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Outcome after Cessation of Therapy in Childhood Acute Lymphoblastic Leukaemia

Momcilo Jankovic, Donatella Fraschini, Augusto Amici, Maurizio Aricò, Alberto Arrighini, Giuseppe Basso, Roberto Colella, Maria Teresa DiTullio, Riccardo Haupt, Pierantonio Macchia, Saverio Magro, Antonia Mancini, Carla Manganini, Giovanna Meloni, Roberto Miniero, Paolo Rosso, Paola Saracco, Benedetto Terracini and Giuseppe Masera on behalf of the Associazione Italiana Ematologia ed Oncologia Pediatrica (AIEOP)

A total of 2192 children with acute lymphoblastic leukaemia who had reached cessation of therapy in complete remission were followed for a median time of 52 months after treatment suspension. Of the 485 relapses observed, 62.3% occurred in the first year off therapy and 68.9% involved the bone marrow. Eight relapses were reported more than 5 years (62–143 months) after treatment withdrawal. Males fared worse than females consistently, experiencing 1.5 times more relapses ($P < 0.0001$). Thirteen patients died in continuous complete remission, 5 because of non-neoplastic central nervous system complications. There were 11 second solid malignancies, 8 of them in the central nervous system; 9 subjects presented an haematopoietic malignancy after ALL. The projected event-free survival at 8 years is 73%. Twenty-two of the 171 young adults (age > 20 years) were married and 16 have had 21 healthy children. Twenty-four per cent of patients experienced an unfavourable event. Relapses accounted for 93% of failures. Central nervous system late effects and second malignancies were the major causes of non-leukaemic morbidity and mortality.
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INTRODUCTION

A NOTABLE PROPORTION of children with acute lymphoblastic leukaemia (ALL) arriving at treatment suspension (off-therapy) are cured. In the late 1970s the proportion of off-therapy children experiencing relapse was approximately 25% [1–3] but, with the introduction of more aggressive front-line therapeutic protocols, it now appears to be declining [4, 5].

As a consequence of the improvement in life expectancy, an evaluation of late effects in relation to the type of therapies received is now mandatory to assess the quality of cure. Specifically, second solid malignancies (SSM) are a major concern in ALL long-term survivors, especially central nervous system tumours [6–12].

In 1980 the Associazione Italiana Ematologia ed Oncologia Pediatrica (AIEOP) established a national registry for patients off-therapy after childhood cancer (ROT) [13]. One of the main goals of the ROT was to collect information about long-term off-therapy survivors under a common format.

The results of the fifth ROT update regarding the ALL population are presented.

PATIENTS AND METHODS

The Registry collects information on all children with ALL who, independently from subsequent evolution, reach treatment suspension in complete remission (CR). Data collection utilises standard flow sheets specifically designed to record long-term side-effects and information about progeny.

Thirty-eight Italian centres collaborate with the Registry. A child with ALL is eligible for registration when, independently from subsequent evolution, he or she reaches treatment suspension in CR. Standard flow-sheets are used for data collection and updating, including long-term complications and information about progeny. On two occasions, complementary *ad hoc* surveys were carried out in order to identify second primary cancers. The present update includes all cases reported as being removed from treatment before 1 January 1989. On this date, the median follow-up time after treatment suspension was 52 months (range 0–269).

Statistical methods

The life-table method and log-rank test were used, respectively, to estimate and compare survival, event-free survival and event-free intervals. Time on study or time to unfavourable event were calculated from the date of treatment suspension to the last follow-up or the date of event, if this occurred.

