatics [43].

In conclusion, chemotherapy of children's ALL has been the object of a monstrous effort, of triumphs, but also of disappointments, at any rate of challenges.

## ALLOGENIC BONE MARROW GRAFT, ADDED TO A RADIO-CHEMOABLATION OF LEUKEMIA AND HEMATOPOIESIS, THE LATTER BEING RESTORED BY THE GRAFT, WHICH ALSO SUPPORTS IMMUNOTHERAPY IN ITS ADOPTIVE FORM, AT THE RISK OF GLOBAL-VERSUS-HOST (GVH) REACTION, PROPOSED IN 1963 TO BE ADDED AS A COMPLEMENT OF HIGH-RISK ALL CHEMOTHERAPY

We discovered experimentally, published in 1958 [44] and 1959 [45], and attempted to apply during these same years, to children with advanced ALL [46, 47] the lytic effect on leukemia cells of allogenic bone marrow graft and lymphocyte transfusions [48].

It was the period where chemotherapy survival curves were starting to became very long, as those shown by the Burchenals in *figure 1* [15], but were not becoming horizontal asymptotes. We hoped that the cytoablation realized by the bone marrow graft conditioning, complemented by the adoptive immunotherapy, which did not seem to obey, as did chemotherapy, first-order kinetics [21, 22, 49], would be able to eradicate the leukemia. We experimentally showed that the condition of this eradication, i.e., of the control of the so-called 'last cells', consisted of a small number of neoplastic cells, as appears by comparing the results published in references [44, 50].

This idea, according to which immunotherapy was only able to eradicate small populations of leukemic cells, conducted us to only apply the human marrow graft in remission. This decision was reinforced by the success of the bone marrow graft treatment of the Yugoslavian victims of a reactor, who had received sublethal or lethal total body irradiation doses [51]. These patients were not infected, contrary to the leukemic patients in whom we had tried and failed our first bone marrow grafts; these patients had died from typical graft-versus-host reactions (then called secondary syndromes) [46, 47].

In 1963, we published the first successful allogenic bone marrow graft in man. It had been applied on a leukemic patient in remission who was not infected *(figure 4)* [52, 53]. He had received, after an 800 rad irradiation, the marrow from several siblings, one of whom had appeared to be the nearest in our so-called 'third man test' [54]. This patient presented a full chimerism *(figure 4)* from this predicted best donor, a tolerance towards his skin graft, a discrete and controllable GvH, and a strong GvL still demonstrable 2 years later [53]. The respective parts of the adoptive immunotherapy, and of cytoablation realized by total body irradiation, could not be determined.

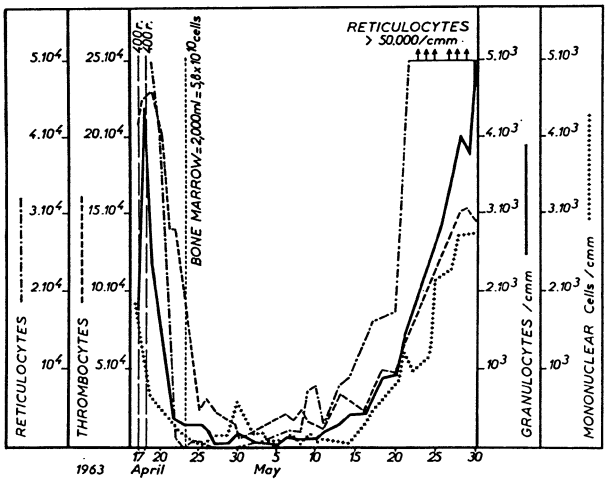


**Figure 4**. Changes in the blood counts in a patient irradiated at 800 rads and treated by allogeneic marrow grafts from six donors. This case represents the first allogeneic bone marrow transplantation success with full chimerism from one of the donors, tolerance toward his skin graft, controlled GvH and successful GvL [52].

