Eur J Cancer Clin Oncol, Vol. 25, No. 11, pp. 1653–1655, 1989.
Printed in Great Britain

0277-5379/89$3.00 + 0.00

Letter to the Editor

Relapse from a Stage IV-S Neuroblastoma and N-*myc* Amplification

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In the staging of neuroblastomas proposed by Evans *et al.* [1], the stage IV-S corresponds to infants having a primary tumor of stage I or II size but showing disseminated disease in the liver, skin and/or bone marrow without involvement of the skeleton. These stage IV-S neuroblastomas exhibit a particularly good prognosis as compared to other stage IV diseases. Indeed, most of them regress spontaneously given the non-aggressive therapy which is commonly used. However, a few tumors display rapid progression or may rarely relapse following an initial complete remission [2]. A retrospective analysis of the IV-S group reveals a disease free survival ranging from 57% to 90% [3]. Thus, the major clinical challenge with these patients is to predict possible tumor progression.

Seeger *et al.* [4] have shown that progression of neuroblastoma is associated with the N-*myc* oncogene amplification. However, few data are available about the relevance of N-*myc* copy number as a prognostic index in stage IV-S neuroblastomas.

Between December 1986 and April 1988, four tumors of stage IV-S were received and analyzed for their N-*myc* genomic content (Fig. 1): one metastatic specimen at relapse (case No. 1) and three primary tumors (cases Nos. 2, 3 and 4). For case No. 1 it has not been possible to obtain a specimen of primary tumor. Cases Nos. 2, 3 and 4 showed a single copy of N-*myc* per haploid genome. They entered complete remission and remained free of disease 11, 13 and 21 months post diagnosis, respectively. In contrast, the specimen obtained from patient No. 1 exhibited an amplification of N-*myc* related to a poor outcome.

Accepted by 5 July 1989.
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This patient was 1 month old at diagnosis and showed a primary tumor of adrenal origin (3 cm in diameter) with metastatic dissemination in the liver. Bone marrow aspirates, radiologic examination of the skeleton and meta-iodobenzyl guanidine scan showed no evidence of bone dissemination. The patient was observed with no treatment for 3 months. During this period, the CT scan showed a marked decrease in the size of the liver lesions. At this time, a complete surgical excision of the primary tumor was performed. On histological examination of this tumor, a typical undifferentiated neuroblastoma was noted. No further therapy was necessary given the total disappearance of the liver metastases within a few months. In spite of this spontaneous and complete remission, this patient relapsed 5 months later, exhibiting a massive skeletal and bone marrow involvement, as well as hepatic and abdominal lymph node metastases. We cultured the bone marrow in order to analyze the N-*myc* genomic content of the metastatic cells. Southern technique analysis revealed that these neuroblastoma cells exhibited a 50-fold amplification of N-*myc* gene (Fig. 1). The patient had been then treated by three cycles of etoposide-*cis*-platinum [5]. After achieving a partial remission, he relapsed and died (2 years after diagnosis).

Given that specimen of primary tumor was not available, this result addresses two hypotheses: N-*myc* oncogene was not amplified in the primary tumor and became during progression of the disease suggesting the emergence of a new clone of malignant cells at recurrence; N-*myc* amplification was present in the primary tumor but the expression of the gene did not correlate with expression of malignancy. The latter hypothesis might be more likely. Indeed in neuroblastomas with N-*myc* ampli-

fication, the gene alteration has been shown to be a stable genetic marker, since it is present in the primary tumor, metastases and recurrences [6], whatever the treatment applied to the patient [7].

The role of N-*myc* amplification in stage IV-S neuroblastoma as an index of poor prognosis has not been firmly stated. Tonini *et al.* [8] reported one case of N-*myc* amplification in a tumor, which was associated with a favorable outcome for the patient as judged 10 months after diagnosis. By contrast, Cohn *et al.* [9] described one case of N-*myc* amplification related to rapid tumor progression. In the particular case of our patient, N-*myc* amplification was not associated with initial rapid progression of the disease but with a relapse following complete remission obtained without any systemic therapy.

Although the evolution of these neuroblastomas is usually favorable, we must keep in mind that a few of them will not be cured. In that respect, N-*myc* amplification would constitute a crucial determinant in identifying this patient subgroup and could lead the physician to apply more aggressive therapy to the N-*myc* amplified stage IV-S neuroblastomas.

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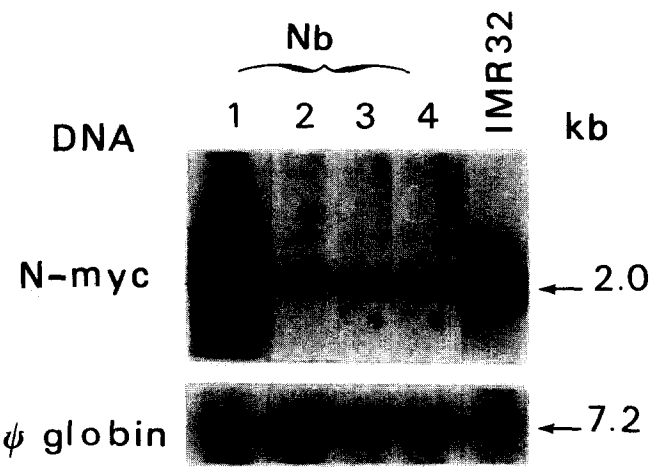

Fig. 1. *Southern blot analysis: DNA was digested with the restriction enzyme* EcoRI *and hybridized with the* ^{32}*P-labeled N-*myc *probe pNb-*1 [4]. *Each lane contains* 6 μg *of DNA. The filter was washed off and rehybridized with the globin pseudogene used as control of a single copy gene. Lane* 1 *contained DNA isolated from a IV-S neuroblastoma at relapse (patient No.* 1). *Scanning laser densitometry showed that this patient's tumor cells had* 50 *copies of N-*myc. *Lanes* 2, 3 *and* 4 *contained DNA isolated from three stage IV-S primary tumors (patients Nos.* 2, 3 *and* 4). *The established human neuroblastoma cell line, IMR-*32 *which exhibited* 15 *copies of N-*myc, *was used as a positive control for amplification (lane* 5).