Correspondence to M. Jankovic at the Department of Pediatrics, Ospedale Nuovo San Gerardo, via Donizetti 106, 20052 Monza (MI), Italy.
M. Jankovic, D. Fraschini, C. Manganini and G. Masera are at the Clinica Pediatrica, Università di Milano, Monza; A. Amici is at the Clinica Pediatrica, Università di Perugia; M. Aricò is at the Clinica Pediatrica, Università di Pavia; A. Arrighini is at the Clinica Pediatrica, Università di Brescia; G. Basso is at the Clinica Pediatrica, Università di Padova; R. Colella is at the Istituto Poucamedra di Pediatria Clinica e Sociale, Università di Bari; M. T. DiTullio is at the Clinica Pediatrica I, Università di Napoli; R. Haupt is at the Istituto G. Gaslini, Genova; P. Macchia is at the Clinica Pediatrica, Università di Pisa; S. Magro is at the Divisione Ematologia, Ospedale di Catanzaro; A. Mancini is at the Clinica Pediatrica III, Università di Bologna; G. Meloni is at the Cattedra Ematologia, Università di Roma; R. Miniero and P. Saracco are at the Clinica Pediatrica I, Università di Torino; P. Rosso and B. Terracini are at the Servizio di Epidemiologia dei Tumori del Dipartimento di Scienze Biomediche e Oncologia Umana, Università di Torino, Italy.
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Unfavourable events were death for survival and relapse, death in continuous remission or in remission unknown and diagnosis of a second malignancy for event-free survival. All patients were evaluated for survival and event-free survival, while 2 patients, lost to follow-up in unknown remission status, were excluded from disease-free interval analysis.

Patients

2192 patients (1126 males and 1066 females) were recruited into the leukaemia ROT. Eight institutions notified more than 100 cases, seven notified 50–99 cases, nine notified 20–49, two notified 10–19 and 12 notified less than 10 cases. Cases were diagnosed between 1960 and 1988: 10% before 1974, the year in which formal protocols were generally adopted in Italy. While in the late sixties only sporadic cases reached treatment suspension, since the mid 1970s, the number of off-therapy patients has steadily increased to reach over 200 new cases per year (Table 1).

2183 patients were diagnosed by the end of 1986; 9 additional patients, diagnosed between January 1987 and June 1988 underwent bone marrow (BM) transplant in the first continuous CR.

Median age at diagnosis was 4 years 8 months (range 1 month–15 years 11 months); infants constituted 1.0% of cases. At the last control, 1.9% were less than 5 years and 8.8% were aged 20 or older.

Patients were treated with different multiple-drug regimens, depending on calendar period, and in 90% of cases according to a formal protocol [14]. In almost all cases standard two-drug induction (vincristine, prednisone—VP) was followed by classical 6-mercaptopurine + methotrexate maintenance reinforced with VP pulses. About 50% received in addition a third drug in induction and/or a consolidation phase. Since 1982 patients have received a reinduction treatment. The majority of children (84%) received cranial irradiation as part of central nervous system (CNS) prophylaxis: almost half of these (42%) received 24 Gy or more. Median duration of treatment was 34 months; 12.4% of cases were treated for 4 years or longer. 27 patients underwent BM transplantation while on therapy, 7 during the first continuous CR and 20 after an on-therapy relapse.

116 (5.3%) patients reached treatment suspension in second or subsequent remission, having experienced one or more relapses on therapy.

RESULTS

The remission status is shown in Table 2. Seventy-six per cent of patients were alive in continuous CR (CCR), and 22.1% had experienced at least one off-therapy relapse. 11 patients developed a second solid malignancy (SSM) in CCR, and 8 of these have since died.

Survival and event-free survival are shown in Fig. 1. Cumulative proportions (with 95% confidence limits—CL) surviving in CCR and without SSM were 79.0% (77–81 95% CL), 74.6% (73–77 95% CL) and 72.8% (70–75 95% CL) at 2, 5 and 8 years from treatment withdrawal, respectively.

Table 1. Distribution of patients by year of birth, diagnosis and off-therapy (OT)

	No. of patients		
	Birth	Diagnosis	OT
1950–1969	415	39	4
1970–1979	1427	970	491
1980–1988	350	1183	1697

Table 2. Remission status of the population

	n	%
Total population	2192	100
Alive in CCR	1672	76.2
Relapses	485	22.1
Bone-marrow ± other	334	(68.9)*
CNS ± other	69	(14.2)
Gonads	75	(15.5)
Other	7	(1.4)
Solid second malignancy	11	0.5
Haematopoietic second malignancy	9	0.4
Deaths		
In CCR†	13	0.6
In unknown CR status	2	0.1

*Percentage of relapses by site (parentheses) calculated on total number of relapses. †CCR = Continuous complete remission.

