



# Alveolar Rhabdomyosarcoma in a 68-Year-Old Patient Identified by Cytogenetic Analysis of Bone Marrow

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**ABSTRACT:** *Pancytopenia and fulminant disseminated intravascular coagulation in a 68-year-old woman suggested an acute hematologic malignancy. However, cytogenetic analysis on a bone marrow sample revealed a near-tetraploid karyotype with an isochromosome 1q and a translocation (2;13) (q35;q14), which was suggestive of an alveolar rhabdomyosarcoma (ARMS). This diagnosis was subsequently confirmed by indirect immunohistochemistry. ARMS has not yet been observed in a patient of this age. Thus, our case underlines the importance of cytogenetics, to establish an a priori unexpected tumor diagnosis.* © Elsevier Science Inc., 1998

## INTRODUCTION

Rhabdomyosarcoma is the most common malignant soft tissue tumor in children [1]. The two major forms of childhood rhabdomyosarcoma are embryonal and alveolar rhabdomyosarcoma (ARMS), but poorly differentiated forms also are common. In adults over 40 years, ARMS is extremely rare, with only a few cases observed. Histological diagnosis of rhabdomyosarcoma often remains difficult. Because a variety of solid tumors with round cell morphology including rhabdomyosarcoma, neuroblastoma, Ewing sarcoma, and some cases of non-Hodgkin lymphoma cannot be differentiated by light microscopy alone, additional immunohistochemistry or electron microscopy or both are needed for further tumor classification. To confirm the diagnosis of ARMS, cytogenetic analysis has become an important diagnostic tool. In a number of cases, a close relation was shown between the translocation (2;13) (q35;q14) and alveolar rhabdomyosarcoma [2–9], which is known to have a poorer prognosis than other histologic subtypes. This unfavorable prognosis is related to the propensity for early and wide tumor dissemination and poor response to chemotherapy. The t(2;13) has not been associated with any other tumors and has been detected in

70% of published ARMS cases [10]; thus, this translocation represents a specific marker for ARMS.

In the present report, we describe a tumor of unclear origin in an elderly woman, in which cytogenetic analysis performed on a bone marrow sample unequivocally led to the diagnosis of ARMS.

## CASE REPORT

A 68-year old woman presented at a local hospital with signs of anemia, hemorrhagic diathesis (with multiple hematomas, epistaxis, and gross hematuria), generalized pain, and a short history of weakness. The whole blood count revealed severe thrombocytopenia and anemia. Because acute leukemia was suspected, the woman was transferred for further evaluation to the Department of Internal Medicine, Division of Hematology of the University Hospital in Vienna. On admission, there was a normochromic anemia (hemoglobin, 8.5 g/dL), thrombocytopenia (15,000/µL), and a pronounced left shift in the differential white blood count (leucocytes, 6030/µL; blasts, 4%; promyelocytes, 2%; myelocytes, 9%; metamyelocytes, 5%; stabs, 3%; and segs, 53%). The presence of numerous normoblasts (10%) pointed to a severe disturbance of the bone marrow/blood barrier. Low haptoglobin and fibrinogen, coagulopathy with highly elevated activation markers (D-dimer, thrombin-antithrombin III, prothrombin-fragment F1+2), and lactate dehydrogenase indicated massive disseminated intravascular coagulation (DIC) based on a generalized, putatively malignant process.

Bone marrow examination by pelvic biopsy and aspiration revealed an infiltration with loosely arranged, poorly differentiated round or oval-shaped malignant tumor cells. This relatively uniform cell population completely displaced the bone marrow. Small areas of necrosis and large

