



Salivary Gland Mucoepidermoid Carcinoma is a Clinically, Morphologically and Genetically Heterogeneous Entity. A Clinicopathologic Study of 40 Cases with Emphasis on Grading, Histological Variants and Presence of the t(11;19) Translocation.

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A Clinicopathologic Study of 40 Cases with Emphasis on Grading,
Histological Variants and Presence of the t(11;19) Translocation.**

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ABSTRACT

Aims: To correlate World Health Organization (WHO) grade, patient's outcome and presence of t(11;19) to histological tumor variants in 40 well characterized mucoepidermoid carcinomas (MECs) out of a series of 290 salivary gland carcinomas.

Methods and Results: MECs were classified as *classical* based on the presence of equal proportions of the three cell types or the dominance ($\geq 50\%$) of mucous cells beside at least one other cell type, and as *variant* if composed of $\geq 80\%$ single cell type. *Classical* MECs were more common (n=23). *Variant* MECs had predominant squamoid (n=9), eosinophilic (n=5), or clear cell (n=3) morphology. 27 tumors were WHO grade 1, 3 grade 2 and 10 grade 3. The t(11;19) was detected in 82%, 35% and 0% of *classical* MEC, *variant* MEC and non-MEC, respectively. *Classical* MECs were significantly associated with age ≤ 60 years ($p < 0.001$), grade 1 ($p < 0.001$), and t(11;19) ($p = 0.003$). Short overall survival was significantly associated with age > 60 years ($p = 0.001$) and UICC stage $> I$ ($p = 0.031$), residual tumor ($p < 0.001$), tumor grade > 1 ($p = 0.001$) and squamoid variant ($p = 0.002$) in Kaplan-Meier analysis.

Conclusions: The results underscore the great histological diversity of MEC, the reproducibility of the WHO grading and the value of histological subtypes as an additional prognostic factor.

KEY WORDS

Mucoepidermoid carcinoma; variant; t(11;19); salivary gland; grading.

INTRODUCTION

Mucoepidermoid carcinoma (MEC) is the most common primary carcinoma of major and minor salivary glands accounting for about one third of all salivary carcinomas.^{1, 2} MECs are composed of three cell types in varying proportion: mucous cells, epidermoid (squamoid) cells and undifferentiated small cells (intermediate cells). Any of these cell types may dominate the histological picture in a given tumor, thus posing great diagnostic difficulties.¹ Traditionally, special stains like Meyer's mucicarmine and periodic acid-Schiff (PAS) are used for detection of sparse mucous cells.

Currently, immunohistochemistry proved also of value in recognizing foci of intermediate and epidermoid cells and, in particular, in differentiating MECs from other salivary gland carcinomas that they may closely mimic.^{3, 4}

Owing to the highly variable biological behavior of MEC, a variety of prognostic factors have been evaluated on previous large series to predict aggressive tumor behavior and hence the necessity of more aggressive treatment.^{5, 6} Of these, histological grading has received special attention and several grading systems have been proposed.⁷⁻⁹ While the difference in behavior of grade 1 and grade 3 MEC was universally confirmed in different studies, the results with regard to intermediate-grade MEC have been inconsistent and varied from low-grade behavior¹⁰ to an aggressive course comparable to high-grade tumors¹¹ or something in between.⁷ The Armed Forces Institute of Pathology (AFIP) system showed a higher reproducibility and was considered valid in the current World Health Organization (WHO) classification of head and neck tumors.^{1, 7, 8} However, Brandwein et al. suggested that the AFIP grading system may downgrade some MEC.¹² They proposed considering histological features including infiltration pattern at the invasive front, vascular invasion and bony infiltration in the assessment of tumor grade.

The translocation t(11;19) has been detected in approximately half of MECs in several studies.¹³⁻¹⁵ However, data on the detection of this molecular alteration in Warthin tumor have been conflicting in different studies.¹⁶⁻¹⁸ Presence of t(11;19) was found to correlate with a better outcome in MEC,^{13, 19, 20} but the explanation for this is still lacking. To our knowledge, the correlation of well defined histological variants with WHO grading, patient outcome and the presence or absence of the translocation t(11;19) has not been evaluated previously. This was the objective of this study.

MATERIAL AND METHODS

Patients and tumor samples

Fourty MECs were retrieved from a consecutive collection of well characterized primary salivary gland carcinomas of both major and minor salivary glands (total n=290) with complete follow-up treated at the Universities of Erlangen and Regensburg, Germany, or at the City Hospital of Nuremberg, Germany, between 1990 and 2007. Clinical information was obtained from the clinical tumor registries of

Erlangen-Nuremberg and Regensburg as well as from the salivary gland carcinoma registry of Erlangen. The registries and the related translational research activities are covered by ethical vota of the medical faculties of the Universities of Regensburg and Erlangen-Nuremberg.

Histology and classification

On the basis of Hematoxylin and Eosin (H&E) and PAS stained slides from at least subtotally embedded tumors all of the 290 tumors have been independently reviewed by two pathologists experienced in salivary gland tumor pathology (S. S. & A. A.) without knowledge of initial histological diagnosis, FISH results or clinical follow-up. A few cases (n=4) were either found to represent other types of salivary gland carcinomas or they were lacking unequivocal MEC areas, hence they were excluded. An agreement was achieved in 40 cases which represented the cohort of the current study.

MECs were diagnosed according to the well established WHO criteria.¹ Furthermore, tumors have been categorized as either *classical MEC* or *variant (non-classical) MEC*. *Classical* MECs were tri- or biphasic neoplasms composed of well recognizable mucous cells (prominent single goblet cells or contiguous goblet cells forming mucinous gland-like or cystic spaces) in addition to variable proportions of epidermoid (squamoid) and intermediate cells. In contrast to true squamous cells, epidermoid cells lacked intercellular bridges and intracellular keratinization. Intermediate cells were small undifferentiated cells with round nuclei and scant basophilic cytoplasm. In difficult cases additional immunohistochemical staining for low (CK7)²¹ or high molecular weight (CK5) cytokeratins was used to identify mucous, intermediate and epidermoid differentiation, respectively. CK5 antibody was purchased from Zytomed systems Ltd., Berlin, Germany, CK7 antibody from DCS Innovative Diagnostik-Systeme Dr. Christian Sartori Ltd., Hamburg, Germany. Immunostaining was performed on 5 µm sections according to the manufacturer's instructions. Generally, all three components were well recognizable in each low-power field in classical MECs. Biphasic tumors were considered *classical* if a prominent mucous component (≥50%) was present beside another one (either intermediate or epidermoid cell component). The presence or absence of a prominent cystic component did not influence this classification. The presence of a focus of *classical* MEC in an otherwise *variant* MEC was judged according to the extent of

both components. Tumors with $\geq 80\%$ *variant* histology were considered *variant* MECs in this study. Applying the WHO criteria¹ all tumors have been graded independently by the two pathologists, thereby showing a complete reproducibility in all cases.