The majority of relapses (68.9%) involved the BM, of which 82.3% was isolated, while CNS was the site of relapse in 14.2%. Other isolated extramedullary relapses occurred in 16.9% of patients, the testicular site being the most frequent (Table 2).

Distribution of relapses by site and time from treatment suspension is reported in Table 3. The majority of relapses (62.3%) occurred in the first year off therapy. There were eight relapses after more than 5 years off therapy; two at 62 months (1 BM, 1 gonadal) and 1 each at 63 months (BM), 66 months (BM), 67 months (BM + other), 85 months (CNS), 112 months (CNS) and 143 months (BM + CNS). These 8 patients were in first CR.

Of the 116 patients reaching treatment suspension in second remission, 37 relapsed: the pattern of second relapse is shown in Table 4. In 13 of the 25 cases with relapse on therapy in a sanctuary site (CNS, testis or eye), the off-therapy relapse involved the bone marrow.

There were no differences in disease-free survival for calendar periods of treatment (before 1974: 216 cases; 1974–1982: 1153 cases; 1982 on: 823 cases).

Males experienced 1.5 times more relapses than females (26.6% males and 17.5% females). Disease-free interval curves

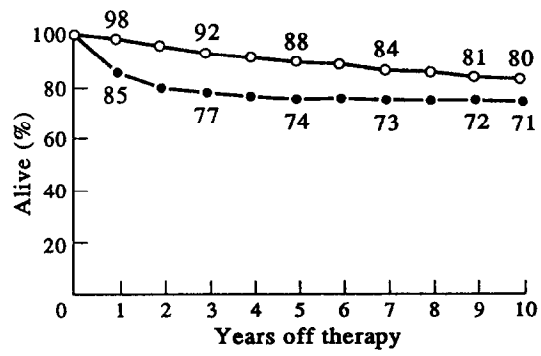

Fig. 1. Survival (SUR ○) and event-free survival (EFS ●) after treatment suspension in childhood acute lymphoblastic leukaemia. The number above the curve represents the number of patients at risk for each period.

Table 3. Off-therapy relapses by site and time from treatment suspension

Site	Months off-therapy 0–11	12–23	24–35	36–47	48–59	60+	Total
Bone marrow ± other*	196	84	27	13	9	5	334
CNS ± other†	49	13	2	3	—	2	69
Gonads							
Testis	50	14	4	—	2	1	71
Ovary	1	3	—	—	—	—	4
Eye	5	—	—	—	—	—	5
Bone	1	1	—	—	—	—	2
Total	302	115	33	16	11	8	485

*Including, in some cases, the gonads. †Two testis (1st and 2nd year) and one kidney + skin.

Table 4. Pattern of first off-therapy relapses according to previous on-therapy relapse site

On-therapy relapse site	Off-therapy relapse site BM ± other	CNS	Eye	Bone	Total
BM ± other	10	2	—	—	12
CNS	8	9	1	—	18
Eye	1	—	1	—	2
Testis	4	—	—	1	5
Total	23	11	2	1	37

by sex are shown in Fig. 2. There was a statistically significant difference between the two sexes ($P < 0.0001$). At 7 years 70.3% (67.4–73.2 95% CL) of males and 80.1% (77.6–82.6 95% CL) of females were alive in CCR.

Disease-free interval for CNS relapses was comparable for patients receiving cranial irradiation as a prophylactic measure or not.