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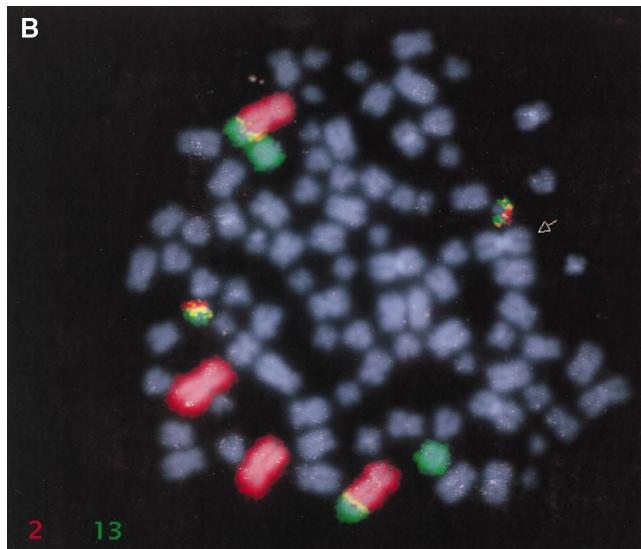
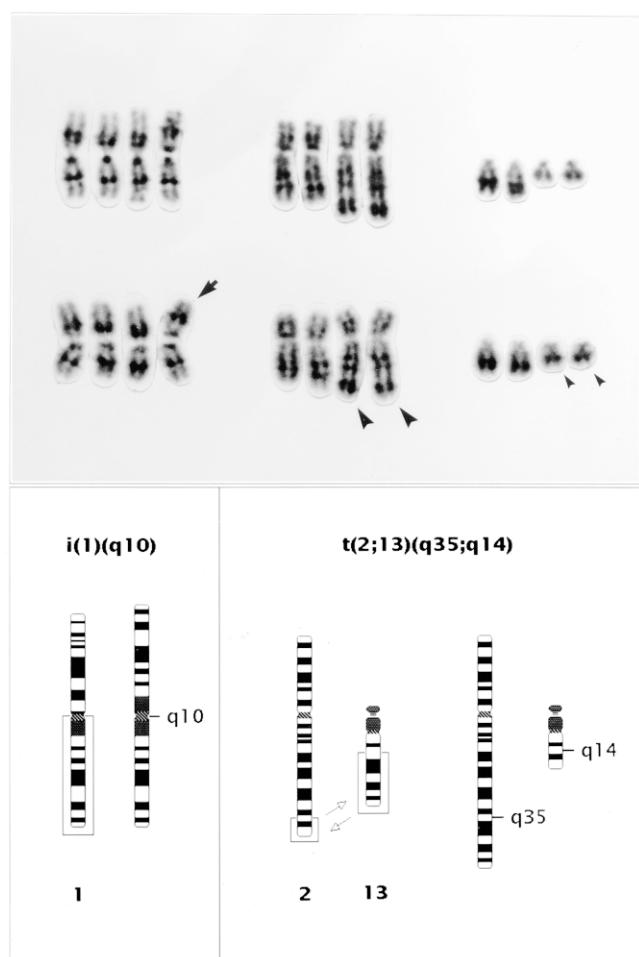


**Figure 1** MR tomography of the cervical and upper thoracic vertebral spine (sagittal view, T2-weighted spin-echo sequence). The arrows mark the soft tissue part of the tumor mass, extending from C5 to T1 and consisting of a prevertebral and intraspinal component (large arrows). There is a direct contact of tumor and myelon (small arrow) with displacement of surrounding liquor.

