Neuroblastoma (NB) is the most common solid tumour of early childhood and accounts for the main cause of cancer in infants (Bernstein et al., 1992). Indeed, nearly one-third of NBs are diagnosed in children younger than 1 year. Half of the patients present with localised disease (Hartmann et al., 1993; Evans et al., 1984) and gross surgical excision is considered as the main requirement for cure (Evans et al., 1976; Le Tourneau et al., 1985). Primary surgery can be performed in about 50% of these children and reported survival rates are high (De Bernardi et al., 1995). Conversely, unresectable tumours usually have a poorer outcome, unless secondary radical excision can be performed (Haase et al., 1989; Tsuchida et al., 1989). Consequently, the efficacy of primary chemotherapy in allowing subsequent resection is of outstanding importance (Garaventa et al., 1993; West et al., 1993). Recently, we reported improved outcome in such patients after primary chemotherapy including carboplatin and etoposide with a 90% survival rate and children with unresectable NB and no N-myc amplification fared as well as those undergoing primary surgery (Rubie et al., 1998). Moreover, such a strategy resulted in survival rates over 95% in infants (Rubie et al., 2001). However, immediate haematological toxicity and transfusion-related risks and possible long-term side effects of such treatment administered in young children may limit its use, although preliminary results of their follow-up showed neither renal nor audition deterioration (Bergeron et al., 2000). Furthermore, this regimen included anthracyclines and we are concerned about the possible long-term consequences on cardiac function. Those considerations prompted us to evaluate the efficacy of a presumably less toxic chemotherapy with low-dose cyclophosphamide and vincristine (CV) in order to attempt a safe surgical excision and ultimately decrease the risks of long-term sequelae (NBL 94 study).
PATIENTS AND METHODS

Patient population
All consecutive and untreated children younger than 12 months at diagnosis, and referred to SFOP institutions, were eligible in the study. Evaluation at diagnosis included CT scan or MRI, MIBG and extensive bone marrow staging (at least two evaluable aspirates and two trephine biopsies if possible). Tumour volume was calculated with the product of the three largest diameters (height, width and thickness). Urinary catecholamines, serum NSE, ferritin and LDH were also measured. Histology of the primary (with either surgical biopsy or tru-cut biopsy) was mandatory to allow both NB diagnosis according to INSS recommendations (Brodeur et al 1993) and MYCN analysis. Only patients with localised NB and no N-myc amplification (less than 10 copies per haploid genome) were eligible in the study.

Surgery
Participating institutions were provided with guidelines for surgical procedures. Resectability was defined according to imaging data. Procedures that would have resulted in the removal of major organs were not recommended unless primary chemotherapy had been administered before any attempt of excision. Tumours defined as unresectable were lesions that crossed and infiltrated the mid-line structures, usually encasing large vessels, and tumours that because of size structure or location, were deemed as difficult to resect without a high risk of rupture of the tumour or of major surgical complications. All children presenting with life-threatening symptoms (cardiac failure, acute respiratory distress, etc) or dumb-bell tumours and neurological symptoms were urgently assigned to more intensive chemotherapy using a combination of carboplatin and etoposide. The appropriateness of treatment allocation was systematically reviewed. Postoperative imaging (CT scan or MRI) was required in all patients 1 month after resection. MIBG scan was recommended in case of treatment failure. Post-surgical staging was defined on the basis of surgical, pathological reports and postoperative imaging data.

Chemotherapy
First-line chemotherapy consisted of two courses of low-dose cyclophosphamide (5 mg kg\(^{-1}\) for days 1–5) and vincristine (0.05 mg kg\(^{-1}\) on day 1) (CV) given at a 2-week interval (Figure 1). Following a first evaluation after those two courses, two more CV courses were to be administered if tumour shrinkage was over 25% and if the tumour was still unresectable. Resectability was then assessed and surgery was attempted in the absence of risk. In case of either no tumour response after the two first courses of CV, or persisting preoperative risk after four courses, or life-threatening symptoms, or symptomatic dumb-bell at diagnosis, a combination of carboplatin (6.6 mg kg\(^{-1}\) on days 1–3) and etoposide (5 mg kg\(^{-1}\) on days 1–3) (CE) was given, followed if necessary by two courses of vincristine (0.05 mg kg\(^{-1}\) days 1 and 5), cyclophosphamide (10 mg kg\(^{-1}\) days 1–5) and doxorubicin (1 mg kg\(^{-1}\) on days 4 and 5) (CAdO) as previously described (Rubie et al, 1998). Drug doses were always reduced by 30% in babies under 1 month of age. No postoperative treatment was recommended whatever the quality of surgical excision.