13 patients died in CR of the following causes: 3 cerebral coma of unknown origin (2 suspected encephalitis and 1 suspected second brain malignancy), 1 hydrocephalus, 1 acute viral hepatitis, 2 liver cirrhosis (1 after BM transplantation in first CR),

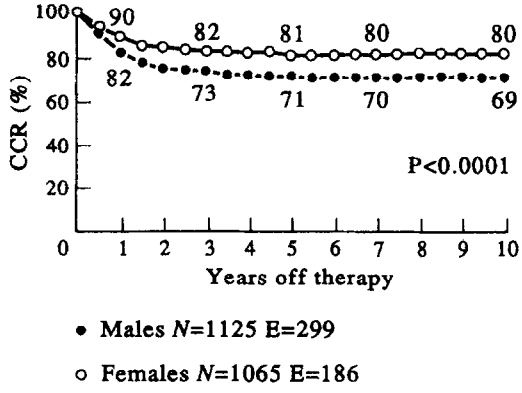

Fig. 2. Disease-free interval by sex after treatment suspension in childhood acute lymphoblastic leukaemia ($P < 0.0001$). The number above the curve represents the number of patients at risk for each period.

Table 5. Solid second malignancy

No.	Sex	Age at ALL diagnosis (years)	SSM	Time off therapy (months)	Cranial irradiation (Gy)	Current status
1	M	2.4	Thyroid carcinoma	78	24	Alive
2	F	4	Thyroid carcinoma	101	34	Lost to follow-up
3	M	3.7	Astrocytoma	97	18	Deceased
4	M	3.6	Astrocytoma	95	24	Alive
5	M	6.7	Astrocytoma	83	24	Deceased
6	F	8.6	Astrocytoma	73	24	Deceased
7	F	7.7	Astrocytoma	76	24	Deceased
8	M	4.4	Astrocytoma	76	24	Deceased
9	F	3.8	Glioblastoma	12	18 + 24	Deceased
10	M	3.6	Oligodendroglioma	63	24	Deceased
11	M	10.9	Osteosarcoma	54	25	Deceased

M, male; F, female. SSM, solid second malignancy.

1 pneumococcal sepsis, 1 myocardiopathy, 1 post-traumatic subdural haematoma and 3 road accident.

The 11 SSM are described in Table 5 and the 9 suspected haematopoietic second malignancies in Table 6. All solid tumours but one who presented a CNS relapse on therapy and received a cranial and spinal irradiation were diagnosed after the fourth year off therapy. There were 8 cases of CNS second malignancy, none of them in the 352 patients who did not receive cranial irradiation (188 observed for more than 4 years and 54 for at least 10 years).

According to the Registry, 22 (5 males, 17 females) of the 171 patients older than 20 years are married; 16 (3 males, 13 females) have a total of 21 healthy children.

Clinical evident sequelae were reported in 149 (7%) patients. Severe complications by system/apparatus were as follows: liver 7, CNS 10, reproductive 4, cardiovascular 5, endocrine 4, other 3.

DISCUSSION

The number of children with ALL reaching treatment suspension recruited by the Italian Registry has grown steadily, reaching over 200 new cases/year since 1980. This uneven distribution of cases by calendar year presumably reflects the increasing

Table 6. Haematopoietic second malignancy after ALL

No.	Sex	Age at ALL diagnosis (years)	Haematopoietic second malignancy	Time off therapy (months)
1	M	11.10	AML	5
2	M	12.4	AML	5
3	F	4.9	AML	14
4	M	5.2	AML	21
5	F	5.7	CML	2
6	F	7.4	CML	2
7	M	9.2	NHL	44
8	F	11.3	NHL	57
9	M	6.6	NHL	106

M, male; F, female. AML, acute myelocytic leukaemia; CML chronic myelocytic leukaemia.

effectiveness of therapeutic regimens. In fact, Italy started in the mid 1970s to adopt what is now considered a minimal standard treatment [14]. A further contributing factor could be that participating centres are progressively draining larger numbers of cases, who were presumably treated before in non-specialised settings. Greater sensitisation of the centres themselves to the registry's demands and the retrospective nature of recruitment for cases stopping treatment before 1980 may also play an important role.

In contrast, the relapse rate after treatment suspension remains constant. Again, treatment is probably the most important factor in this pattern. While in the last 10 years the therapeutic strategies adopted in Italy have brought progressively larger numbers of patients to therapy cessation, these same treatments have not been sufficiently effective to guarantee long-term remission. The use of more aggressive therapies greatly enhances the probability of long-term disease-free survival [4, 5, 15, 16].