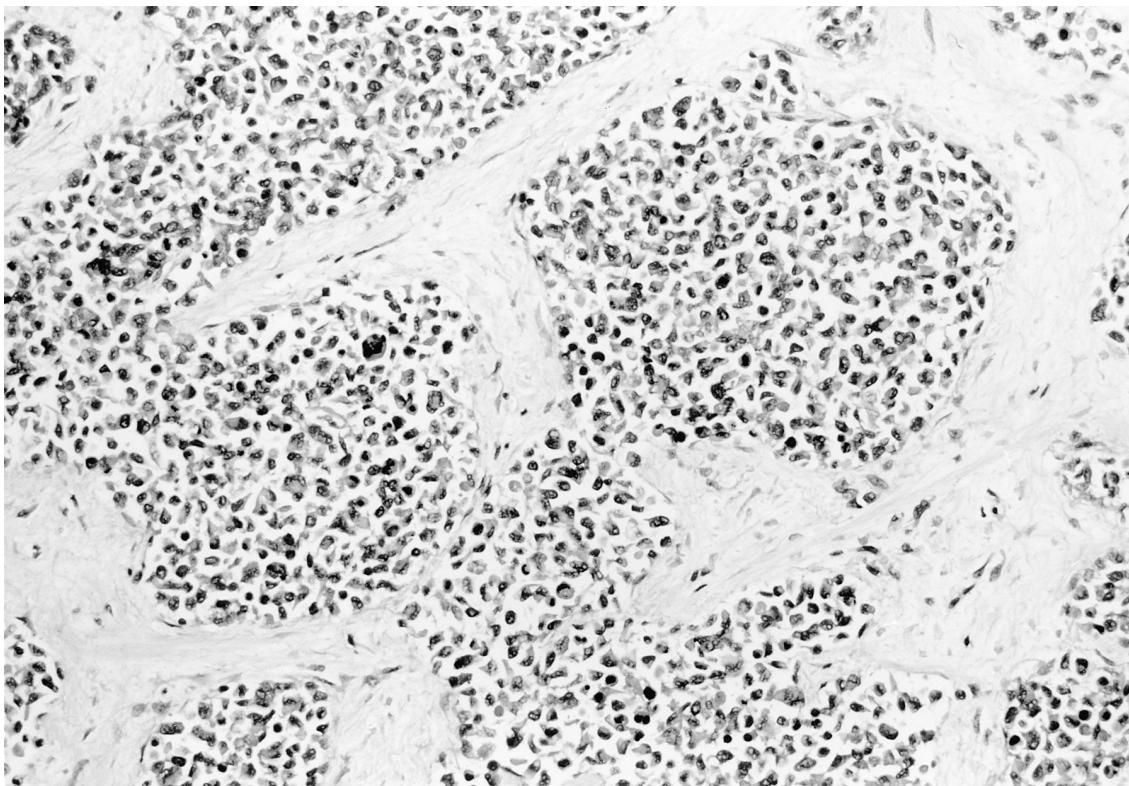
numbers of apoptotic cells were present throughout the tumor. Immunohistochemistry with a standard panel of antibodies excluded the presence of a hematological malignancy but could not provide convincing clues to the origin of the tumor. A scanty positive reaction for cytokeratin (CAM 5.2) and strong positive reaction for vimentin suggested the diagnosis of a poorly differentiated malignoma of putatively mesenchymal origin. Computerized tomography (CT) of the chest revealed an upper thoracic prevertebral mass and a pathological costal fracture. A perivertebral solid mass extending from vertebral bodies C5 to Th1 with epidural and subarachnoidal infiltration was revealed on the MRI scan of the cervical vertebral spine (Fig. 1). A course of chemotherapy including cyclophosphamide, doxorubicin, and cisplatin was given. However, rapid disease progression led to clinical deterioration, and the woman died 11 days after admission.

#### Cytogenetics

With the use of cytogenetic methods described previously [11], chromosome analysis was performed on Giemsa-banded metaphases from a bone marrow specimen. Seven of 15 metaphases were characterized by the translocation (2;13)(q35;q14), strongly indicating a diagnosis of ARMS [2–9]. These 7 metaphases were near-tetraploid, with a



**Figure 2** (A) Partial karyotypes from two near-tetraploid metaphases showing  $t(2;13)(q35;q14) \times 2$  (arrowhead) and  $i(1)(q10)$  (arrow). (B) Tetraploid metaphase hybridized with painting probes for chromosomes 2 (red) and 13 (green) showing the reciprocal translocation (2;13) and the isochromosome (1)(q10) (arrow).



**Figure 3** Aggregates of round tumor cells separated by fibrous trabeculae.

chromosome number ranging between 80 and 92, and showed an isochromosome of the long arm of chromosome 1—i(1)(q10). Both findings, aberrations of chromosome 1 and tetraploidy, had been reported to be associated with ARMS [4, 12]. In Figure 2A, partial karyotypes of representative tumor cells are shown, including a schematic diagram. The translocation (2;13) occurred in duplicate, whereas the isochromosome (1)(q10) was single, indicating that the translocation was formed before and the isochromosome after the duplication event. The remaining 8 metaphases had a normal female karyotype. In view of the uncommon diagnosis of ARMS in a patient of this age, an examination of t(2;13) by fluorescence in situ hybridization (FISH) with the use of whole-chromosome probes and the technical protocol of ONCOR was performed. Indeed, in two chromosome pairs, t(2;13) was demonstrated by FISH (Figure 2B), confirming the cytogenetic diagnosis of ARMS.

