# Polymorphous low grade adenocarcinoma with distant metastases and deletions on chromosome 6q23–qter and 11q23–qter: a case report

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**Abstract**
**Polymorphous low grade adenocarcinomas (PLGAs) are thought to be indolent tumours that are localised preferentially to the palate and affect the minor salivary glands almost exclusively. Metastases to locoregional lymph nodes occur in only 6–10% of cases. Recently, two cases of PLGA with microscopically confirmed distant metastases have been reported. This study reports a third case of PLGA with histologically and immunohistochemically confirmed distant metastases. It is the first case with multiple pleural, as well as pulmonary parenchymal, metastases and metastases in cervical and paraoesophageal lymph nodes. In most cases, PLGAs are salivary gland tumours with limited potential to metastasise and a good prognosis after local treatment. However, the recently reported cases reveal that the tumour can give rise to widely spread metastases. To obtain more information about the incidence of distant metastases, periodic chest *x* ray examination during follow up is desirable.**
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In 1984, Evans and Batsakis reported a subset of malignant tumours among the until then heterogeneously composed group of adenocarcinomas arising in the minor salivary glands and named them "polymorphous low-grade adenocarcinomas" (PLGAs).[1] Almost at the same time, Freedman and Lumerman reported 12 cases of what they referred to as "lobular carcinomas" of the minor salivary glands.[2] Although the latter term did not survive, both series describe PLGAs. Indolent local behaviour with little tendency to metastasise was considered to be characteristic for this neoplasm. Neither distant spread nor death from this tumour had been reported and radical locoregional surgery was thought to be the curative treatment. Recent review articles on the clinical outcome of PLGA confirm this policy, with local recurrence rates ranging from 10% to 20%, and regional metastases in up to 10% of the cases.[3][4] Nevertheless, two histologically confirmed cases of distant metastases from a PLGA have been reported so far.[5][6] We report an additional case of PLGA that has widely metastasised to the pleura, lung,

mediastinum, and cervical lymph nodes. Our case is the first with metastases to the pleura, and the first where cytogenetical analysis of the tumour genome has been performed by means of comparative genomic hybridisation.

**Case report**
A 60 year old woman was referred to the department of maxillofacial surgery at the University Hospital Nijmegen, the Netherlands. The referring ear, nose, and throat surgeon had attempted to excise a palatal lesion that was diagnosed as "adenocarcinoma NOS (not otherwise specified)". Revision of the sections at our pathology department revealed an incompletely resected PLGA. After a complete examination, the tumour was staged $cT_xN_0M_0$. Two months after the initial biopsy, a wide resection of the tumour was performed. Histopathological examination confirmed the diagnosis and ample resection margins. The patient was enrolled in a routine follow up with periodical physical examination as well as chest *x* rays. The follow up was uneventful until 34 months after the initial treatment a routine chest *x* ray revealed several pleural lesions in the right sinus that were confirmed by computed tomography scan to be suspicious of a neoplasm (fig 1). Both clinical history, especially with respect to pulmonary complaints, as well as mammography and thyroid scan were normal. The patient was not known to have been in contact with asbestos. Several biopsies were performed through thoracoscopy. After confirmation of the metastatic origin of the lesions, a lobectomy was
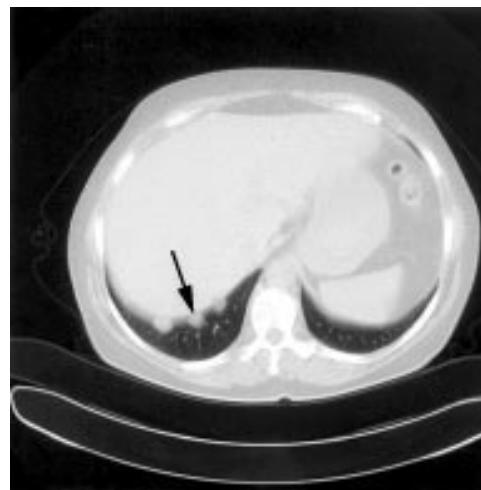


*Figure 1 Thoraco-abdominal computed tomography scan showing three pleural metastases in the right sinus (arrow).*