Evaluation of response to therapy
Response to therapy was assessed according to INRC criteria (Brodeur et al, 1993). The value of tumour response, based on reduction of volume that was considered more significant than that of urinary catecholamine excretion, was evaluated during induction therapy (every two courses) before and 1 month after surgery, and then at least every 3 months. As those children were young, response was considered as significant if the tumour shrinkage was over 25% of the volume at diagnosis (objective response – OR) instead of the usual shrinkage of 50% (partial response – PR). Indeed, such an objective effect was considered as encouraging to continue the treatment.

Statistical analysis
To prevent selection bias, all consecutive patients with newly diagnosed localised NB in the participating centres were included in the analysis, whatever the treatment actually administered. The probabilities of survival were calculated from the time of diagnosis to death or last follow-up according to the Kaplan – Meier product-limit method (Fleiss, 1981). In the event-free survival (EFS), disease progression or relapse and death, whatever the reasons, were considered as events. Comparisons between mean doses of chemotherapy and proportions were performed with Student’s \(t\)-and \(\chi^2\)-tests corrected for heterogeneity or Fisher’s exact test, respectively (Peto et al, 1977). The analysis of EFS times was performed by Cox’s proportional hazards models and differences between curves were tested for statistical difference by the log-rank test (Cox, 1977). The multivariate model was not used as no prognostic factor was found. All tests were two-tailed.

RESULTS
From January 1995 to December 1999, a total of 301 consecutive children with confirmed localised NB were registered in the study. Among them, 134 were infants of whom 82 underwent primary surgical excision. Among the 52 remaining infants presenting with and unresectable primary, N-myc was not evaluable in 13 and not amplified in the other 39. Therefore, the present analysis (NBL 94 study) deals with 39 infants with localised unresectable tumour
and no N-myc amplification, and reports their outcome as of October 2002, 33 months after the last patient’s inclusion.

PATIENT CHARACTERISTICS (TABLE 1)

Most primary tumours were abdominal (64%), INSS stage 3 (97%) and neuroblastoma (97%). As compared to the previous cohort (NBL 90 study), patient characteristics were similar regarding age, gender, location of the primary, stage, histology and N-myc status (Table 1). The only difference was patient’s eligibility. Actually, as compared to the NBL 94 study, histology was not mandatory at diagnosis in the NBL 90 study, explaining that more children could be included in the former one.

Primary chemotherapy (Table 2)

A total of 29 children were to be given low doses of CV according to the protocol. Among them, one patient presented with an antenatal diagnosis and spontaneous regression of the tumour was observed and neither chemotherapy nor surgery was realised. Thus, 28 received two courses of CV. OR was observed in 14, which led to two additional courses to 11 of them before surgery. The remaining three children presenting with a moderately symptomatic dumb-bell tumour at diagnosis had no significant improvement or response of the intraspinal component after the two first CV courses and received subsequently the CE regimen. Four additional patients were not evaluated correctly after the two CV courses, three of whom were given two additional courses. No significant response was observed in 10 and all received CE (Table 2). According to the protocol recommendations, neurosurgical resection was considered as useless (due to the duration of symptoms or no significant response of the intraspinal component after the two first CV courses) and eight children with moderate to severe neurological impairment. Eight children were given CE as first-line chemotherapy either because of symptomatic dumb-bell NB was observed in 10 out of 33 evaluable (see text). Among them, seven received CE according to the protocol, eight children with moderate to severe neurological impairment.

Table 1: Patient characteristics in comparison with the previous cohort (Rubie et al, 2001)

| NBL 90 study | NBL 94 study |
|--------------|--------------|
| Number       | %            | Number | %            | P |
| Registered   | 338          | 385    |
| Eligible     | 316/338      | 93.5   | 301/385      | 78.2 | <0.001 |
| Infants      | 152/316      | 48.1   | 134/301      | 44.5 | NS    |
| Unresectable | 52/152       | 34.2   | 52/134       | 38.8 | NS    |
| Evaluable N-myc | 37/52   | 71.2   | 39/52        | 75.0 | NS    |
| No N-myc amplification | 35/37   | 94.6   | 39/39        | 100  | NS    |
| Median age   | 5.4 (1–11)   | 5.3 (0–11) | NS |
| (months, range) |          |        |              |      |
| Dumb-bell tumour | 14     | 40     | 15           | 38   | NS    |

NS = not significant

Deficit) or could be avoided in all but one and neurological recovery was observed in five of them. As a whole, the observed response rate (RR) according to first-line chemotherapy was 61% (n = 20 out of 33 evaluable) and 58% in patients receiving only CV (n = 14 out of 24). Furthermore, RR to first- and second-line chemotherapy planned by the protocol was 82% (31 out of 38). No significant toxicity, particularly haematological, was observed after CV and the time elapsing between two courses was always less than 2 weeks. The toxicity of CE+CAdO was mainly haematological and easily manageable as already reported (Rubie et al, 1998, 2001). In summary, 14 children received only CV (four courses), 11 were given CV and CE (two courses each), five had CV–CE and CAdO (courses each) and eight received CE and CAdo (two courses each). The cumulative doses of potentially harmful drugs (i.e. doxorubicin–etoposide and carboplatin) was significantly lower in the present protocol than that of the previous regimen given in the NBL 90 study particularly for doxorubicin (2.31 vs 4.29 mg kg⁻¹, P < 0.0005) and carboplatin (31.98 vs 41.3 mg kg⁻¹, P < 0.05).

