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1p/19q loss within oligodendroglioma is predictive for response to first line temozolomide but not to salvage treatment

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ABSTRACT

Background: Combined loss of 1p/19q predicts an almost 100% response rate to first line procarbazine, CCNU and vincristine chemotherapy (PCV) chemotherapy in oligodendroglial tumours. We assessed the impact of 1p and 19q loss on the outcome to first line temozolomide (TMZ) chemotherapy and to second line PCV or TMZ in progressive oligodendroglial tumours.

Materials and methods: Tumour samples from patients included in two prospective EORTC studies on first line and second line TMZ chemotherapy in recurrent oligodendroglioma were used for this study. Most patients in the first line TMZ trial received PCV at further progression. Loss of 1p and 19q was assessed on paraffin embedded tumour samples by fluorescent in situ hybridisation with locus specific probes for 1p36 and 19q13.

Results: Losses of 1p and 19q were mainly observed in morphologically classical oligodendrogliomas (OD). Thirteen out of 18 patients with 1p loss (72%) responded to first line temozolomide ($p < 0.01$). Both response to second line salvage PCV or to second line temozolomide was limited, even in patients with combined 1p/19q loss. Patients with tumours with 1p loss treated with salvage PCV had improved PFS ($p < 0.05$). More patients with 1p loss were alive at 60 and 120 months after initial surgery ($p < 0.001$).

Conclusion: Combined 1p/19q loss is mainly observed in classical OD. Responses to first line temozolomide are strongly correlated to loss of 1p. Response to second line alkylating treatment is modest even in tumours with 1p/19q loss. For further improvement of outcome in OD novel treatments are needed.

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1. Introduction

High grade oligodendrogliomas (AOD) and mixed oligoastrocytomas (AOA) are sensitive to chemotherapy. Especially the activity of PCV chemotherapy (consisting of procarbazine, CCNU and vincristine) and temozolomide (TMZ) has been clearly established, with objective response rates ranging from 55% to 70%.[1–4] Beneficial effects of PCV chemotherapy have particularly been shown in oligodendroglial tumours with combined loss of the short arm of chromosome 1 (1p) and the long arm of 19 (19q), with 90–100% of patients responding.[5] This combined loss of 1p and 19q occurs in up to 60% of patients with histologically classic oligodendroglioma (OD), but is less frequent in oligoastrocytoma (OA).[5–8] Combined loss of 1p/19q also correlates with a more indolent behaviour of tumours and prolonged overall survival.[5,9]

For most patients with recurrent disease after radiotherapy, salvage chemotherapy represents the only alternative option for further treatment. Since temozolomide is better tolerated this drug has almost completely replaced PCV chemotherapy as first line treatment.[10–12] It is unclear though if combined 1p/19q loss also predicts outcome to first-line chemotherapy with TMZ or second line chemotherapy (TMZ or PCV). We used two EORTC Brain Tumour Group trials (EORTC study 26971 and EORTC study 26972) on first- and second-line chemotherapy with TMZ on recurrent AOD and AOA to assess the impact of 1p/19q loss on the outcome to first line TMZ and to second line treatment with either PCV or TMZ.[7,13]

2. Materials and methods

2.1. Patients

For this study all patients were considered that: (a) had been included in two prospective multi-centre phase II EORTC trials on first line and second line TMZ chemotherapy in recurrent OD or OA (EORTC 26971 and 26972); and (b) with sufficient unstained paraffin embedded tumour tissue available to determine 1p and 19q loss. The clinical details of these studies have been published elsewhere.[2,7,13] A separate retrospective study assessed the outcome to second line PCV in all EORTC 26971 patients progressing during or after TMZ treatment.[7] Since these studies required enhancing disease as an entry criterion, even previously low grade tumours will have transformed into anaplastic tumours at the time of study entry.

Patients were eligible for the prospective clinical studies if they had: (1) histological proven (anaplastic) oligodendroglioma or oligoastrocytoma provided that at least 25% of oligodendroglial elements were present, (2) for EORTC study 26971: progressive disease after radiation therapy without prior chemotherapy; for EORTC study 26972 progressive disease after radiation therapy and one line of chemotherapy, either given adjuvant or at first recurrence (and regardless of the response to prior chemotherapy), (3) measurable disease requiring a contrast-enhancing lesion with a diameter of at least 1 cm on magnetic resonance imaging (MRI) or computed tomography (CT) scan. Patients were treated with 150 mg/m^2 (EORTC 26972) or 200 mg/m^2 (EORTC 26971) TMZ administered in the standard days 1–5 in 28-d cycles for a maximum of 12 cycles. All centres used the standard PCV schedule as described previously.[7,13]

Response was evaluated preferably with MRI, but CT was allowed provided that the same imaging modality was used throughout the entire treatment. For response assessment the bidimensional Macdonald's criteria were used, with central scan review of all responding patients.[14] For the purpose of this study only complete response (CR) and partial response (PR) were considered true responses. Overall survival was measured from 1st surgery (OS) and progression free survival (PFS) from the start of 1st or 2nd line chemotherapy.

