

Tumour Review

Diffuse intrinsic pontine gliomas: A systematic update on clinical trials and biology

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ABSTRACT

Patients with diffuse intrinsic pontine gliomas (DIPG) have a poor prognosis. Although DIPG constitute only 10–15% of all pediatric brain tumors, they are the main cause of death in this group. Despite 26 clinical trials in newly diagnosed DIPG in the past 5 years (including several targeted agents), there is no clear improvement in prognosis. However, knowledge on DIPG biology is increasing, mainly due to the (re)introduction of biopsies and autopsies, the possibility of gene expression profiling, and the development of in vivo models. Translation of this knowledge into clinical trials in combination with improved drug distribution methods may eventually lead to more effective treatment of this devastating disease.

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Background

Effective treatment of diffuse intrinsic pontine gliomas (DIPG) in children remains elusive. DIPG comprise 10–15% of childhood brain tumors but are the main cause of death in this young group. Despite several treatment regimens being studied over the last 25 years, prognosis has not improved and <10% of patients are alive two years after diagnosis.¹

In 2006, Hargrave et al. reviewed all clinical studies performed in DIPG patients from 1984 to 2005: 29 studies were reported including a total of 973 patients.¹ Most studies were non-randomized and comparison was difficult due to unclearly defined inclusion criteria. In these studies, the median overall survival (OS) ranged from 7–16 months; when only studies with detailed clinical and radiological eligibility criteria were included, the median

OS ranged from 8–11 months with a progression-free survival (PFS) of 5–9 months. The use of hyperfractionated radiotherapy, pre-irradiation chemotherapy, concurrent chemo-radiotherapy and radiosensitizers has not increased long-term OS. In addition, adjuvant chemotherapy and high-dose chemotherapy regimens have also failed to improve long-term prognosis.

Historically, DIPG were diagnosed radiologically and biopsies were not performed due to perceived morbidity.² This was later shown to be overestimated and biopsies are now more widely practiced.^{3–5} Biopsies, together with autopsy material, have paved the way for biological studies in DIPG. Studies published in the past five years will probably be the basis for a more rational drug treatment of these tumors.

The present study is an update on the clinical trials (including recently completed and ongoing studies) and presents current knowledge on DIPG biology with regard to potential future targeted therapy.

Methods

A search was made in PubMed, the Cochrane Central Register of Controlled Trials and Embase covering the period from 1 January 2005 until 1 March 2011. The following terms were used (with synonyms and closely related words): “brain stem” and “gliomas” or “tumors” and “RCT’s” or “reviews” or “meta-analyses” or “systematic reviews” and “children”. Two reviewers (MJ and GJK) independently screened the references for eligible articles by reading

Abbreviations: CNS, central nervous system; DIPG, diffuse intrinsic pontine gliomas; EGFR, epidermal growth factor receptor; HGG, high-grade glioma; ITH, intratumoral hemorrhage; IV, intravenous; MTD, maximum tolerated dose; OS, overall survival; PDGFR, platelet-derived growth factor receptor; PFS, progression-free survival; PO, orally; VEGFR, vascular endothelial growth factor receptor.

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the title and abstract. Full-text versions of these articles were then obtained. The full search strategy can be requested from the first author.

Inclusion criteria: only prospective clinical trials including and separately analyzing pediatric patients (aged 0–19 years) with newly diagnosed DIPG were eligible for review. Investigated parameters were: inclusion criteria of the studies, baseline characteristics (age, gender, life expectancy at diagnosis, symptoms at diagnosis and duration of symptoms, performance scores, percentage of tumor involving the pons, histological diagnosis) and outcome (response on MRI, progression-free and overall survival time). Overall rates were composed of the range of the medians.

To evaluate ongoing trials, a search was done using the search terms “brain stem glioma” “brainstem glioma” and pontine glioma on <http://apps.who.int/trialsearch/> and <http://clinicaltrials.gov/> and the Abstract Books of ISPNO, SNO, SIOP, AACR and EACR from 2005–2010.

Results

The systematic search on clinical trials yielded 584 publications dating from 2005 (Fig. 1). Unfortunately, although some studies included newly diagnosed DIPG patients, they did not analyze them separately and were therefore excluded from this review.^{6,7}

Table 1 summarizes the inclusion criteria and baseline characteristics, and Table 2 the survival time and response rates for all published clinical studies.