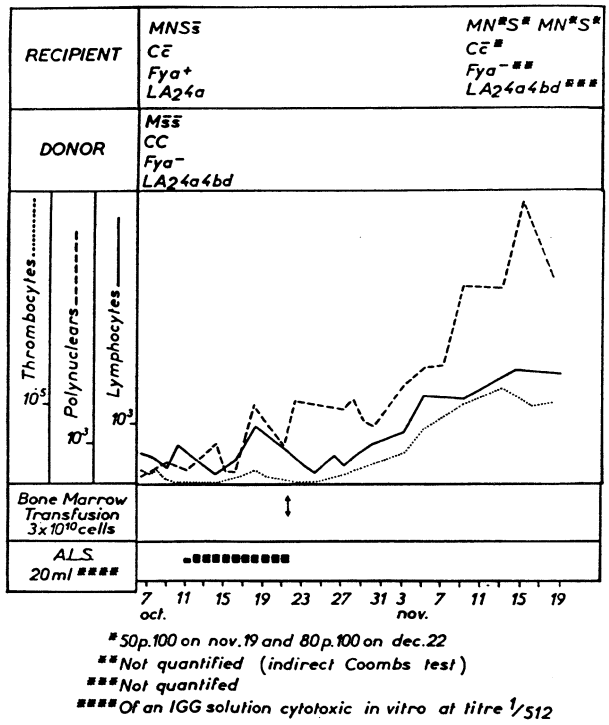


**Figure 5**. An example of allogeneic bone marrow graft after conditioning leukemic recipient and donor by ALS [66].

Our group became one of the first members of the Advisory Committee Bone Marrow Transplant Registry [55], which confirmed our original results and collected the data from all over the world. One of its members had the merit to replace the irradiation by cyclophosphamide [56], and later, many chemical or radiochemical conditionings types would be used [57]. Later as well, progress would be accomplished by the replacement of the marrow by blood stem cells [58] and matched unrelated donors would be as successfully used as matched sibling ones [59].

We had established an early project with Ferrebee and Thomas [60], but the latter, suddenly refusing to cooperate with anybody, especially Ferrebee, decided to devote a special institute to bone marrow graft, and also refused any cooperation with the National Registry Group. His team confirmed the graft of allogeneic marrow in ALL in 1979 [61] (publishing seven survivals out of 22 children), and, statistically, the antileukemic role of GvL by comparing allogeneic to autologous grafts [62].

We had demonstrated its reality by observing GvH and GvL manifestations in patients having received allogeneic lymphocyte transfusions [63]. The successes of identical twin donor and autologous grafts [64] may not only result from the role of leukemia cytoablation: we had shown that these syngeneic grafts induce a GvL reaction from cells that we demonstrated to be auto-reactive [65]. There were more reasons to dread GvH from autologous marrow regraft than to control it from an allogeneic transplant.

We tried in 1968 and succeeded in realizing allogeneic bone marrow grafts in man *(figure 5)* without GvH: myeloablation conditioning was replaced by anti-lymphocyte serum application to the recipient and to the donor [66, 67]. A mixed chimerism was realized, as in our Yugoslavian reactor victims [51], with double tolerance, as Starzl has observed in liver transplantation recipients [68].

This type of bone marrow graft without myeloablation conditioning is ideal for non-malignant indications, as GvL was reduced in parallel of GvH. Foss [69] published that he was able to restore in such a chimerism the antineoplastic effect by the means of photopheresis.

Contrary to the general idea of the inseparability of GvH and GvL, we have been able to reduce the first and enhance the second by using the immunodominancy of viral antigens [70], i.e., by vaccinating the allogeneic donors against the virus which had

induced the recipient leukemia [71]: we suppressed most GvH and saved from leukemia seven out of the 31 induced by Friend's virus.

## ACTIVE IMMUNOTHERAPY CURING POTENTIAL. ITS TUMOR VOLUME AND HOST HLA-RESTRICTED INDICATIONS. ITS ABSENCE OF TOXICITY AND CAPACITY FOR PREVENTING THE HANDICAPPING OR MALIGNANT LATE SIDE EFFECTS OF LONG CHEMOTHERAPIES

It was only a few years later that we attempted to induce an immunotherapeutic effect from the leukemic patients' own lymphocytes. We called it active immunotherapy [72]. We used as specific activation means, in vitro irradiation-killed leukemic cells of allogeneic donors suffering from ALL and as non-specific adjuvant, BCG [73].