While insufficiently aggressive treatment can account for early off-therapy relapses, the relationship between very late events (> 5 years off therapy) and first-line therapies is less probable. In this series relapses occurring as long as 12 years after treatment suspension were observed. Unfortunately, the biological evaluation of these patients at diagnosis is lacking or extremely poor, thus making any comparison of cellular characteristics at onset and relapse impossible. Also, in the face of an ALL recurrence after so many years of complete and untreated remission, the possibility of a second ALL should be considered.

As already recognized [1, 13, 17, 18], the relapse rate of male patients is higher than that of females.

As follow-up time increases, the incidence of CNS complications in our series became greater. With a median follow-up of only 52 months, six deaths in CCR due to neurological problems and eight CNS second malignancies were observed. Tumours of the CNS accounted for 60% of observed SSM and occurred only in previously irradiated patients. CNS complications in leukaemia patients are mainly attributable to CNS prophylaxis [19], at least for sequelae such as leuko-encephalopathy. However, though the oncogenic role of radiation is well established [20], in the case of CNS second malignancies other possible biological interrelations cannot be excluded. In fact the excess of neurological primaries observed in ALL patients [6–8] has a counterpart in studies on patients with CNS tumours, where data on second malignancies and on cancer in relatives seem to indicate a relationship between haematological and neurological neoplastic diseases [21, 22].

The main message of this study is that 24% of children successfully reaching therapy suspension (between one fifth and one fourth) will still experience either relapse, death in CCR or SSM. Relapses are responsible for the vast majority of these failures (94%), indicating that the front line strategies adopted were generally insufficient to eradicate the disease, especially in the male population. The prognosis for relapsed patients is still unsatisfactory [23] and hindered by the toxicity of second-line treatments. In addition, a more "tailor-made" CNS prophylactic approach is needed in order not to expose unnecessarily patients at low biological risk for CNS relapse to the risk of CNS complications.

The Registry was established to unify the many small series in Italy. This allows not only the evaluation of the final outcome of ALL on large numbers, but also provides a surveillance program of relevant but infrequent late complications, like those emerging in the last years (particularly CNS second malignancies and heart failure).