Tissue microarray construction

A tissue microarray (TMA) was constructed from formalin-fixed paraffin-embedded tissue blocks of these 40 patients, and of additional 250 patients with salivary gland carcinomas others than MEC as well as of 60 cases of normal salivary gland tissue, as previously described.^{22, 23} One tissue cylinder with a diameter of 2.0 mm was punched from morphologically representative tissue areas of each donor tissue block and brought into a recipient paraffin block using a homemade semiautomated tissue arrayer. TMA sections were mounted on charged slides (SuperFrostTMPlus; Menzel GmbH, Braunschweig, Germany). H&E-stained TMA sections were used for reference histology.

Fluorescence *in situ* hybridization

Fluorescence *in situ* hybridization (FISH) was performed on 5 μm sections of the TMAs using commercially available, directly labeled DNA break-apart probes to detect the translocation t(11;19) (ZytoVision Ltd., Bremerhaven, Germany). The probes identify two locus specific sequences on chromosomes 11 (orange/green) spanning the break point at 11q21 and resulting in a yellow appearing fusion signal. A translocation was evident if the fusion signal was split into an orange and a green signal. TMA sections were dewaxed for 40 min in an incubator at 72 °C and for 2 \times 10 min in xylene. After being rehydrated in a graded ethanol series and rinsed in distilled water, slides were placed in 0.01 M sodium citrate and steamed for 40 min in a water bath. Cell structures were digested in 0.1% pepsin (Sigma) and 0.01 M HCL for 10 min at 37 °C. After washing in 2 \times standard sodium citrate (SSC) (1 \times SSC is 150 mM sodium chloride and 15 mM sodium citrate, pH 7) and water, slides were dehydrated in graded alcohols and air-dried. 10 mL of the DNA probe set were applied to the TMA area of each section. Sections were cover-slipped and the edges sealed with rubber cement. For co-denaturation of the probe and target DNA, slides were placed on a hotplate preheated to 73 °C for 5 min and transferred to a warmed hybridization chamber at 37 °C overnight.

After hybridization, the rubber cement was removed and the slides were immersed in 4×SSC + 0.3% Igepal (Serva), 2×SSC, and 1×SSC for 10 min at 50 °C, respectively. The slides were rinsed briefly in Millipore water and air-dried. Nuclei were counterstained with anti-fading DAPI (4',6-diamidino-2-phenylindole) Vectashield (Vector Laboratories, Burlingame, CA, USA) and were analyzed by epifluorescence microscopy.

Microscopy, FISH scoring, and digital imaging slides were imaged with an Axio Imager Z.1 (Zeiss, Göttingen, Germany) equipped with specific filter sets for DAPI (excitation 365 ± 20 nm, emission 450 ± 25 nm; Zeiss), green (excitation 500 ± 10 nm, emission 535 ± 15 nm), and red fluorescence (excitation 545 ± 15 nm, emission 610 ± 35 nm; AHF, Tübingen, Germany). Fluorescence images were obtained with a Plan-Apochromat lens (63×, 1.4) and recorded with a charge-coupled device (CCD) camera AxioCam MRm (Zeiss). FISH scoring was performed by counting fluorescence signals in 50 malignant, non-overlapping cell nuclei for each case by two independent interpreters (CS, MM). A tumor was considered positive if > 50% of the cells harbored the translocation.

Statistical analyses

All clinicopathologic and cytogenetic data were analyzed with SPSS for Windows, version 15.0 (SPSS, Erkrath, Germany). Relationships between dichotomized histological or cytogenetic markers and clinicopathologic factors were examined using Fisher's exact probability test (significant association with $p < 0.05$). Overall survival (= primary outcome measure) was calculated as the time from the date of diagnosis to death from any cause or the date the patient was last known to be alive. Patients lost to follow-up were treated as censored cases based on the date they were last known to be alive. Survival curves were generated by using the Kaplan-Meier method, and log-rank tests compared the distributions between groups. For multivariate analysis a Cox proportional hazards model was used in a backwards, stepwise elimination approach. At each step the least significant factor with $p > 0.10$ was eliminated with reassessment of each factor in the model at each step. A limit for the factors to be included was set at 5%.

RESULTS

Clinical features of MEC

The clinicopathologic parameters are summarized in Tables 1 and 2. Using the current WHO criteria for grading salivary gland MEC,¹ 27 cases were low grade (G1), 3 cases were intermediate grade (G2) and 10 cases were high grade (G3). *Classical* MEC was significantly associated with age ≤ 60 years ($p = <0.001$), grade 1 ($p = <0.001$) and presence of the translocation t(11;19) ($p = 0.003$).

Mean follow up was 64.5 months (range 1.6-224 months). Ten patients (25%) died of their tumors at a mean interval of 25.2 months (range 1.6-70.9 months).

Histological features of MEC

Classical MEC

Twenty-three tumors fulfilled the above criteria of *classical* MEC. These revealed variable cystic areas; a prominent cystic component ($>20\%$) was seen in 7 tumors. Mucous cells were prominent in each low-power field and gland-like spaces or aggregates of contiguous mucous cells were well discernible even at low power magnification (Fig. 1A). Focal unusual features in classical MEC included minor components with microvesicular cytoplasm suggesting abortive sebaceous differentiation (two cases; Fig. 1B), focal syncytial overgrowth of spindled intermediate cells reminiscent of myoepithelial differentiation but lacking expression of p63 and CK5 (expressing CK7 diffusely) (Fig. 1C-D) and circumscribed tumor nests composed of peripherally located polygonal myoepithelial-like clear cells punctuated by centrally located small microcystic glands mimicking epithelial-myoepithelial carcinoma (EMC) (Fig. 1E-F). However, the lumina were lined by flattened epithelial cells and lacked the well discernible glands of EMC. Furthermore and interestingly, immunohistochemistry showed the reverse of EMC with CK5 being expressed in the luminal cells and CK7 in the outer layer, thus adopting an “inverted EMC pattern” (Fig. 1E-F). A common finding in several cases was the presence of atypical looking preexisting salivary duct showing both bland-looking ciliated epithelium and crypt-like mucous cell hyperplasia resulting in atypical budding of mucous cell-rich ductules and small solid nests of intermediate cells (Fig. 1G-H). These changes were often associated with an avascular papillary epithelial tufting.