We observed that the notion of immunity amplification was not limited to its application before the neoplasia implantation, but was also acting when applied after it *(figure 6)* [72]. But, as adoptive immunotherapy, it was only effective, whether eradicating them or not, on small numbers of tumor cells: $10^5$ in mice [72].

We applied it under the form of $4 \times 10^7$ allogeneic irradiation-killed ALL cells, applied in i.d. injections and/or 150 mg living BCG [73] of the Pasteur Institute, each week, to 20 patients [74]. Ten served as controls. The 20 treated patients were divided into three groups, one receiving BCG, one the cells and one receiving both. One sees in *figure 7* that our ten controls, left without treatment after a relatively short length and poor cytostatic power post-remission chemotherapy, relapsed in 130 days [74]. Grouped together, the patients under immunotherapy presented a totally different event-free survival curve: five relapsed before the controls. Four relapsed between the thirtieth day and the one hundredth day. The last four to relapse did so respectively on the two hundred and tenth, three hundred and fifteenth, three hundred and twenty-fourth and the nine hundred and fiftieth days *(figure 7)*. Seven had not relapsed at the twelfth year. This cure rate was highly significant.

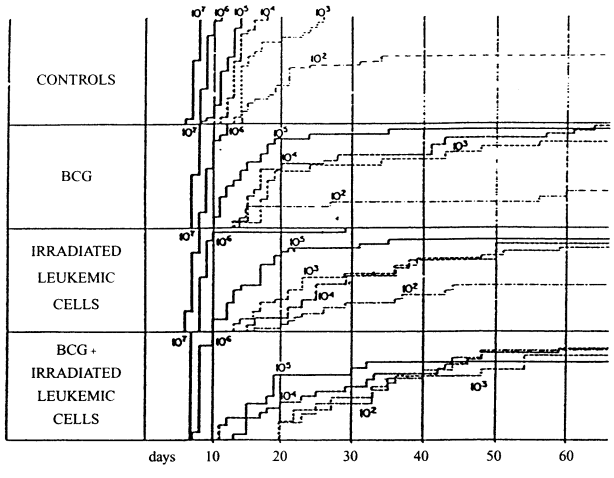


**Figure 6**. Growth curve populations of tumors obtained from mice treated with BCG without or with irradiated cells, the day after transplantation of $10^6$ L1210 cells [72].

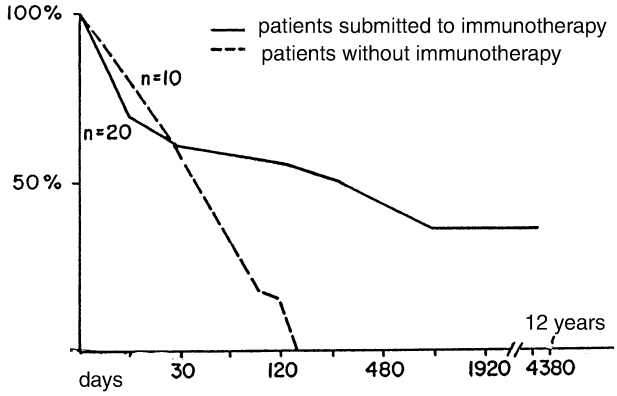


**Figure 7**. Event-free survival curves of acute lymphoid leukemia patients submitted to the first trial of active immunotherapy by BCG with or without irradiation killed ALL cells. The details are given in the text. Note that the abcissa represents 12 years [74].

*Figure 8* shows that specific lymphocyte cytotoxicity, studied by Oldham [75], was significantly increased in the active immunotherapy group, compared to different controls, and that this increase had only appeared after a certain time of immunotherapy. Cytotoxicity was decreased in some patients under chemotherapy or chemotherapy interspersed with immunotherapy.