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| Antibody/antigen | Source | Dilution | Palatal lesion | Pleural lesions |
|---|---|---|---|---|
| Cytokeratin (AE1/AE3) | Biogenex | 1/200 | + | + |
| CAM 5.2 | Becton Dickinson | 1/20 | + | + |
| Vimentin | Biogenex | 1/200 | + | + |
| LeuM1 | Becton Dickinson | 1/10 | + | + |
| Mib1 | Immunotech SA | 1/100 | + (Borders only) | + (10–15%) |
| EMA | Dako | 1/30 | – | – |
| S-100 | Dako | 1/400 | + | + |
| Ber-EP4 | Dako | 1/150 | – | – |
| Cytokeratin 7 | Biogenex | 1/200 | – (Some spots +) | – |
| HMFG 2 | Novocastra | 1/10 | – | – |
| Chromogranin | Boehringer | 1/1000 | – | – |
| B72.3 | Biogenex | 1/250 | – | – (Some spots +) |
| CEA | Biogenex | 1/160 | – | – |
| α-SM1 | Sigma | 1/15 000 | – | – |
| HHF35 | Dako | 1/15 000 | – | – |

CEA, carcinoembryonic antigen; EMA, epithelial membrane antigen.

performed. The specimen showed widely distributed parenchymal and pleural PLGA metastases, also involving the mediastinum.

Subsequently, cervical metastasis of the PLGA was identified and confirmed by ultrasound guided fine needle aspiration.

## Material and methods

TISSUES

Formalin fixed, paraffin wax embedded tissue samples from the initial excision, the subsequent palatal resection, and the pleural biopsies were prepared and stained with haematoxylin and eosin. The palatal resection specimen was routinely decalcified before sectioning. A part of the pleural biopsy material was stored in liquid nitrogen. The lobectomy specimen, containing a para-oesophageal lymph node, was also formalin fixed, paraffin wax embedded, and routinely stained with haematoxylin and eosin.
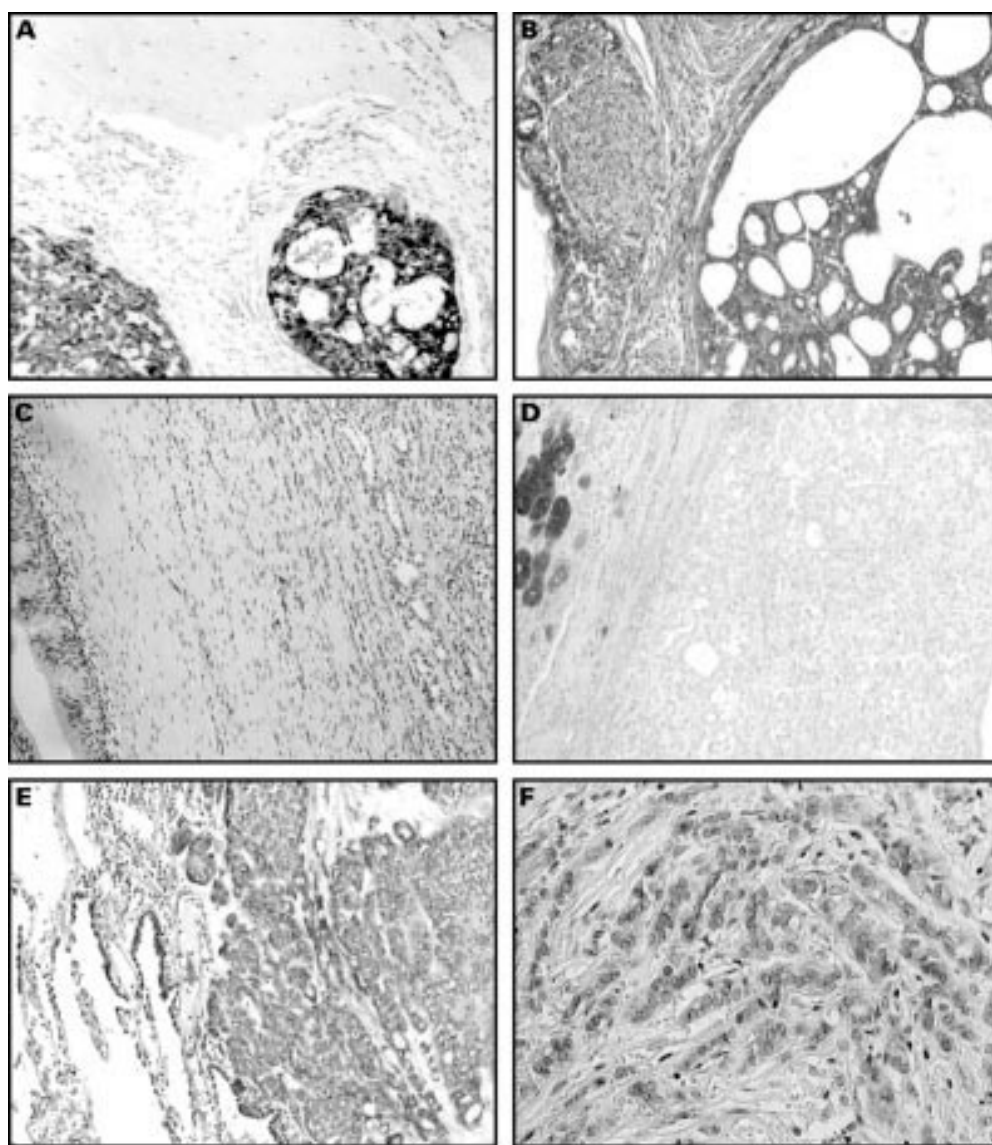