Squamoid (epidermoid) MEC

Squamoid tumors ($n=9$) showed variable degree of squamoid differentiation with horn cysts and sharp intercellular junction, but prominent intercellular bridges as seen in

typical squamous cell carcinoma were lacking. Occasional cytoplasmic vacuoles, possibly representing abortive glandular differentiation, were seen. A few tumors revealed a focal or prominent acantholytic (pseudoglandular or pseudovascular) pattern. Another pattern seen in this group was the presence of differentiated squamoid sheets with numerous microcystic lumina filled with blue mucin and imparting a pseudocribiform pattern to the tumor (Fig. 2A-B). One tumor classified into this subtype showed solid sheets of medium-sized to relatively small basophilic cells with histological and immunohistochemical features of intermediate cells with minor component of mucous cells (Fig. 2C-D). This tumor would be better categorized as *variant MEC of intermediate cell type*, but no more cases were found to warrant an additional subtype and the case was hence discussed under the subheading of squamoid MEC.

Eosinophilic (oncocytoïd) MEC

This represented the second most common type of *variant MEC* (n=5). Tumors in this group showed a predominance of medium-sized to large cells with deeply eosinophilic cytoplasm lacking equivocal squamoid differentiation (Fig. 3A-B). Tumor cells revealed either a prominent glandular differentiation that closely resembled mature striated ducts (Fig. 3C) or they were arranged into solid sheets and possessed prominent centrally located nuclei, thus strikingly mimicking an oncocytic neoplasm (Fig. 3D). Occasionally, both features were seen side-by-side. In addition, all tumors showed either minor *classical MEC* areas, focal epidermoid/squamoid differentiation or scattered mucous cells, thus establishing the diagnosis of MEC (Fig. 3E-F). The tumor illustrated in Fig. 3A was composed of large atypical cystic glands lined by oncocytic partially ciliated cells with occasional papillary pattern reminiscent of Warthin tumor. Single striated duct-like glands seemed to bud off the cyst. However, mucous cells and other typical features were evident in small areas of the tumor. Furthermore, a lymphoid component was lacking and, instead, a hypervascularized stroma was seen.

Clear cell MEC

Although focal clear cell areas were quite common in MEC in general, the clear cell variant was the least common variant in our study (n=3). Clear cell variants were composed of solid sheets of medium-sized polygonal cells with cytoplasmic clearing,

usually with peripheral cytoplasmic retraction with occasional lightly eosinophilic staining tumor cell groups (Fig. 4A-B). The appearance of some of these tumors was reminiscent of the chromophobe renal cell carcinoma (Fig. 4C). The typical nesting and hyaline bands of clear cell carcinoma, not otherwise specified (NOS), was absent. Unequivocal mucous cells or minor foci of typical MEC were seen, thus establishing the diagnosis (Fig. 4B, D).

Distribution and prognostic role of the translocation t(11;19) in MEC

The t(11;19) was detected in 25 of the 40 MEC (62.5%). This corresponded to 19/23 classical MEC (82.6%) and 6/17 variant MEC (35%). Among the different variant MEC types, the clear cell variant showed the highest, and the squamoid variant the lowest positivity rate (100% versus 11%, respectively). The eosinophilic variant showed an intermediate value (40%). All of the non-MEC salivary carcinomas were translocation-negative. The closer association of the translocation with the classical type of MEC was significant ($p=0.003$) as was the negative association with the squamoid variant ($p=0.001$). As given in Table 2 the translocation significantly associated with low grade tumors ($p=0.01$), but not with age, stage, presence of residual tumor, sex and localization. Although not significant, the occurrence of t(11;19) was a factor of better overall survival ($p=0.098$).

Associations of the histological variants with clinicopathologic parameters

The different variants of MEC have been correlated with clinical parameters (age and tumor stage) as well as with tumor grade. The *classical* type predominated in younger patients with a mean age of 42 years (range 11-80 years, $p<0.001$) whereas the squamoid variant usually occurred in the elderly with a mean age of 68 years (range 37-94 years, $p=0.03$). MECs of *classical* type were typically low grade (G1, $p<0.001$), the squamoid variant was of intermediate or high grade ($p<0.001$). Squamoid variant tended to occur more frequently in the major glands (parotid and submandibular gland) than in the minor glands of the oral cavity ($p=0.055$). An association of the variants with sex and stage could not be demonstrated.

Prognostic significance of clinicopathologic parameters in MEC

To examine the prognostic significance of important clinicopathologic parameters in MEC, Kaplan-Meier analyses were applied (Figure 5). Here, short overall survival

was significantly associated with age >60 years ($p=0.001$), high UICC stage (II, III, IV vs. I; $p=0.031$), residual tumor after primary surgery ($p<0.001$) and higher tumor grade (G2 and G3 vs. G1; $p=0.001$). Sex and localization did not significantly influence survival. The analysis of known clinicopathologic parameters was supplemented by a Kaplan-Meier analysis of the aforementioned histological variants of MEC. Here, the *classical* type of MEC was associated with best prognosis, whereas the *non-classical* MEC variants were significant negative prognosticators ($p=0.002$).

The univariate Kaplan-Meier analysis was complemented by a multivariate Cox regression to examine the independence of the prognostically relevant clinicopathologic parameters. As the tumor variant was strongly associated with tumor grade (Table 1), the Cox regression analysis could not include both parameters. Thus, two analyses were performed: one with grade (grade 1 *versus* others) and one with variant (squamous *versus* others). The other parameters to include into the analysis were those which proved to be of prognostic significance in univariate Kaplan-Meier analysis (Figure 5): age, stage and residual tumor. The Cox regression model (Table 3) was adjusted within two reverse steps in order to exclude a non significant parameter (stage). High age, higher grade, presence of residual tumor and squamous variant remained significant prognostic factors with hazard ratios between 4.7 and 18.0.

DISCUSSION

In this study, we analyzed the full histological spectrum of MEC in relation to tumor site, WHO-grade, stage, survival and the presence of the translocation t(11;19) in 40 well characterized MECs of major and minor salivary glands. Our findings underscore the diverse morphology of MEC which results from the striking variability in the proportion of the three cell types comprising the tumor. In its classical form, MEC shows a comparable proportion of three or of at least two cell types, hence the diagnosis of this classical variant is straight forward and usually poses no difficulty. However, a single cell type may constitute the bulk of the tumor, thus giving rise to monophasic variants of MEC, the diagnosis and true categorization of them may be challenging, even for those with great experience in salivary gland pathology. We

have subclassified *variant* MEC into squamoid, eosinophilic and clear cell subtypes based on the presence of >80% variant pattern in the tumor.