Inclusion criteria and baseline characteristics

In total, 26 prospective clinical trials, including 561 children (40% boys and 60% girls) with newly diagnosed DIPG, were published in 23 manuscripts and were eligible for review.^{8–30} The median number of patients per study was 20 (range 7–63). The median age was 6.2 (range 2.5–9.2) years; one study included patients up to age 3 years only.¹⁵ There was considerable inter-study variability in eligibility criteria, including performance state, life expectancy, symptoms at diagnosis and laboratory findings. A Karnofsky performance state of $\geq 40\%$ was requested in two stud-

ies,^{10,28} $\geq 50\%$ in seven studies,^{9,11,14,16,17,21,25} $\geq 60\%$ in one study,²⁶ and $\geq 70\%$ in two studies.^{13,19} A life expectancy of six weeks or more was requested in five studies.^{9,11,13,22,28} The presence of one typical symptom at diagnosis (cranial nerve deficits, long tract signs or ataxia) was an inclusion criteria in two studies^{23,28} and five studies requested two or more of these symptoms.^{8,17,18,21,26} Seven studies also restricted the period of symptom duration at diagnosis in an attempt to exclude less aggressive tumors.^{8,18,21,23,26,28,30} In total, 18 studies stated that only typical DIPG patients were included based on MRI criteria, but only eight of these further specified the MRI criteria, i.e. six requested tumor involvement of $\geq 50\%$ of the pons^{8,9,17,18,24,30} and two stated a minimal involvement of 67%.^{11,21} Seven studies also included gliomas other than DIPG: four included newly diagnosed pediatric high-grade glioma (HGG),^{14,28–30} one included recurrent pediatric HGG,²⁵ and two included other (refractory) brain tumors.^{13,15} However, in all these studies DIPG patients were analyzed separately.

Biopsies were generally obtained in case of uncertainty about MRI diagnosis. In one study, biopsy was an inclusion criterion. Thirteen studies reported a total of 108 biopsies; pathology showed 20 (WHO grade II) diffuse astrocytomas, 1 oligodendroglioma grade II, 1 oligoastrocytoma grade II, 37 (WHO grade III) anaplastic astrocytomas, 3 oligoastrocytoma grade III, 27 (WHO grade IV) glioblastoma multiforme, 15 not further specified malignant gliomas, and 4 were undefined.^{8,10–13,17–20,24,25,28,30}

Survival rates

The median OS ranged from 4–17 months, but was not reported in three studies.^{10,14,16} The 1-, 2- and 3-year OS ranged from 14–70%, 0–25% and 0–10%, respectively. Median PFS ranged from 3–10 months. If only studies which specified MRI criteria for DIPG ($>50\%$ pontine involvement) were included, median OS ranged from 7–14 months and PFS from 5–8 months.^{8,9,11,17,18,21,24,30}

Hypofractionation of radiotherapy

Two studies on hypofractionation of radiotherapy, focusing on non-inferiority with shorter treatment duration, reported median OS rates of 8 and 9 months; this is at the lower end of the published range.^{18,24}

Neo-adjuvant chemotherapy

The study accomplishing the highest median OS (17 months (95% C.I. 11–20)) was conducted by Frappaz et al. In that study, neo-adjuvant high-dose methotrexate, BCNU, cisplatin and tamoxifen was given in cycles to DIPG patients until progressive disease occurred, then followed by radiotherapy.¹² One toxic death was reported. When survival was estimated from the start of radiotherapy, Kaplan–Meier curves were comparable with historical controls (median OS 9 months), suggesting that the 8-month improvement in survival duration was due to upfront chemotherapy. However, the long-term survival was poor, with a 3-year OS of 4%. In addition, Massimino et al. reported on three neo-adjuvant chemotherapy protocols, including one high-dose chemotherapy regimen followed by autologous stem cell transplantation.²² The median OS ranged from 9–13 months. Remarkably, in the study in which vinorelbine was used, two long-term survivors were reported: both alive at 31 and 48 months post-diagnosis, respectively.²²

Temozolomide

Addition of temozolomide to radiotherapy has not resulted in an improved survival rate in DIPG, in contrast to adult glioblastoma multiforme.³¹ The median OS ranged from 9 to 10 months.^{11,17,26} Importantly, no long-term survivors were present in the 63 pa-

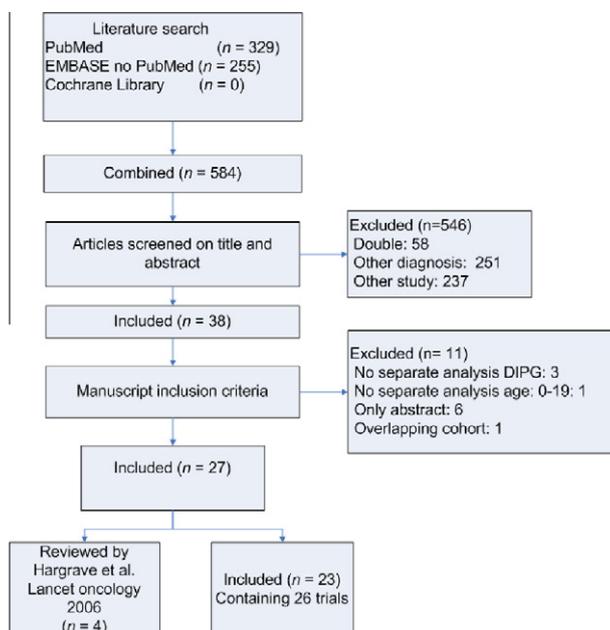


Fig. 1. Flow chart (according to PRISMA) of the systematic search.