#### Postmortem Examination

Autopsy revealed widespread abdominal, thoracic, and cervical metastases: a solid mass in the pancreas measuring 10×5×5 cm initially suggested a primary pancreatic carcinoma. A prevertebral mass of similar size extended from C5 to T1, and multiple smaller tumors were found in the thoracic wall and in the submucosa of the stomach. The larger masses contained widespread, macroscopically identifiable necroses. Ill-defined tumor margins indicated

invasive growth. Aggregates of poorly differentiated, round tumor cells were separated by broad, fibrous trabeculae (Fig. 3). The cytogenetically suspected ARMS was ultimately confirmed by immunohistochemistry (indirect immunoperoxidase on formalin-fixed paraffin-embedded tissue) by using a broad panel of antibodies (Table 1). A strongly positive reaction for muscle-specific actin (HHF 35-antibody) in most tumor cells, which were completely negative for smooth muscle actin (1A4-antibody), established the diagnosis of ARMS. On archived sections of the

**Table 1** Immunostaining results

Antigen/antibody (clone)	Result
Smooth muscle actin $\alpha$ (1A4)	0
Muscle actin $\alpha$ and $\gamma$ (HHF 35)	+++
Desmin (33)	+
S100 (polyclonal)	+
Vimentin (V9)	+++
Cytokeratins 8, 18, 19 (CAM5.2)	+
MIC-2 (12e7)	0
Melanoma-specific antigen (HMB45)	0
Neuron-specific enolase (BBS/NC/VI-H14)	0
Chromogranin-A (LK2 H10)	0
Leukocyte common antigen (2B11 and Pd7/26)	0

*Symbols:* 0, no positive tumor cells; +, <10% of tumor cells positive; ++, 10–50% of tumor cells positive; +++, >50% of tumor cells positive.

bone marrow biopsy, an identical staining pattern with the use of the extended panel of probes (Table 1) proved infiltration by ARMS.

## DISCUSSION

We describe the first case of alveolar rhabdomyosarcoma (ARMS), initially presenting as an acute hematologic disorder that was identified cytogenetically through a translocation (2;13) in the cells of a bone marrow sample. Prompted by the cytogenetic findings, immunohistochemistry was performed by using a special panel of antibodies (Table 1), which confirmed the diagnosis of ARMS [13].

Translocation (2;13) and the variant translocation (1;13) are highly specific for ARMS and have not yet been found in other malignancies [10]. Most so-called tumor-specific chromosome aberrations occur in different malignant disorders or groups of disorders—such as the t(9;22) in chronic myeloid leukemia (CML), acute myeloid leukemia (AML), and acute lymphocytic leukemia (ALL); the t(14;18) in follicular lymphoma and ALL; the loss of 17p in CML and other leukemias but also in solid tumors; trisomy 8 in hematologic disorders and in breast cancer; and the t(11;22) and variants in Ewing sarcoma and primitive neuroectodermal tumor [14]. As yet, only the translocation (15;17) like t(2;13), has shown high specificity, being restricted to one tumor subtype, acute promyelocytic leukemia (AML-FAB type M3). Although t(2;13) is a tumor-specific chromosome aberration, allowing classification of an undifferentiated tumor as an ARMS, isochromosome (1q) occurred as a secondary anomaly in the course of karyotype evolution, indicating tumor progression.

In all ARMS-specific 2;13 translocations, the breakpoints are located at 2q35 and 13q14. Cloning of the breakpoints has revealed transcription-factor-encoding genes *PAX3* at 2q35 and *FKHR* at 13q14. The *PAX3*-*FKHR* fusion protein may function as an oncogenic transcription factor by enhanced activation of normal *PAX3* target genes [15]. Bone marrow failure characteristic of acute leukemia is also typical of ARMS, which frequently presents with bone marrow infiltration [8, 16–21]. The mechanism of fulminant DIC is largely unknown, but it can be assumed that tumor cells may release mediators with a procoagulant activity [22]. In ARMS with t(2;13), it is conceivable that one of the *PAX3* target genes is involved in the coagulation process. The advanced age of the woman was also remarkable, because ARMS generally occurs in young patients under 20 years of age, with a median age of 15 years, as was shown in a large single-center study [1]. There are only a few published ARMS cases in adults over 40 years of age, including one 58-year-old patient [1, 23].

In conclusion, cytogenetic analysis has proved to be a valuable diagnostic tool in malignancies of unclear origin. The present case confirms the high specificity of t(2;13) (q35;q14) for ARMS.

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