The squamoid subtype was the most common *variant* MEC in this study and implied commonly a high-grade tumor with significantly adverse outcome. The diagnosis of this variant may be facilitated by detection of minor foci of *classical* MEC through excessive tumor sampling and demonstration of isolated mucous cells or intracellular mucin assisted by special stains (mucicarmine, PAS). Also, the presence of a population of smaller cells lacking evident squamoid differentiation (intermediate cells) represents an important clue to diagnosis. Immunohistochemistry may help to recognize such minor foci that would remain undetected on initial H&E evaluation. Adenosquamous carcinoma, which is actually not included into the WHO classification of salivary gland tumors, is an important differential diagnosis of the squamoid MEC. Demonstration of unequivocal mucous cells and/or intermediate cells on H&E, mucin stains or by immunohistochemistry represents the main discriminator. In this setting, the t(11;19) may help to confirm the diagnosis of MEC in equivocal cases. Lennerz et al. detected the t(11;19)-related rearrangements in 7 of 7 MECs of the uterine cervix, but in none of 14 conventional cervical adenosquamous carcinomas.²⁴ However, sensitivity of t(11;19) for squamoid MEC is quite low in our study (11%). Immunohistochemistry showed consistent expression of CK5 in squamoid MEC as well as in high grade MEC²⁵. One tumor in this series showed a predominance of small blue-staining CK5-negative intermediate cells, suggesting the existence of an intermediate cell variant, but the clinical significance of this remains to be further studied in larger series. We discussed this single case within the spectrum of the squamoid variant.

Eosinophilic MEC (also known as oncocytoid MEC)^{26, 27} shows increased cytoplasmic eosinophilia irrespective of growth pattern but some cases may appear oncocytoid as a result of prominent striated duct-like differentiation.²⁸ The latter pattern is exceedingly rare and may mimic MEC arising in Warthin tumor.²⁹ Although eosinophilic MEC may show a prominent papillary and cystic pattern, the two-layered pattern of oncocytic cells with centrally located nuclei and a prominent lymphoid stroma typical of Warthin tumor are lacking in *de novo* eosinophilic MEC. Furthermore, MEC ex Warthin tumor is usually of the *classical* type.²⁹ On the other hand, eosinophilic MEC with papillary pattern and tumor-associated lymphoid

proliferation has to be distinguished from benign Warthin tumor with squamous and/or mucous metaplasia and from so-called Warthin adenocarcinoma.³⁰

The clear cell variant was generally reminiscent of the clear cell carcinoma, NOS. However, on careful evaluation, a variable, but generally minor population of intermediate cells or a minute focus of classical MEC may be detected. The t(11;19) proved helpful and sensitive in the current study in detecting this variant (3 of 3), but the small case number does not allow for solid conclusions. Of interest, focal clear cell change associated with a biphasic pattern very reminiscent of EMC was seen in one classical MEC. Immunohistochemistry revealed however an “inverted EMC pattern” with the luminal CK5 positivity and CK7 expression in the outer layer. Awareness of this finding, whether in classical or in clear cell MEC, should help to avoid misinterpretation as EMC.

Our results showed a high reproducibility of the current WHO grading¹ which is based on the AFIP criteria.^{7, 8} However, due to the relatively limited case number we could not assess, whether the WHO system downgrades MEC. In line with the observation of Brandwein et al.,¹² dominant histological pattern/ features of tumors correlated well with the tumor grade and outcome, thus suggesting that defining histological criteria should be included in the scoring/ grading of MEC. *Non-classical* variants tend to be of higher WHO grade than *classical* MEC, the latter were predominantly grade 1 tumors (96%) compared to only 29% grade 1 *variant* MEC. A comparable statistical significance value ($p < 0.001$) was demonstrated for WHO grade 1 and *classical* variant in the association analysis indicating that recognition of the *classical* variant implies a grade 1 tumor.

One interesting finding in this study was the occurrence of atypical crypt-like mucous cell hyperplasia and foci of intermediate-like cells associated with residual salivary duct in several cases of classical MEC. This finding probably represents a putative precursor lesion, but reactive changes induced by the tumor as well as secondary colonization of preexisting ducts by tumor cells can not be reliably excluded. However, in the experience of the authors, this finding is not common in specimens harboring other-type salivary gland carcinomas, suggesting a precursor change specifically associated with mucoepidermoid neoplasms and arguing against a

reactive ductal lesion, but this merits future studies. Williamson et al. described similar foci of squamous and mucous cell metaplasia associated with cytological atypia within oncocytic epithelium of Warthin tumor harboring MEC and speculated that these might represent precursor lesions for MEC.²⁹

In summary, we analyzed 40 salivary MEC, demonstrating a high histological variation and showing that histological variant correlates well with the WHO tumor grade, biological behavior and presence of t(11;19). Future studies on larger series are needed to further verify the value of histological variant in supplementing tumor grading and predicting treatment failure.

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FIGURE LEGENDS

Figure 1 Variations of classical (low-grade) mucoepidermoid carcinoma with unusual features. **A** Classical and partially cystic MEC composed of mucous, epidermoid and intermediate cells. **B** MEC with focal sebaceous-like differentiation characterized by polygonal cells with microvesicular cytoplasm. **C** MEC exhibiting partial overgrowth of spindle shaped intermediate cells mimicking myoepithelial differentiation but negative for myoepithelial markers (p63, not shown) and CK5 (**D**). **E** Classical MEC (G1) with focal clear cell change of intermediate cells, negative for CK5 (**F**), but positive for CK7 (not shown), generating an inverted epithelial-myoeplithelial pattern. **G** Classical MEC entrapping a salivary duct with mucous metaplasia, possibly representing a precursor lesion of MEC. **H**

Higher magnification demonstrating a proliferation of intermediate cells within the duct lumen on the right side and a focal eosinophilic change of epidermoid tumor cells on the left side.

Figure 2 Mucoepidermoid carcinoma of squamoid type. **A** High grade MEC (G3) with mucus filled microcystic spaces and sheets of intermediate cells differentiating to mature squamoid epithelia forming horn pearls. **B** Strong expression of CK5 in the intermediate/squamoid cells. This tumor was t(11;19) negative. **C** High grade MEC (G3) predominantly composed of solid sheets of intermediate cells with interspersed single mucous cells. Partial expression of CK5 (**D**) and negativity of CK7 (not shown) indicate immature/intermediate cells. This tumor was t(11;19) positive (not shown).

Figure 3 Mucoepidermoid carcinoma of eosinophilic (oncocytoïd) type / with striated duct differentiation. **A,B** Cyst wall of a partially cystic MEC of eosinophilic type (G1) resembling a Warthin tumor with mucous metaplasia and without lymphoid stroma. The neoplastic tubules were strikingly similar to striated ducts of normal salivary gland. Translocation t(11;19) was negative. **C,D** Another t(11;19)-positive low-grade MEC with striated duct differentiation (**C**) and solid cell sheets with characteristic oncocytic appearance (**D**). **E** Eosinophilic MEC with predominant sclerosis (sclerosing type eosinophilic MEC, G2). The tumor was composed of solid sheets of CK7-positive (**F**) intermediate cells with interspersed mucous cells and focal positivity for CK5 (not shown). This tumor was t(11;19)-negative.