*Figure 2  Section from the palatal tumour (A–D). (A) Polymorphous low grade adenocarcinoma (PLGA) infiltrating the palatal bone and exhibiting S-100 positivity. (B) PLGA with perineural spread and cribriform growth pattern (haematoxylin and eosin stained). (C) PLGA with cord-like growth and Indian files, next to pre-existing respiratory epithelium from the nasal cavity (haematoxylin and eosin stained). (D) PLGA with ductular growth, no Alcian blue positivity in tumour cells, but Alcian blue positivity in pre-existing salivary tissue. Section from the lung metastasis (E and F). (E) PLGA metastasis with solid fields and some ductular growth (haematoxylin and eosin stained). (F) PLGA metastasis with Indian file configuration (haematoxylin and eosin stained).*
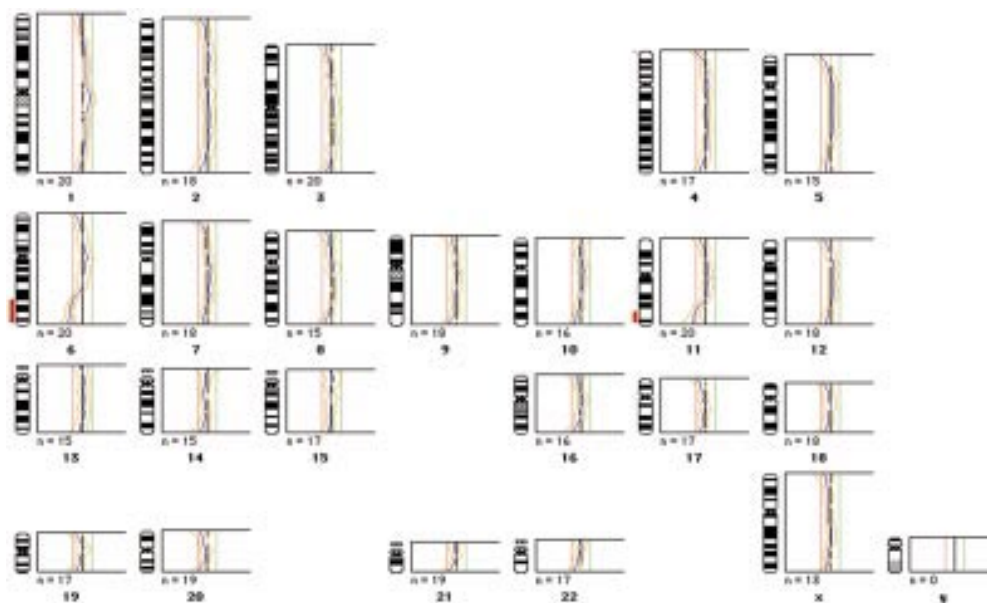
*Figure 3   Comparative genomic hybridisation (CGH) analysis of the pleural metastases. Red to green ratio profiles obtained from CGH analysis of the pleural metastases. Losses on 6q and 11q are indicated by vertical lines to the left of the chromosome ideograms.*

IMMUNOHISTOCHEMISTRY

Immunohistochemical examination was performed on the palatal resection specimen and pleural biopsies with a panel of antibodies (table 1). Routine immunohistochemical procedures as described by Hsu *et al* were used.[7]

COMPARATIVE GENOMIC HYBRIDISATION (CGH)

Normal DNA was prepared from peripheral blood lymphocytes from a healthy male donor. DNA was extracted[8] from the paraffin wax embedded tissue specimens from the primary tumour and the pleural biopsies. There was insufficient fresh material from the small pleural biopsies for use in CGH.

## Results

HISTOMORPHOLOGICAL FINDINGS

Microscopic examination of the excision biopsy specimen of the palatal tumour showed a non-encapsulated, cell rich tumour localised beneath an intact palatal mucosal surface. The tumour cells had weakly eosinophilic cytoplasm and exhibited little nuclear pleomorphism. As well as solid fields, other areas demonstrated ductal, tubular, and papillary growth patterns. Microscopic examination of the palatal resection specimen measuring $3 \times 2.5 \times 2$ cm revealed a well demarcated, but infiltrative tumour destroying the bone of the palate and nasal septum (fig 2A). Perineural spread was seen occasionally (fig 2B). The central part consisted of more solid fields, whereas other parts showed various growth patterns, such as the typical streaming of rows of cells, the so called "Indian files" (fig 2C). Areas with characteristic small ductal structures were seen (fig 2D), as well as cribriform patterns (fig 2B), and trabecular and a few papillary areas. In general, the cells showed uniformity throughout the tumour (fig 2D), with eosinophilic cytoplasm and fine chromatin texture. The tumour areas in the pleural biopsies and lobectomy specimen had the same histomorphological features, with isomorphic cells and, among other growth patterns, Indian files (fig 2E and F).