Surgery

Surgical excision was attempted in all children but one (spontaneous regression of the tumour with continuous complete

Table 2: Response to primary chemotherapy and outcome for all patients (n = 39)

| First-line chemotherapy | Response | Second-line chemotherapy | Overall response | Postoperative status | Relapse | Outcome |
|-------------------------|----------|--------------------------|------------------|---------------------|--------|---------|
| CV, n = 28              | 14 OR    | CV, n = 11               | 11 PR            | 7 CR, 4 VGPR        | 2 local| 3 CR    |
| CV, n = 3*              | 2 PR     | 2 VGPR                   |                  |                     |        |         |
| CV, n = 4               | 2 PR     | 2 VGPR                   |                  |                     |        |         |
| CV, n = 6               | 3 PR     | 2 CR, 1 VGPR             |                  |                     |        |         |
| CV, n = 5               | 3 NR     | 2 CR, 1 VGPR             |                  |                     |        |         |
| CV, n = 8               | 2 PR     | 2 CR                    |                  |                     |        |         |
| CV, n = 9               | 2 NR     | 2 CR                    |                  |                     |        |         |
| CV, n = 10              | 3 NR     | 2 CR                    |                  |                     |        |         |
| CV, n = 11              | 4 NE     | CV, n = 3               | 2 PR, 1 NR       | 3 CR                | 3 CR   |         |
| CV, n = 12              | 4 NE     | CV, n = 4               | 1 PR             | 1 CR                | 1 CR   |         |
| CV, n = 13              | 4 NE     | CV, n = 5               | 1 PR             | 1 CR                | 1 CR   |         |
| CV, n = 14              | 4 NE     | CV, n = 10              | 1 PR             | 1 CR                | 1 CR   |         |
| CV, n = 15              | 4 NE     | CV, n = 11              | 1 PR             | 1 CR                | 1 CR   |         |
| CV, n = 16              | 4 NE     | CV, n = 12              | 1 PR             | 1 CR                | 1 CR   |         |
| CV, n = 17              | 4 NE     | CV, n = 13              | 1 PR             | 1 CR                | 1 CR   |         |
| CV, n = 18              | 4 NE     | CV, n = 14              | 1 PR             | 1 CR                | 1 CR   |         |
| CV, n = 19              | 4 NE     | CV, n = 15              | 1 PR             | 1 CR                | 1 CR   |         |
| CV, n = 20              | 4 NE     | CV, n = 16              | 1 PR             | 1 CR                | 1 CR   |         |
| CV, n = 21              | 4 NE     | CV, n = 17              | 1 PR             | 1 CR                | 1 CR   |         |
| CV, n = 22              | 4 NE     | CV, n = 18              | 1 PR             | 1 CR                | 1 CR   |         |
| CV, n = 23              | 4 NE     | CV, n = 19              | 1 PR             | 1 CR                | 1 CR   |         |
| CV, n = 24              | 4 NE     | CV, n = 20              | 1 PR             | 1 CR                | 1 CR   |         |
| CV, n = 25              | 4 NE     | CV, n = 21              | 1 PR             | 1 CR                | 1 CR   |         |
| CV, n = 26              | 4 NE     | CV, n = 22              | 1 PR             | 1 CR                | 1 CR   |         |
| CV, n = 27              | 4 NE     | CV, n = 23              | 1 PR             | 1 CR                | 1 CR   |         |
| CV, n = 28              | 4 NE     | CV, n = 24              | 1 PR             | 1 CR                | 1 CR   |         |
| CV, n = 29              | 4 NE     | CV, n = 25              | 1 PR             | 1 CR                | 1 CR   |         |
| CV, n = 30              | 4 NE     | CV, n = 26              | 1 PR             | 1 CR                | 1 CR   |         |
| CV, n = 31              | 4 NE     | CV, n = 27              | 1 PR             | 1 CR                | 1 CR   |         |
| CV, n = 32              | 4 NE     | CV, n = 28              | 1 PR             | 1 CR                | 1 CR   |         |
| CV, n = 33              | 4 NE     | CV, n = 29              | 1 PR             | 1 CR                | 1 CR   |         |
| CV, n = 34              | 4 NE     | CV, n = 30              | 1 PR             | 1 CR                | 1 CR   |         |
| CV, n = 35              | 4 NE     | CV, n = 31              | 1 PR             | 1 CR                | 1 CR   |         |
| CV, n = 36              | 4 NE     | CV, n = 32              | 1 PR             | 1 CR                | 1 CR   |         |
| CV, n = 37              | 4 NE     | CV, n = 33              | 1 PR             | 1 CR                | 1 CR   |         |
| CV, n = 38              | 4 NE     | CV, n = 34              | 1 PR             | 1 CR                | 1 CR   |         |
| CV, n = 39              | 4 NE     | CV, n = 35              | 1 PR             | 1 CR                | 1 CR   |         |