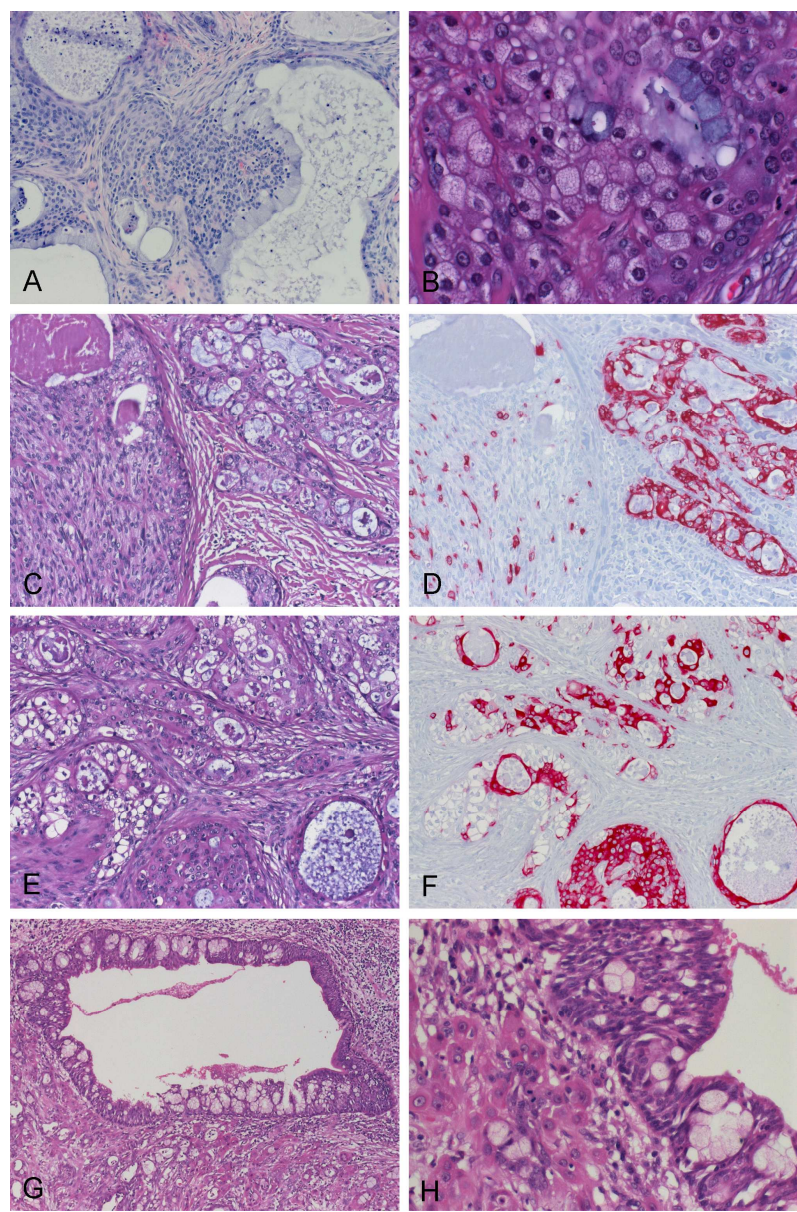
Figure 4 Mucoepidermoid carcinoma of clear cell type. **A** low-grade MEC predominantly composed of solid sheets of clear cells with inconspicuous nuclei and “chromophobe” appearance. **B** a small focus with classical morphology detected on further sampling. **C** FISH using break-apart probes demonstrated translocation t(11;19) indicated by split orange and green signals. **D** High grade clear cell MEC with higher nuclear pleomorphism and focal well differentiated areas (**E**). **F** FISH demonstrated polysomy 11 and bi- or tri-allelic translocation t(11;19).

Figure 5 Univariate Kaplan-Meier analyses of clinicopathologic parameters (sex, age, localization, type, UICC stage, residual tumor classification, grade, and translocation t(11;19) regarding overall survival (in months). P values according to log rank statistics.

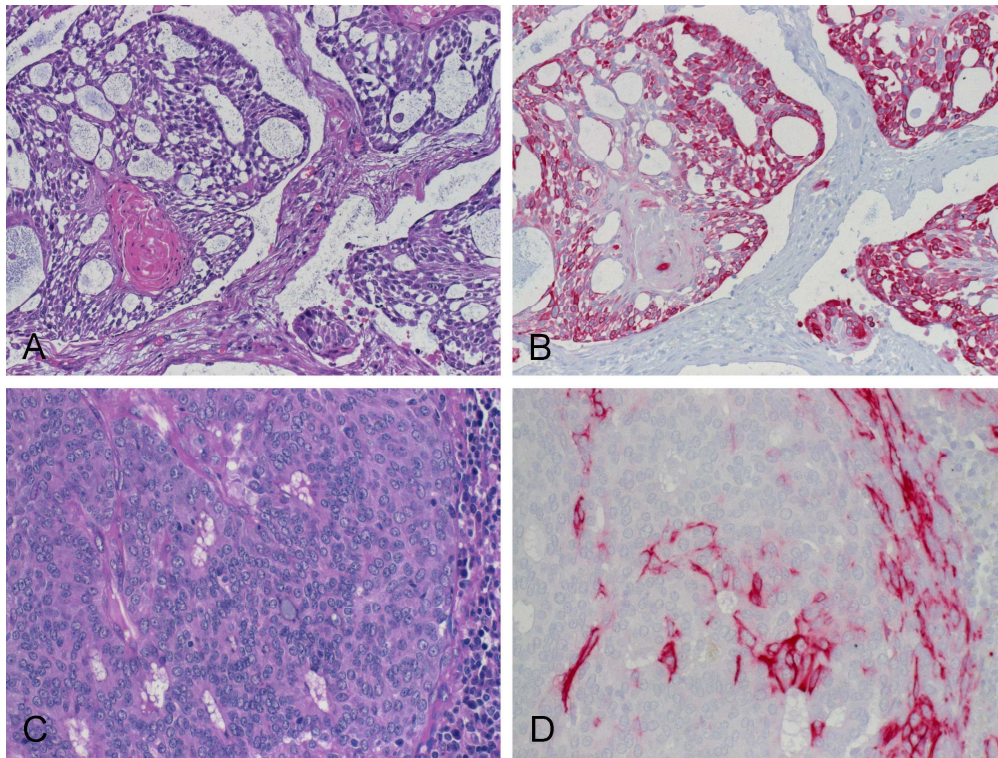
Table 1 Clinicopathologic parameters related to the four subtypes of MEC (Significance of associations was evaluated by Fisher exact test).

Table 2 Clinicopathologic parameters related to presence or absence of t(11;19). Significance of association was evaluated by Fisher exact test.