IMMUNOHISTOCHEMICAL FINDINGS

Both the primary and pleural tumours were positive for cytokeratin (AE1/AE3), CAM 5.2, vimentin, S-100 (fig 2A), and LeuM1. No immunostaining was seen for HHF35, α-Sm1, carcinoembryonic antigen (CEA), B72.3, Ber-EP4, and epithelial membrane antigen (EMA). Intracytoplasmic mucous production in the tumour cells was not found with Alcian blue, whereas pre-existing salivary gland tissue was positive (fig 2D). These immunohistochemical findings indicate that the tumour is composed of both epithelial and myoepithelial cells and are consistent with the diagnosis of polymorphous low grade adenocarcinoma. The absence of EMA and HMFG2 and the immunopositivity for S-100 excludes the possibility of a malignant pleural mesothelioma (table 1).

COMPARATIVE GENOMIC HYBRIDISATION

Figure 3 shows the CGH results of the pleural biopsy, identifying a relative loss of material on chromosome 6q23–qter and 11q23–qter. The same results were obtained in several consecutively repeated assays. However, repeated attempts could not produce a good CGH result on DNA isolated from the primary tumour.

## Discussion

To the best of our knowledge, this is the first case of a PLGA with both multiple pleural and pulmonary parenchymal metastases and metastases in para-oesophageal and cervical lymph nodes confirmed by microscopical and immunohistochemical examination. It is also the first PLGA in which deletions of chromosome 6q23–qter and 11q23–qter are described.

In 1993, Slootweg was the first to describe a patient with PLGA, who died with lesions suspicious of pulmonary and vertebral metastases, but these lesions were not confirmed as PLGA metastases by microscopic examination.[9] More recently, two PLGAs with microscopically confirmed distant metastases have been reported.[5][6] Tanaka and colleagues[5] described a patient with PLGA and distant metastases to the lung parenchyma, whereas Thomas *et al* described a patient with PLGA who had orbital and skin metastases.[6] No cytogenetical data from these two patients are available. We feel that in our patient both the primary tumour and metastases are well established PLGAs. Although the clinical implications of the genetic analysis of a single tumour are limited, we think that in the perspective of the growing insight into the genetic aberrations associated with tumour progression, our findings may be of benefit.

Our observation of 6q deletion is of special interest because the most consistent rearrangements in malignant salivary gland tumours are deletions, or more rarely translocations, involving the long arm of chromosome 6.[10] The breakpoints in tumours with 6q deletions clustered within the 6q22–25 region, and the minimal common region lost in most of the tumours, was 6q25–qter. The 6q deletions are more common in high grade malignant salivary gland tumours such as adenoid cystic carcinoma, high grade mucoepidermoid carcinoma, and undifferentiated carcinoma than in the lower grade malignancies of the salivary glands, such as acinic cell carcinoma and low grade mucoepidermoid carcinoma.[10] These cytogenetic observations were confirmed in a recent loss of heterozygosity study performed on 19 salivary gland carcinomas.[11] This study, using 23 polymorphic markers for chromosome 6, revealed that the most frequently deleted regions were 6q27 and 6q21–23.3. Chromosomal aberrations in the 11q region in malignant salivary gland tumours are less frequently reported. Non-random rearrangements on 11q are exclusively reported in mucoepidermoid carcinomas.[12]

To the best of our knowledge, cytogenetical data are available in the literature on only four PLGAs, and in none of these cases deletions on 6q and/or 11q were reported.[13–15] The 6q deletion in our widely metastasised PLGA is of special interest because several candidate tumour and/or metastasis suppressor genes have been mapped to the long arm of chromosome 6.[11]

We conclude that there is growing evidence that PLGA is a low grade malignancy, with the potential occasionally to metastasise to distant organs. We suggest the periodic follow up of patients with PLGA, including chest *x* rays, to obtain more information on the prevalence of distant metastases. In addition, molecular cytogenetical investigations of larger series of metastasised and non-metastasised PLGAs are needed to acquire data on both the occurrence of chromosome 6q deletions in this tumour and the relation of this chromosomal aberration to the biological behaviour of PLGA.

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