CR = complete remission; VGPR = very good partial remission; PR = partial remission; OR = objective response; NR = no response. *Those three children received CE because persisting neurological symptoms or no intraspinal component shrinkage after CV courses (see text).
Consequently, primary chemotherapy allowing resection is of outstanding importance. The outcome of infants with localised but unresectable tumours is rarely reported and represents a challenging issue to limit the risk of immediate morbidity and treatment-related sequelae. Indeed, the poor prognosis usually associated with unresectable primaries should be balanced with the favourable outcome reported in infants (Evans et al., 1976; Hartmann et al., 1983).

We reported recently in the NBL 90 study a survival rate over 95% in infants presenting with localised and unresectable NB and with no N-myc amplification, but chemotherapy was intensive (Rubie et al., 2001). These excellent results may have been due first to a strict selection of patients having a favourable type of NB (i.e. extensive staging including MIBG to eliminate metastatic disease). Secondly, it has been demonstrated that N-myc amplification had a major prognostic value in localised NB, including in infants; indeed, although rare in that subset of patients, N-myc amplification has a strong negative influence on outcome (Rubie et al., 1997). Last, primary chemotherapy using a combination of two courses of CE followed by two courses of CAdO proved to be very efficient as all infants could undergo safe and satisfying surgical excision as shown in our previous NBL 90 study. However, haematological toxicity was significant, although always manageable, carrying transfusion-related risks. Moreover, although subsequent follow-up showed neither auditory nor renal toxicity (Bergeron et al., 2000), the risk of long-term sequelae due to that chemotherapy administered in young children cannot be definitely ruled out. This is the reason why the efficacy of a low-dose CV regimen, a first-line therapy was investigated in that subset of patients, providing no urgent situation (i.e. life-threatening sign or symptomatic dumb-bell tumour) as that regimen is thought to have a lower or delayed efficacy as compared to CE combination.

The stratification of treatment could not be performed on other risk factors such as tumour size or location or LDH, since small primaries may cause threatening symptoms. Thus, treatment allocation was based on clinical tolerance of the tumour. Indeed, 20% of patients presented with a symptomatic dumb-bell tumour. In such a situation, any treatment has to be delivered in emergency to avoid neurologic sequelae and we already reported the efficacy of that chemotherapy as an alternative to decompressive laminectomy in order to prevent orthopaedic sequelae as well (Plantaz et al., 1996). This strategy also seems appropriate for children presenting with slight neurological symptoms. Indeed, in this study three of four children were given CE as second-line chemotherapy, suggesting that CV is not to be recommended in that situation. Although the number of patients is small, we confirm the relevance of such an approach in this series. As far as other patients are concerned, this strategy proved to be efficient as a safe resection could be attempted in nearly 50% of these children. Is it possible to go further in the de-escalation process? The usefulness of neoadjuvant treatment may be questioned in such a subgroup of patients. A recent report suggests that apart from surgical excision adjuvant treatment is not necessary, providing no N-myc amplification has been found (Kushner et al., 1996). However, this is a single-centre study that included only four patients with true INSS stage 3 disease and no dumb-bell tumour, and such results may be difficult to achieve in a multicentre setting. Furthermore, surgery may be also harmful, especially in multicentric studies. We already reported a 3% death rate after postoperative complications among 186 patients who underwent primary surgical excision (Rubie et al., 1997). As spontaneous regression of the disease may be observed in very young children, some investigators are evaluating the feasibility of observation only in infants presenting
with localised but unresectable NB. Recently, the German group reported spontaneous regression of the primary after biopsy or partial resection in infants with unresectable NB, but the cohort was small and the follow-up still too short to draw any definitive conclusions (Hero et al., 2002). Those studies are still underway and their results, provided they are confirmed, may be considered to establish future strategies in multicentric prospective studies.

In conclusion, low-dose chemotherapy is efficient in about half of infants presenting with unresectable NB and no N-Myc amplification, allowing safe surgical resection and preventing long-term late side effects. Those patients have to be strictly selected (absence of threatening symptom) and followed cautiously in order to benefit from such a de-escalation strategy without jeopardising the excellent outcome. This approach is presently awaiting confirmation in a larger European study.

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