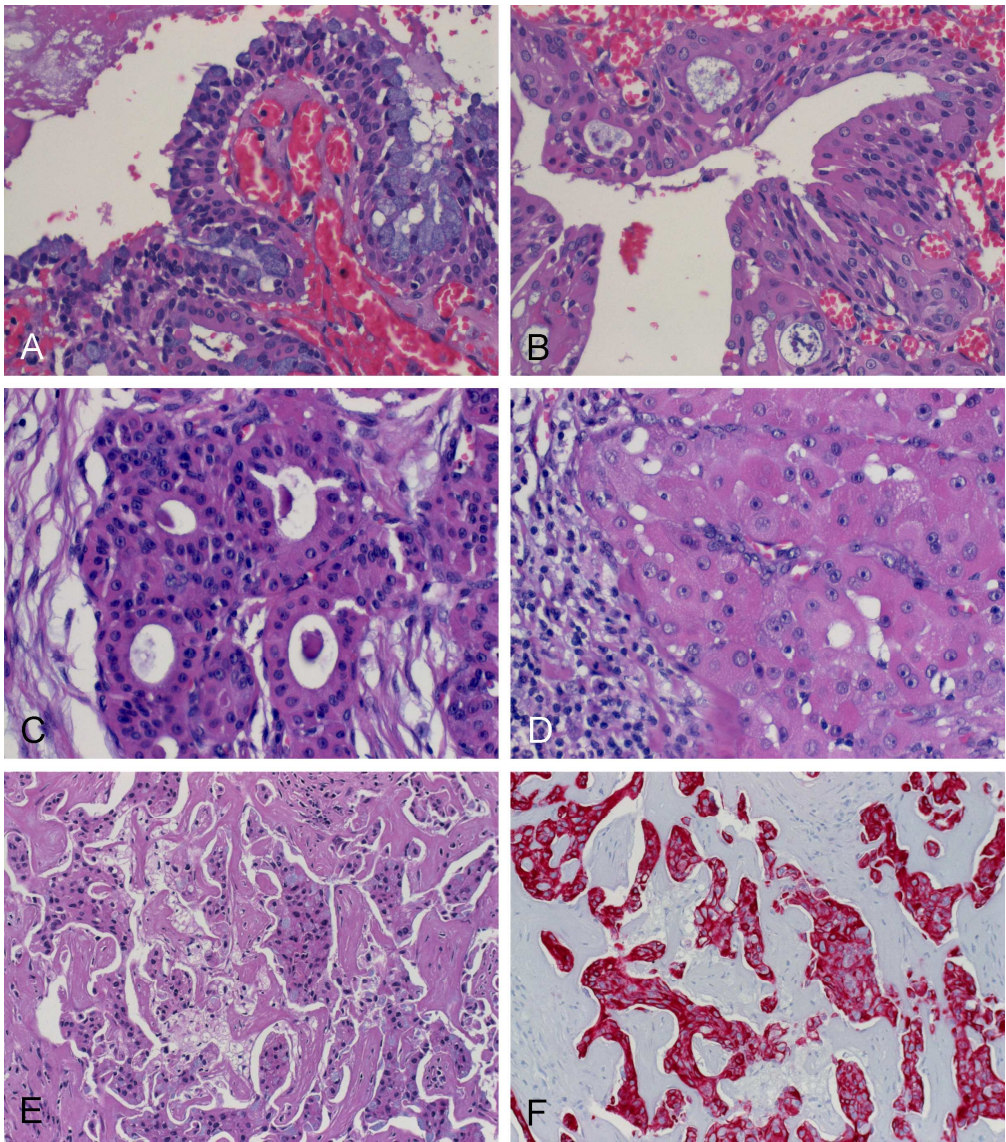
Table 3 Univariate and multivariate Cox regression with reverse stepwise selection. In the upper half age, stage, grade, and residual tumor are included, in the lower half grade is replaced by tumor type (squamous vs. others).



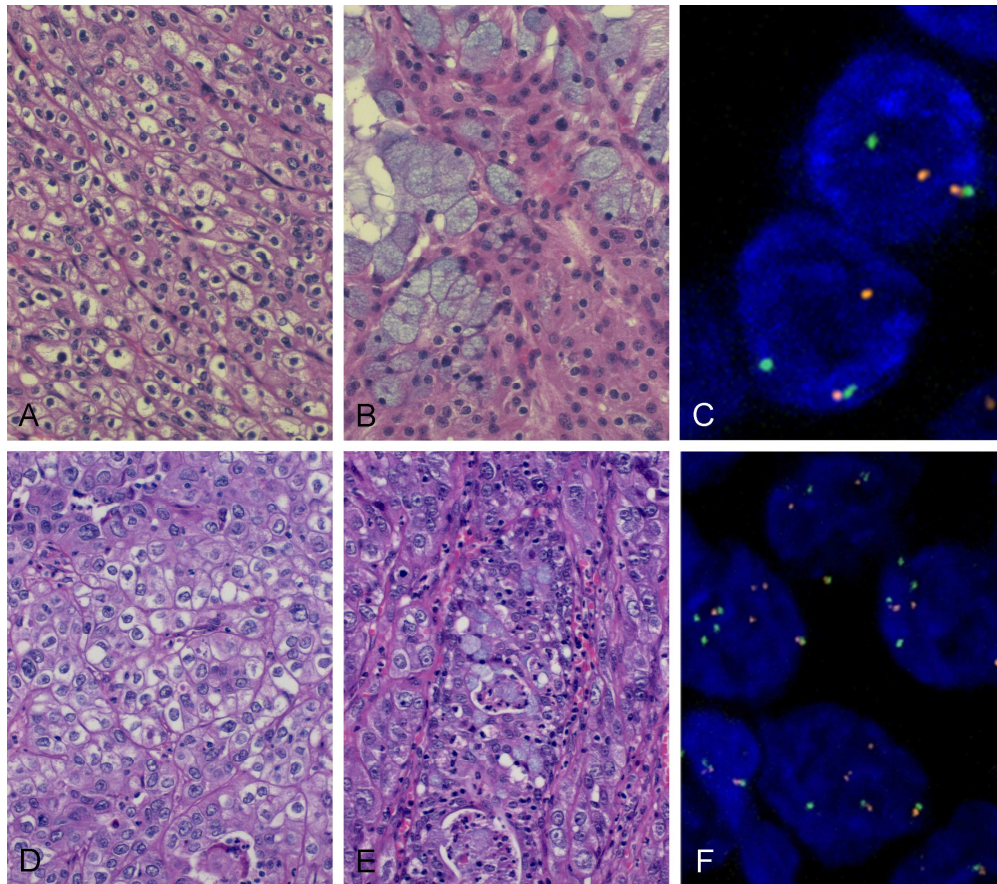
Variations of classical (low-grade) mucoepidermoid carcinoma with unusual features. A Classical and partially cystic MEC composed of mucous, epidermoid and intermediate cells. B MEC with focal sebaceous-like differentiation characterized by polygonal cells with microvesicular cytoplasm. C MEC exhibiting partial overgrowth of spindle shaped intermediate cells mimicking myoepithelial differentiation but negative for myoepithelial markers (p63, not shown) and CK5 (D). E Classical MEC (G1) with focal clear cell change of intermediate cells, negative for CK5 (F), but positive for CK7 (not shown), generating an inverted epithelial-myoeplithelial pattern. G Classical MEC entrapping a salivary duct with mucous metaplasia, possibly representing a precursor lesion of MEC. H Higher magnification demonstrating a proliferation of intermediate cells within the duct lumen on the right side and a focal eosinophilic change of epidermoid tumor cells on the left side.
650x987mm (79 x 79 DPI)