But the most original and interesting observation concerns the HLA phenotypes of children who were surviving more than 8 years after active immunotherapy, compared to those of the general population, and to children suffering from acute lymphoid leukemia and having long-term survival after che

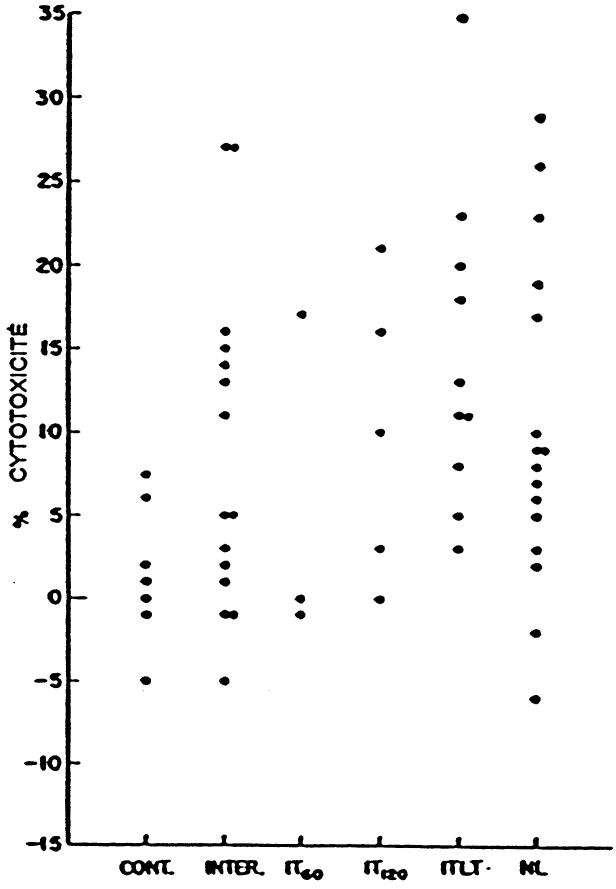


**Figure 8**. Study by R. Oldham [75] of lymphocyte cytotoxicity in our patients submitted to active immunotherapy for various times (IT50: 50 days, IT 120: 120 days, ITLT: longer diration) and of other subjects (cont: controls under maintenance chemotherapy; INTER: ALL chemotherapy interspersed with immunotherapy; NL, controls without any treatment).

motherapy only [76] *(table III)*. Those who had been submitted for 9–18 years to killed allogeneic leukemic cells and BCG (after a short chemotherapy of 6–15 months) appeared in a study conducted by Tursz [76], a study including the tests for 31 HLA-A and -B antigens, to present a high and significant predominance of HLA-AW33 and HLA-B17. The differences of the frequencies of these two antigens are highly significant with the normal population controls, and especially with those of the patients in long survival under chemotherapy. It is noteworthy that no patient carrying HLA-AW33 or HLA-B17 was found in the chemotherapy group. This observation is of great interest, as the role of major histocompatibility 1complex is known to be fundamental for the

**Table III.** HLA B17 and HLA-A33 antigen incidences in active immunotherapy long-term survivors, compared to the same number of patients submitting to chemotherapy only.

| | *BCG* | *Chemotherapy only* | P |
|---|---|---|---|
| HLA-B17 | 42.8% | 7.3% | < 0.01 |
| HLA-A33 | 35.7% | 1.2% | < 0.01 |
| HLA-B17 and -A33 | 71.4% | 8.1% | < 0.001 |

function of cytotoxic-T-lymphocytes [77]. HLA typing provides an attractive procedure for selecting the ALL children for active immunotherapy. The selected ones have a good chance of cure.

Not less interesting are the facts that the immunotherapy curing effect does not depend on the length of adjuvant chemotherapy and that horizontal asymptotes of the event-free survival in first remission can be obtained after non-intensive and relatively short chemotherapies *(figure 9)* [78].