2.2. Pathology review

All tumours were histologically reviewed by a single pathologist (JMK), who was kept unaware of the clinical data. For genotyping the most representative tumour area was selected. Selection of individual areas was based on tumour content and predominant tumour morphology present in each individual case. In cases diagnosed as (A)OD, the pathologist stated whether he considered the tumour a 'classical' (A)OD or not. Only those tumours composed of neoplastic cells with round nuclei and perinuclear halos arranged in a honeycomb fashion were considered classical (A)OD. Tumours consisting of cells without an orderly arrangement, with less round nuclei and/or without perinuclear halos while lacking astrocytic features or with astrocytic cells, were considered atypical (A)OD. By definition, oligoastrocytomas were defined as atypical (A)OD.

2.3. Fluorescent in situ hybridisation (FISH)

Probes to 1p36 (D1S32), centromere 1 (CEP1; pUC1.77), 19p (equivalent amounts of human BAC RPCI-11 959O6, 957I1 and 153P24; BacPac Resources, Oakland, CA) and 19q13 (RPCI-11 426G3; Research Genetics, Huntsville, AL) were labelled with digoxigenin-16-dUTP (pUC1.77, 959O6, 957I1 and 153P24), biotin-16-dUTP (D1S32; both Roche Diagnostics, Mannheim, Germany) or spectrum orange-dUTP (426G3; Vysis Inc., Downers Grove, IL), respectively.[15,16] Tumour sections were deparaffinised, dehydrated, microwave treated in citrate buffer (pH 6.0) for 5–10 min and digested in pepsin solution (Sigma–Aldrich, St Louis, MO; 0.005% in 0.1 M NaCl, pH 1.5–2.0) for 10–15 min at 37 °C and dehydrated. Subsequently, paired probe solutions were dispensed at 10–20 μl per slide, depending on the surface area of tissue. Tumour sections and probes were co-denaturated by placing slides upon the metal surface of a slide moat preheated to 80 °C for 3–5 min. Slides were then cooled on ice and incubated at 37 °C during 48 h in a moistened chamber. After incubation, slides were washed in 1.5 M urea/0.1× SSC at 45 °C for 15–30 min and rinsed in 2× SSC. Probes were detected using anti-digoxigenin conjugated with fluorescein isothiocyanate (FITC; Roche Diagnostics, Mannheim, Germany) or CY3-conjugated avidin (Brunschwig Chemie, Amsterdam, the Netherlands) antibodies at a concentration of 4 and 15 μg/ml diluted in PBS, respectively. Nuclei were counter stained with DAPI in antifade solution (Vector Laboratories, Burlingam, CA).

2.4. Data analysis

Locus-specific FISH probes were enumerated in 60 non-overlapping nuclei per hybridisation utilizing a Leica DM-RXA fluorescence microscope (Leica, Wetzlar, Germany). Images were captured in five different levels with 0.5 μm interval utilising a COHU 4910 series monochrome CCD camera (COHU, San Diego, CA) attached to the fluorescence microscope equipped with a PL Fluotar 100×, NA 1.30–0.60 objective and I3 and N2.1 filters (Leica) and Leica QFISH software (Leica Imaging Systems, Cambridge, UK). Ratios were calculated for 1p *versus* CEP1 or 19q *versus* 19p by dividing the number of signals of the marker by the number of signals of the reference; a ratio below 0.80 was considered an allelic loss. In case of an inconclusive ratio, *e.g.* ratios between 0.75 and 0.90, 200 additional non-overlapping nuclei were counted.

3. Results

A total of 32 and 38 eligible patients were registered, respectively, in EORTC study 26972 and EORTC study 26971; 24 patients included in the latter study received second line PCV. Of 54 patients sufficient material was available for genotyping by FISH. At review, forty of these patients were diagnosed with oligodendroglioma (32 AOD, 8 OD), 11 with AOA and three patients with astrocytoma (A). Among samples with an oligodendroglial phenotype 28 AOD and 6 OD had a classical morphology.

3.1. Genotypical alterations occur frequently in tumours with a classic oligodendroglial phenotype

Loss of 1p was observed in 65% (35/54) of the investigated samples. In 20 patients combined 1p/19q loss was observed (20/54, 37%). A solitary loss of 19q was observed in only 1 (2%) patient, 1 had no loss of 19q without data on 1p (2%), while 19q was found present in 17 (33%) samples. In the presence of a classic (A)OD phenotype 1p and 19q loss was more frequent (30/34, 88% and 17/34, 50%, respectively) compared to tumours without characteristic (A)OD morphology (5/20, 25% and 4/20, 20%; Table 1).