Mucoepidermoid carcinoma of squamoid type. A High grade MEC (G3) with mucus filled microcystic spaces and sheets of intermediate cells differentiating to mature squamoid epithelia forming horn pearls. B Strong expression of CK5 in the intermediate/squamoid cells. This tumor was t(11;19) negative. C High grade MEC (G3) predominantly composed of solid sheets of intermediate cells with interspersed single mucous cells. Partial expression of CK5 (D) and negativity of CK7 (not shown) indicate immature/intermediate cells. This tumor was t(11;19) positive (not shown).
647x487mm (96 x 96 DPI)



Mucoepidermoid carcinoma of eosinophilic (oncocytoid) type / with striated duct differentiation. A,B Cyst wall of a partially cystic MEC of eosinophilic type (G1) resembling a Warthin tumor with mucous metaplasia and without lymphoid stroma. The neoplastic tubules were strikingly similar to striated ducts of normal salivary gland. Translocation t(11;19) was negative. C,D Another t(11;19)-positive low-grade MEC with striated duct differentiation (C) and solid cell sheets with characteristic oncocytic appearance (D). E Eosinophilic MEC with predominant sclerosis (sclerosing type eosinophilic MEC, G2). The tumor was composed of solid sheets of CK7-positive (F) intermediate cells with interspersed mucous cells and focal positivity for CK5 (not shown). This tumor was t(11;19)-negative.
647x736mm (96 x 96 DPI)



Mucoepidermoid carcinoma of clear cell type. A low-grade MEC predominantly composed of solid sheets of clear cells with inconspicuous nuclei and "chromophobe" appearance. B a small focus with classical morphology detected on further sampling. C FISH using break-apart probes demonstrated translocation t(11;19) indicated by split orange and green signals. D High grade clear cell MEC with higher nuclear pleomorphism and focal well differentiated areas (E). F FISH demonstrated polysomy 11 and bi- or tri-allelic translocation t(11;19).
736x647mm (96 x 96 DPI)

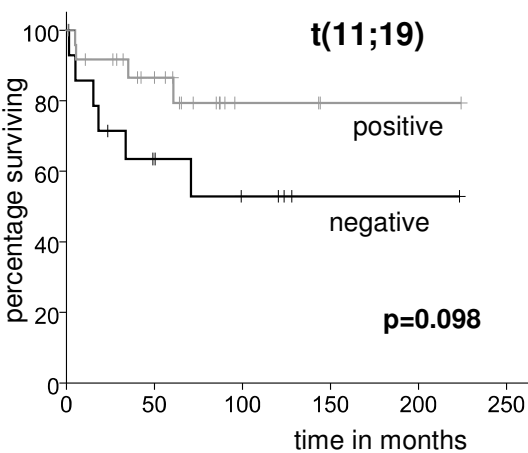
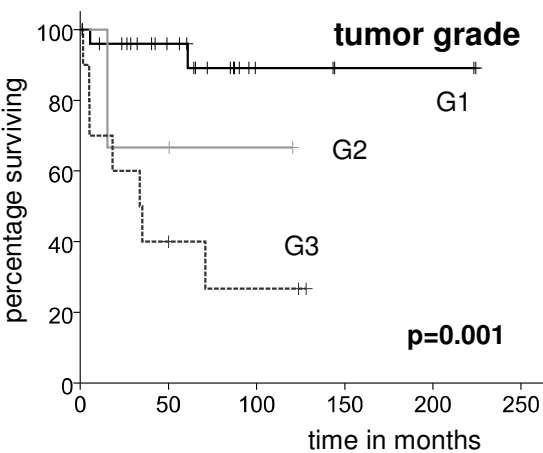
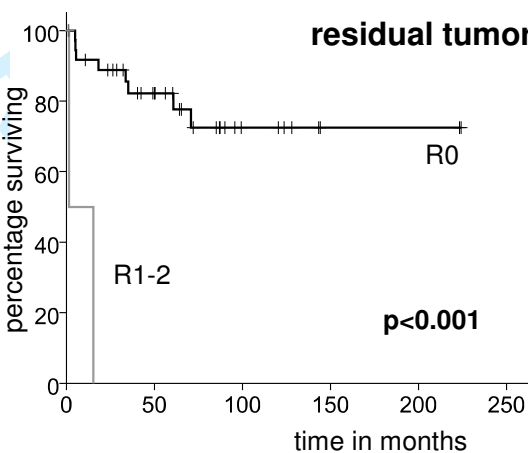
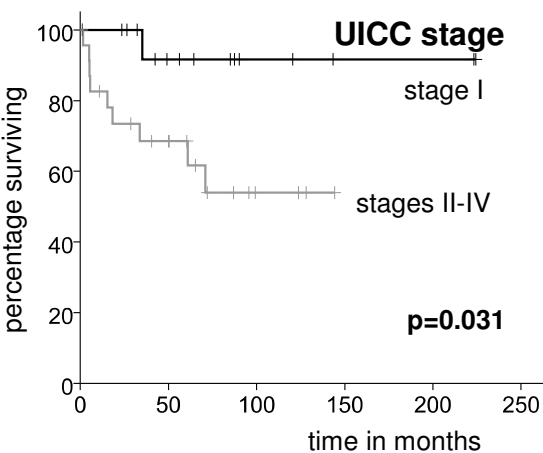
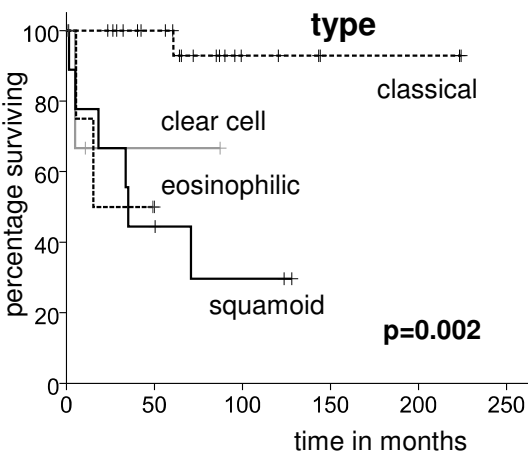
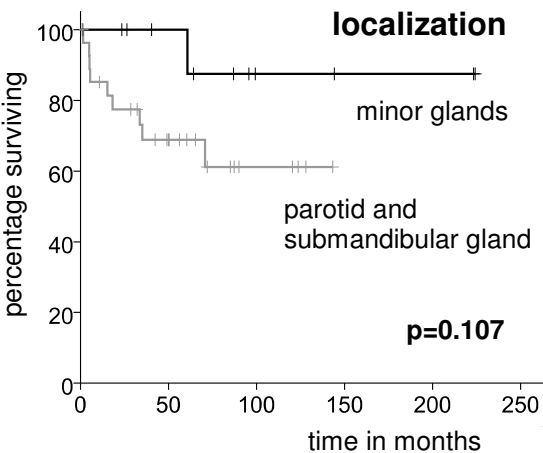
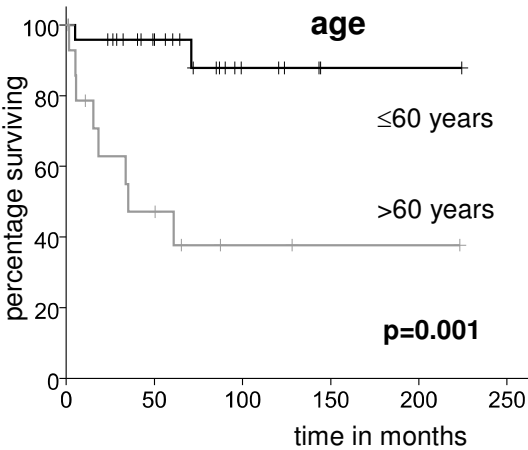
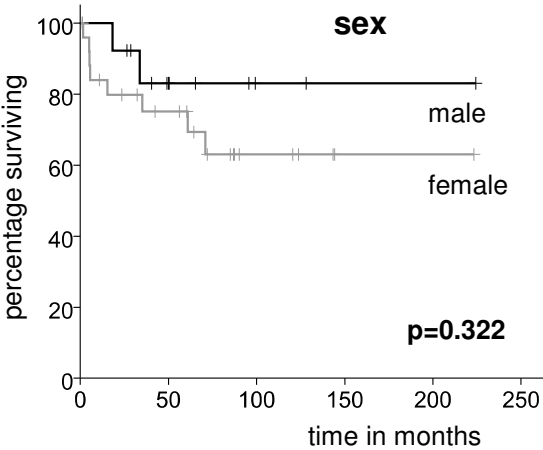