*Figure 10* shows that once active immunotherapy is completed, no relapse is observed in Sakurai's

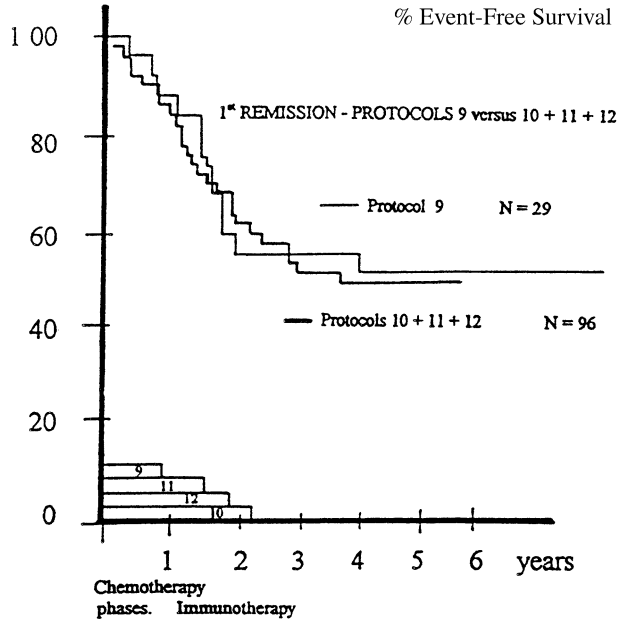


**Figure 9**. Relapse-free survival curves after the initiation of active immunotherapy in our acute lymphoid leukemia children, belonging to two groups of protocols, which differed by the lengths of pre-immunotherapy-chemotherapy (all patients categorized as good and as poor prognosis are included). The lengths of the two types of chemotherapy, short for protocol 9 (1970–1973), and longer for protocols 10, 11 and 12, are visible in the lower left part of the figure.

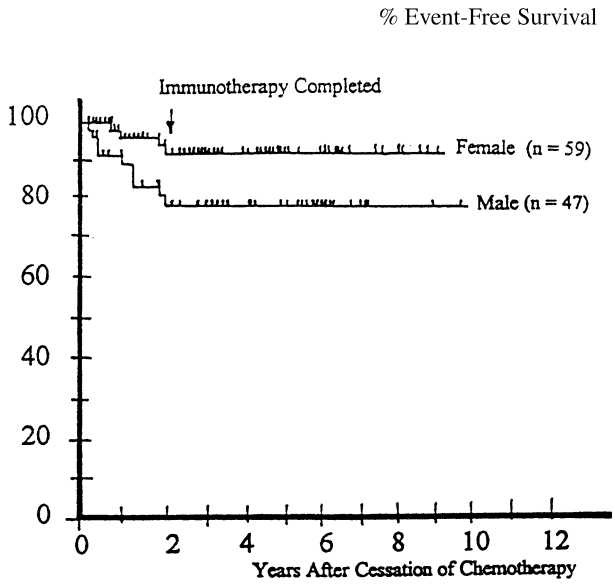


**Figure 10**. Gender-sex relation to the duration of event-free survival after cessation of immunotherapy in Sakurai's experience [79].

experience during the next 10 years [79]. Relapses are possible after maintenance chemotherapy [79].

Immunotherapy appears to realize an immunologic screening in conjunction with MCH1, whose effect may participate in the elimination of the 'last cells' of residual disease if they are in small numbers.

## FROM THE BCG ERA TO THE PROMISE OF THE HLA CLASS I ANTIGEN

Before modern therapeutic immunomanipulations are applied at least to the patients suffering from the least malignant types of ALL and belonging to some HLA phenotypes, a search for immunologic defects in the case of this disease is necessary. It has been accomplished by Yotnda et al. [80], who found in the active phase a decrease in specific T-cytotoxic responses. This proves that the tumor antigens are recognized, and denounces an immunologic insufficiency. Yotnda's study has to be repeated during remission. It is in agreement with our observations according to which active immunotherapy only eradicates leukemic neoplasias made of a few cells only [72, 74].

Yotnda et al. [80] also described several abnormalities: the absence of CD 40 and of CD 25 markers in T cells, the predominance of Th2 over Th1, and an important rate of apoptosis.

As far as immunomodulators other than BCG, we have discussed them recently in the third edition of Oldham's *Principle of Cancer Biotherapy* [81].