3.2. Genotypic alterations predict response to first line TMZ

Loss of 1p (either solitary or in combination with 19q) was highly correlated with a response to first line TMZ ($p < 0.01$; Table 2). Nine out of 11 patients with combined 1p/19q loss had a response, the remaining two had stable disease (SD) as the best response. In 19 patients without 19q loss (independent of 1p status) only 8 responded, and 3 had progressive disease (PD). At 12 months 11 of 18 patients (61%) with 1p loss were still free from progression, in contrast to 3 of the 12 patients (25%) without 1p loss ($p = 0.025$; Fig. 1A). No correlations were observed between PFS and age at inclusion with the presence or absence of 19q loss.

3.3. Genotype does not correlate with response to second line chemotherapy

Samples of 19 patients treated with salvage PCV after prior TMZ treatment were available for FISH. In only 3/19 (16%) patients a response to PCV was observed. Nine patients had combined 1p/19q loss, only 2 (22%) of which responded to salvage PCV treatment. No correlations were observed between losses 1p and/or 19q loss and response to salvage PVC treatment.

Of 23 PCV pre-treated patients subsequently treated with 2nd line TMZ, samples were available for FISH. In 4/23 (17%) patients a PR was observed. Two patients with a response to salvage TMZ had 1p loss (1 also 19q loss), while three out of four (75%) patients had intact 19q. Only 1 of 9 patients with combined 1p/19q loss responded.

3.4. Overall survival upon salvage PCV in TMZ pre-treated patients

Sixty and 120 months after first surgery 81% and 45% of the patients in EORTC study 26971 were still alive. Considering tumours' genotype, 4/18 patients with 1p loss were still alive 10 years after initial surgery, while all patients ($n = 12$) that retained 1p had deceased. Moreover, prolonged survival correlated highly to loss of 1p at both time points ($p < 0.001$; OS-60 94 *versus* 58% and OS-120 60 *versus* 22%; Fig. 1B). Although patients with classic oligodendroglial tumours also seemed to have longer OS at 120 months compared to patients without such classical histology (52 *versus* 28%) the difference was only of borderline significance ($p = 0.063$). Age (⩽45 years or >45 years) or presence or absence of the 19q allele was not of statistical significance. Neither genotype nor histology was correlated to overall survival in EORTC study 26972.

4. Discussion

The diagnosis of oligodendroglial tumours has been hampered by lack of specific immunohistopathological markers

Table 1 – Genotypical alterations occur frequently in tumours with a classic oligodendroglial phenotype

	$1p^{loss}19q^{no\ loss}$	$1p^{loss}19q^{loss}$	$1p^{no\ loss}19q^{loss}$	$1p^{no\ loss}19q^{no\ loss}$	Total
Classical OD	13 (24%)	17 (31%)	0	4 (7%)	34
Non-classical OD	2 (4%)	3 (6%)	1 (2%)	14 (26%)	20
Total	15 (28%)	20 (37%)	1 (2%)	18 (33%)	54

Tumours with a classical oligodendroglial (OD) histology as described in Section 2 frequently had loss of 1p either alone (13/34) or in combination with 19q loss (17/34). In contrast, tumours without such characteristic phenotype generally had an unaltered genotype (14/20).

Table 2 – Response to first line temozolomide (TMZ) is correlated to loss of 1p

	$1p^{loss}$	$1p^{no\ loss}$	Total
CR	9 (50%)	0	9 (30%)
PR	4 (22%)	4 (33%)	8 (27%)
SD	5 (28%)	5 (42%)	10 (33%)
PD	0	3 (25%)	3 (10%)
Total	18	12	30

Higher proportions of patients with tumours displaying 1p loss had a response (complete (CR) or partial (PR)) to first line TMZ (EORTC trial 26971) compared to patients without such genotypic alterations (72 *versus* 33%). In contrast, patients without a response to first line TMZ (stable (SD) or progressive disease (PD)) mainly had tumours without 1p loss (28 *versus* 67%; $p < 0.01$).