1. George SL, Aur RJA, Mauer AM, Simone JV. A reappraisal of the results of stopping therapy in childhood leukemia. *N Engl J Med* 1979, **300**, 269–273.
2. Johansen OJ, Eilersten ME, Moe PJ. Relapse rate after cessation of therapy in childhood leukaemia. *Acta Paediatr Scand* 1983, **72**, 171–174.
3. Mandelli F, Amadori S, Cantù Rajnoldi A, *et al.* Discontinuing therapy in childhood acute lymphocytic leukemia. *Cancer* 1980, **46**, 1319–1323.
4. Lampert F, Henze G, Langermann HJ, *et al.* Acute lymphoblastic leukemia: current status of therapy in children. *Recent Results Cancer Res* 1984, **93**, 159–181.
5. Niemeyer CM, Hitchcock-Bryan S, Sallan SE. Comparative analysis of treatment programs for childhood acute lymphoblastic leukemia. *Semin Oncol* 1985, **12**, 122–130.
6. Meadows AT, Baum E, Fossati-Bellani F, *et al.* Second malignant neoplasms in children: an update from the late effects study group. *J Clin Oncol* 1985, **3**, 532–538.
7. Gutjahr P. Nonleukaemic second malignancies following childhood acute lymphocytic leukemia. *Helv Paediat Acta* 1985, **40**, 449–459.
8. Rimm IJ, Li FC, Tarbell NJ, Winston KR, Sallan SE. Brain tumors after cranial irradiation for childhood acute lymphoblastic leukemia: a 13 years experience from the Dana-Farber Cancer Institute and the Children's Hospital. *Cancer* 1987, **59**, 1506–1508.
9. Terracini B, Pastore G, Zurlo MG, *et al.* Late deaths and second primary malignancies among long-term survivors of childhood cancer: an Italian multicentre study. *Eur J Cancer Clin Oncol* 1987, **23**, 499–504.
10. Ron E, Modan B, Boice JD, *et al.* Tumors of the brain and nervous system after radiotherapy in childhood. *N Engl J Med* 1988, **319**, 1033–1039.
11. Jankovic M, Masera G, Cristiani ML, Nardi E, Arrighini A. Brain tumors as second malignancies in children treated for acute lymphoblastic leukemia. *Neuro-Oncology* 1991, 365–369.
12. Neglia PJ, Meadows AT, Robison LL, *et al.* Second neoplasms after acute lymphoblastic leukemia in childhood. *N Engl J Med* 1991, **325**, 1330–1336.
13. Zurlo MG, Pastore G, Masera G, *et al.* Italian registry of patients off-therapy after childhood acute lymphoblastic leukemia. *Cancer* 1986, **57**, 1052–1055.
14. Paolucci P, Masera G, Vecchi V, Marsoni S, Pession A, Zurlo MG. Treating childhood acute lymphoblastic leukemia (ALL): summary of ten years' experiences in Italy. *Med Ped Oncol* 1989, **17**, 83–91.
15. Hitchcock-Bryan S, Gelber R, Cassady R, Sallan SE. The impact of induction anthracycline on long-term failure-free survival in childhood acute lymphoblastic leukemia. *Med Pediatr Oncol* 1986, **14**, 211–215.
16. Clavell LA, Gelber RD, Cohen HJ, *et al.* Four-agent induction and intensive asparaginase therapy for treatment of childhood acute lymphoblastic leukemia. *N Engl J Med* 1986, **315**, 657–663.
17. Sather H, Miller D, Nesbit M, *et al.* Differences in prognosis for boys and girls with acute lymphoblastic leukemia. *Lancet* 1981, **1**, 739–743.
18. Gustaffson G, Kreuger A. Sex and other prognostic factors in acute lymphoblastic leukemia in childhood. *Am J Pediatr Hematol Oncol* 1983, **5**, 243–250.
19. Bleyer A, Poplack D. Prophylaxis and treatment of leukemia in the central nervous system and other sanctuaries. *Semin Oncol* 1985, **12**, 131–149.
20. Meadows AT. Second malignant neoplasms. *Clin Oncol* 1985, **4**, 247–261.
21. Farwell J, Flannery JT. Second primaries in children with CNS tumors. *J Neurooncol* 1984, **2**, 371–375.
22. Farwell J, Flannery JT. Cancer in relatives of children with CNS neoplasms. *N Engl J Med* 1984, **311**, 749–753.
23. Butturini A, Rivera GK, Bortin MM, Gale RP. Which treatment for childhood acute lymphoblastic leukemia in second remission? *Lancet* 1987, **1**, 429–432.

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Paolucci), Clinica Pediatrica, Brescia (A.G. Ugazio), Clinica Pediatrica, Cagliari (P. Biddau), Divisione di Ematologia Pediatrica, Catania (S.P. Dibenedetta), Osp. Regionale, Catanzaro (S. Magro), Ospedale di Chiari (E. Mazzoleni), Clinica Pediatrica III, Firenze (C. Guazzelli), Istituto G. Gaslini, Genova (L. Massimo), Osp. Regionale, Mantova (S. Miccolis), Osp. Buzzi, Milano (U. Formica), Osp. Fatebenefratelli, Milano (M. Masarone), Osp. Niguarda, Milano (A. Nicolini), Osp. Regina Elena, Milano (L. Brunelli), Osp. San Carlo, Milano (S. Razon Veronesi), Clinica Pediatrica, Modena (F. Massolo), Clinica Pediatrica, Monza (G. Masera), Clinica Pediatrica I, Napoli (S. Cutillo), Osp. Cardarelli TERE, Napoli (R. Cimino), Osp. Cardarelli Pediatria, Napoli (F. De Maddi), Ops. S. Paolo, Napoli (U. De Bellis), Osp. Pausillipon, Napoli (M. F. Pinta), Clinica Pediatrica II, Padova (L. Zanesco), Clinica Pediatrica, Palermo (M. Lo Curto), Osp. Regionale, Parma (G. Izzi), Clinica Pediatrica, Pavia (F. Severi), Clinica Pediatrica, Perugia (A. Amici), Clinica Pediatrica III, Pisa (P. Macchia), Osp. Bambino Gesù, Roma (A. Don Francesco), Cattedra Ematologia, Roma (F. Mandelli), Clinica Pediatrica, Roma (G. Digilio), Clinica Pediatrica, Siena (A. Acquaviva), Clinica Pediatrica III, Torino (E. Madon), Clinica Pediatrica II, Torino (W. Gabutti), Clinica Pediatrica, Trieste (P. Tamaro), Osp. Regionale, Varese (L. Nespoli).