Interleukin-2 (IL2), at high dose and in IV bolus infusion ($8 \times 10$ [6] UI/m$^2$) every 8 hours, increases the numbers of activated T-lymphocytes and NK cells [82]. But the final effect is dominated by its severe toxicities, especially thrombocytopenia. At low doses (4.5 million IU, 5 days per w for 12 consecutive weeks), IL2 increased NK cell numbers and activated lymphocyte-activated killing activity (LAK), but the relapse risks of the treated patients who are in remission, hence in the best condition to receive it, are not different from those of controls in Gonzalez-Barcas's experiment [83].

We have a more rewarding experience of two immunomodulators, of which one, tuftsin, is an Ig oligopeptide spontaneously produced by the spleen in two enzymatic steps, the cleavage by an endocarboxy-peptidase at the carboxyterminal arginin, and by leukokinase at its amino terminal threonine *(figure 11)* [84]. The other, bestatin, is a peptide *(figure 12)* extracted by Umezawa et al. [85] from *Actinomycetes olivoreticuli*. Both share, in young animals, the same increase of CD4 action, of the immunity-killing power and of macrophage functions, while at small doses they decrease the

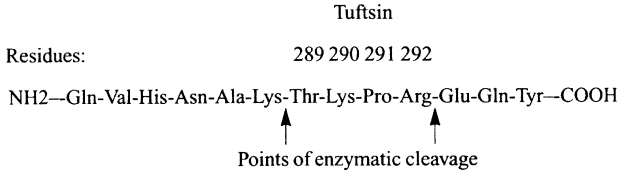


**Figure 11**. Cleavage of tuftsin as a part of the Fc fragment of the heavy chain of IgG [84].

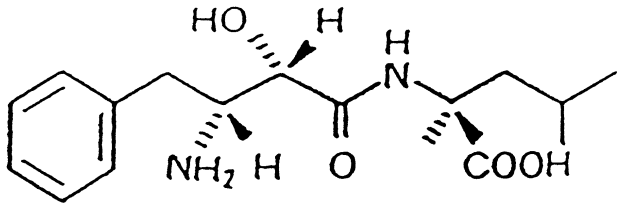


**Figure 12**. Chemical structure of bestatin [85].

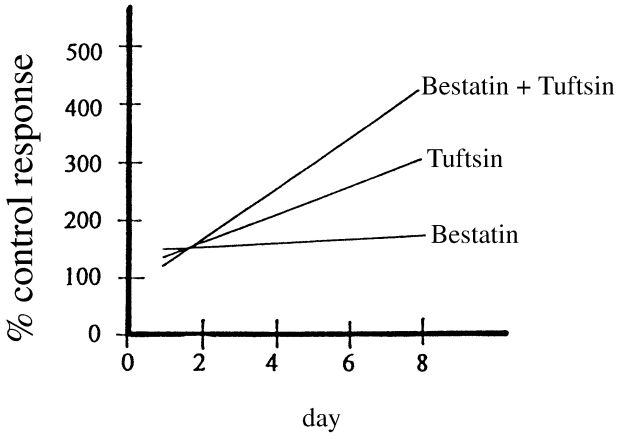


**Figure 13**. Peritoneal cell activation after bestatin or tuftsin, or their combination applied to mice.

T-suppressor cell numbers and functions [86]. Both are immunorestorators in aged animals [87]. Their combination induces this additive effect *(figure 13)* [81]. In man, we have obtained significant actions in patients who were immunodepressed [88], and Ota obtained an increase of acute myeloid leukemia survivors in remission, but affecting only the population of the patients older than 40 years [89]. Their main action seems to cover immunorestoration.

Finally, we are greatly interested by Borgmann's experience of immunotherapy of ALL by vaccination with autologous leukemic cells transfected with a cDNA expression plasmid coding for an allogeneic HLA class I antigen combined with IL2 [88]. This manipulation, for which he only described the protocol, meets two of our observations: the use in ALL immunotherapy of allogeneic ALL-killed cells, and the two HLA class 1 antigens which appear, when possessed by the patients, to render them sensitive to active immunotherapy.

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