Table 1: Clinicopathologic parameters related to the four subtypes of MEC (Significance of associations was evaluated by Fisher exact test).

		classical type (n=23)	clear cell variant (n=3)	eosinophilic variant (n=5)	squamoid variant (n=9)	Fisher exact Test classical vs. others	Fisher exact Test squamous vs. others
age	≤60 years (n=24)	19	1	2	2	p<0.001	p=0.03
	>60 years (n=16)	4	2	3	7		
sex	males (n=13)	7	0	2	4	n.s.	n.s.
	females (n=27)	16	3	3	5		
site	parotid gland (n=24)	10	2	4	8	p=0.125 ^a	p=0.055 ^a
	submandibular gland (n=4)	2	1	0	1		
	oral cavity (n=12)	11	0	1	0		
stage	I (n=16)	12	1	2	1	p=0.107 ^b	p=0.061 ^b
	II (n=12)	5	1	2	4		
	III (n=6)	2	0	1	3		
	IV (n=6)	4	1	0	1		
grade	1 (n=27)	22	2	3	0	p<0.001 ^c	p<0.001 ^c
	2 (n=3)	1	0	1	1		
	3 (n=10)	0	1	1	8		
residual tumor	0	22	3	4	8	n.s. ^d	n.s. ^d
	1	0	0	1	0		
	2	1	0	0	1		
t(11;19)	negative	4	0	3	8	p=0.003	p=0.001
	positive	19	3	2	1		
number of cases		23	3	5	9		

^a major glands vs. minor glands^b stage I vs. stages II, III and IV^c grade 1 vs. grades 2 and 3^d residual tumor 0 vs. residual tumor 1 and 2

Table 2: Clinicopathologic parameters related to presence or absence of t(11;19). Significance of association was evaluated by Fisher exact test.

		t(11;19) negative	t(11;19) positive	Fisher exact Test
age	≤60ys (n=24)	6	18	p=0.094
	>60ys (n=16)	9	7	
sex	m (n=13)	6	7	n.s.
	f (n=27)	9	18	
site	parotid gland (n=24)	9	15	n.s. ^a
	submandibular gland (n=4)	2	2	
	oral cavity (n=12)	4	8	
stage	I (n=16)	5	11	n.s. ^b
	II (n=12)	5	7	
	III (n=6)	3	3	
	IV (n=6)	2	3	
grade	1 (n=27)	5	22	p=0.001 ^c
	2 (n=3)	3	0	
	3 (n=10)	7	3	
residual tumor	R0 (n=37)	13	24	n.s. ^d
	R1 (n=1)	1	0	
	R2 (n=2)	1	1	
number of cases		15	25	

^a major glands vs. minor glands
^b stage I vs. stages II, III and IV
^c grade 1 vs. grades 2 and 3
^d residual tumor 0 vs. residual tumor 1 and 2

Table 3. Univariate and multivariate Cox regression with reverse stepwise selection. In the upper half age, stage, grade, and residual tumor are included, in the lower half grade is replaced by tumor type (squamous vs. others).

		univariate	multivariate step 1	step 2	hazard ratio	95% confidence interval
age	≤60ys (n=24) vs. >60ys (n=16)	0.005	p=0.06	p=0.05	5.1	1.0-26.9
stage	stage I (n=16) vs. stages II-IV (n=24)	0.064	p=0.32			
grade	grade 1 (n=27) vs. grades 2-3 (n=13)	0.004	p=0.11	p=0.04	5.4	1.1-28.4
residual	R0 (n=37) vs. R1-2 (n=3)	0.001	p=0.13	p=0.10	4.7	0.74-30.0
age	≤60ys (n=24) vs. >60ys (n=16)	0.005	p=0.09	p=0.10	4.3	0.8-24.0
stage	stage I (n=16) vs. stages II-IV (n=24)	0.064	p=0.31			
variant	squamous (n=9) vs. others (n=31)	0.007	p=0.12	p=0.05	4.7	1.0-20.7
residual	R0 (n=37) vs. R1-2 (n=3)	0.001	p=0.02	p=0.01	18.0	2.1-152.8