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Cell Cycle Modifications of Breast Cancers During Neoadjuvant Chemotherapy: a Flow Cytometry Study on Fine Needle Aspirates

Y. Remvikos, M. Jouve, P. Beuzeboc, P. Viehl, H. Magdelenat and P. Pouillart

Breast cancer cells from 92 patients were obtained by repeated fine needle sampling and analysed by flow cytometry for cell cycle modifications during neoadjuvant chemotherapy. Modifications of the histograms were observed for 47 of the 71 informative cases (66%), the most frequent concerning S-phase (increase or decrease) and G_2M accumulation. These modifications correlated well with the efficacy of cytotoxic chemotherapy ($P < 0.0001$). A significant relationship between clinical regression and pretreatment proliferative activity was also observed, with 31/35 (89%) responders in the high proliferation group (S-phase fraction > 5% or BrdU labelling index > 3.3%) compared to 20/36 (56%) in the low proliferation group ($P < 0.002$). For patients undergoing chemotherapy including doxorubicin, a high incidence of G_2M accumulation was observed (33%), a modification which was rare (4.5%) for a regimen with no anthracycline, for which S-phase was the most frequently modified cell cycle compartment (64%). The measurement of the pretreatment tumour proliferative activity as well as the early kinetic modifications, as indicators of response, may prove interesting parameters for the future management of neoadjuvant chemotherapy.
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INTRODUCTION

NEOADJUVANT CHEMOTHERAPY was first proposed as part of multimodality treatments for locally advanced breast cancer [1–3], but was soon extended to operable tumours [4]. Unfortunately, up until now there have been no results from randomised trials demonstrating an advantage of the chemotherapy-first sequence, although it has been claimed to improve breast conservation [5].

The systematic research for prognostic factors led us and others to develop a method for DNA flow cytometric analysis on fine needle samples without aspiration [6, 7] at the time of diagnosis. Thus we were able to show that pretreatment proliferative activity, expressed in terms of S-phase fractions (SPF), was highly correlated with response to neoadjuvant chemotherapy [8].

In the present study, we applied sequential fine needle sampling to follow the course of breast cancers during neoadjuvant chemotherapy. All samples were analysed by flow cytometry for DNA content and the more recent ones for BrdU incorporation [9] in an attempt to achieve early prediction of the efficacy of treatment in individual patients.

PATIENTS AND METHODS

Fine needle cytological samples (FNS) of breast cancers were obtained from 92 patients before and during neoadjuvant chemotherapy, with their informed consent. The patients were treated for stage II or IIIA disease. All were premenopausal. FNS were performed before the first injection (day 0), after the last injection of the first cycle (day 8) and before starting the second cycle (normally day 28).

Flow cytometry

DNA histograms for ploidy or spectrofluorometry analysis and BrdU labelling indices (BLI) were obtained as previously

Correspondence to Y. Remvikos.
Y. Remvikos is at the CNRS URA 620; H. Magdelenat is at the Laboratoire de Radiopathologie; M. Jouve, P. Beuzeboc and P. Pouillart are at the Departement de Medecine Oncologique; and P. Viehl is at the Laboratoire de Cytologie, Institut Curie, 26 rue d'Ulm, 75231 Paris, France.
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