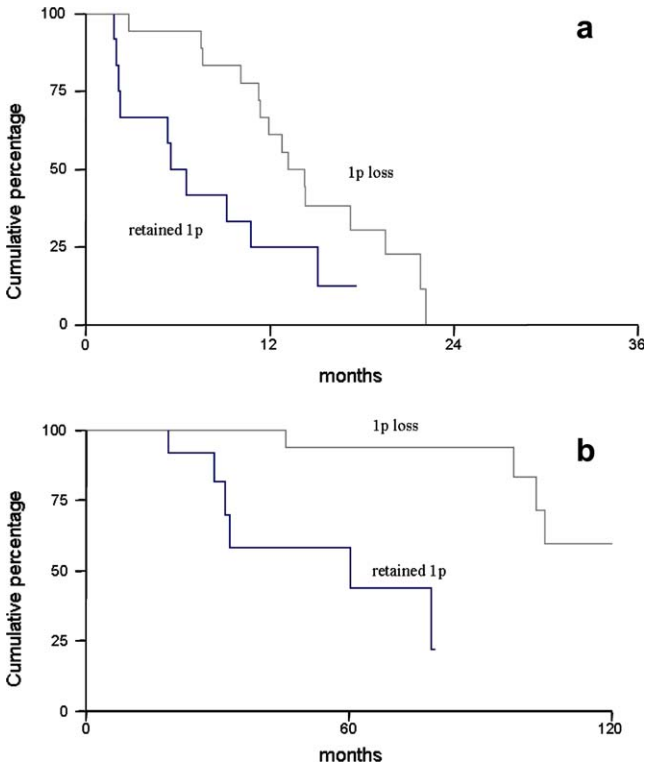

Fig. 1 – Among patients treated with first line temozolomide (TMZ) and subsequently procarbazine, CCNU, and vincristine (PCV) (included in EORTC 26971), a higher proportion of patients with tumours harbouring 1p loss were progression free at 6 (94% *versus* 50%) and 12 (61% *versus* 24%) months after start of salvage chemotherapy compared to patients with retained 1p ($p < 0.05$; panel A). Additionally, patients with 1p loss were more frequently alive at 60 (94% *versus* 58%) and 120 (60% *versus* 22%) months after primary surgery compared to patients with unaltered tumours' genotype ($p < 0.001$; panel B).

and changing classification criteria. This has resulted in a considerable inter-observer variability[17,18] as well as in a marked increase in oligodendrogliomas.[19] The recognition of the sensitivity of oligodendroglial tumours with combined 1p/19q loss to PCV with almost 100% of patients responding has made the identification of a subset of patients with different biological behaviour possible.

In the present set of oligodendroglial tumours 65% had loss of 1p of which 59% also had 19q loss. Within the total patient group 37% of tumours had a combined 1p/19q loss. In previous studies 60–70% of patients with oligodendroglial tumours were shown to have combined 1p/19q loss. This difference and the relatively high number of patients with 1p loss only may reflect limited sensitivity, to which the multi-centre setting of our study may have contributed. Differences between the various institutions in the fixation of tumour specimen make the optimisation of the samples for FISH more difficult, especially for the determination of loss of 19q. We may therefore have underdiagnosed 19q loss, despite the use of multiple probes for this chromosome.

Loss of 1p and 19q is not unique for pure AOD and also occurs in astrocytomas and AOA.[5,9,15,20] Our data confirm that it is mainly observed in oligodendroglioma with classical morphological features. More importantly, our data show that 1p/19q loss also predicts outcome to temozolomide in recurrent oligodendroglioma, with more patients responding and a longer time to progression in patients with 1p loss or combined 1p/19q loss. This gives further evidence for a more general association between genotype alterations and response to various alkylating agents in oligodendroglioma. So far, only data on the impact of the genotype of oligodendroglioma on the response to temozolomide for low grade tumours have been published, which study confirmed a higher response rate of tumours with loss of 1p/19q.[8]

Despite favourable responses to initial treatment, tumours ultimately recur requiring further treatment strategies. The EORTC trial on salvage TMZ (EORTC 26972) but also the retrospective study on salvage PCV on temozolomide pretreated patients show a marginal response rate to second line chemotherapy. In contrast to the almost 100% response rate observed after first line PCV or the 81% response rate to first line temozolomide in this study in 1p/19q loss tumours, our data show a limited 10–20% response rate even in patients with combined 1p/19q loss tumours to second line treatment. Low response rates to salvage PCV or TMZ treatment may suggest the development of cross resistance to consecutive alkylating agents by induction of several DNA-repair enzymes or by selection of tumour clones with alkyltransferase expression.

In conclusion, losses of 1p and 19q are mainly confined to tumours with a classic oligodendroglial phenotype. Response rates to first line chemotherapy are generally high and correlate to losses of 1p with or without 19q loss. Modest results in

second line chemotherapy – even in 1p/19q loss patients – may indicate the development of progressive resistance of tumours to different alkylating agents. Consequently, for further improving the outcome of these patients novel strategies need to be developed.[7]

Conflict of interest statement

None declared.

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