Table 1
Inclusion criteria and baseline characteristics of published studies including newly diagnosed DIPG patients from 2005.

Therapy	Phase	DIPG only	In- and exclusion criteria							Baseline characteristics							Ref No.			
			Symp-toms ^a	Lab	Symptom duration	Karnofsky/Lansky	Life exp.	Tumor > 50%	NF excl.	N	Age (yr)	Range (yr)	Boys/girls	Symptom (mths)	Biopsy	Gr 2		Gr 3	Gr 4	MG ^b
<i>Pre-irradiation therapy</i>																				
HDC (and adj VCR-lomustine)	II	y	n	n	n	n	n	n	n	10	6	-	37%	-	30	6	18	6	-	22
Cisplatin, VP-16 (and adj isotretinoin)	II	y	n	n	n	n	n	n	n	17	6	-	37%	-						22
Vinorelbine	II	y	n	n	n	n	n	n	n	14	6	-	37%	-						22
BCNU, cisplatin, tamoxifen, HD-MTX	II	y	n	n	n	n	n	n	n	23	9	3-20	50%	2	4 ^c	-	-	-	-	12
Carboplatin, VCR, MTX, cyclophosphamide, cisplatin	II	BT	n	n	n	n	n	n	n	7	3	0-3	57%	-	-	-	-	-	-	15
<i>Radiotherapy</i>																				
Hypofractionation	Pilot	y	≥2	n	<3 mths	n	n	y	y	9	6	3-13	44%	2	4	-	-	4	-	18
Hypofractionation	-	y	n	n	n	n	n	y	n	22	6	3-13	55%	1	4	-	3	1	-	24
<i>Chemo-radiotherapy</i>																				
TMZ	II	y	≥2	y	n	≥50%	n	y	n	20	8	3-18	75%	-	8	6	-	-	2	17
TMZ	II	y	≥2	y	<6 mths	≥60%	n	n	n	15	6	2-12	40%	-	-	-	-	-	-	26
TMZ	II	y	n	y	n	≥50%	n	>2mths y > 67%	n	63	8	3-16	48%	-	3	-	1	2	-	11
TMZ and cis-retinoic acid	II	y	n	n	n	n	n	n	n	12	4	3-9	25%	1.5	-	-	-	-	-	27
TMZ and thalidomide	I/II	y	n	y	n	≥70%	n	n	n	17	8	3-16	35%	-	1	-	-	1	-	19
Cisplatin, VP-16, VCR, ifosfamide	II	HGG	n	n	<6 mths	n	n	y	n	37	8	-	57%	-	16	4	8	4	-	30
Tamoxifen	II	y	≥1	n	<6 mths	n	n	n	n	31	7	2-16	42%	0.7	-	-	-	-	-	23
VCR and oral VP-16	II	y	≥2	n	<6 mths	≥50%	n	>6 wks y > 67%	y	30	8	3-14	40%	-	-	-	-	-	-	21
VP-16, cytarabin, ifosfamide, cisplatin, dactinomycin	II	y	n	n	n	n	n	n	n	21	6	-	37%	-	-	-	-	-	-	22
<i>Radiosensitizers</i>																				
Carbogen	Pilot	y	≥2	n	<3 mths	n	n	y	y	10	9	-	-	1	3	-	-	-	3	8
Motexafin gadolinium	I	y	n	y	n	≥50%	n	>8 wks y	n	44	6	2-20	36%	-	-	-	-	-	-	9
<i>Adjuvant chemotherapy</i>																				
Interferon γ and cyclofosfamide	I/II	HGG	n	n	n	n	n	n	n	19	-	-	-	-	-	-	-	-	-	29
<i>Anti-angiogenesis therapy</i>																				
Thalidomide	II	GBM	≥1	n	<3 mths	≥40%	n	>8 wks n	n	12	6	2-14	31%	-	1	-	-	-	1	28
Topotecan, adjuvant thalidomide, celecoxib, etoposide	II	y	n	n	n	n	n	n	n	8	8	3-13	-	-	5	2	3	-	-	20
<i>Targeted therapy</i>																				
Tipifarnib	I	y	n	y	n	≥50%	n	n	n	17	6	4-14	35%	-	-	-	-	-	-	16
Imatinib	I	refr HGG	n	y	n	≥50%	n	n	n	27	7	3-19	51%	-	5	-	1	1	3	25
Gefitinib	I	HGG	n	n	n	≥50%	n	n	n	20	7	3-21	30%	-	2	-	2	-	-	14
Vandetanib	I	y	n	y	n	≥40%	n	n	n	35	6	3-16	43%	-	1	-	-	1	-	10
Erlotinib	I	refr BT	n	y	n	≥70%	n	>8 wks n	n	21	6	2-16	33%	-	21	4 ^d	4 ^e	7	6	13

n = no inclusion criterion, y = yes, inclusion criterion, lab = normal liver, renal and bone marrow function, mths = months, wks = weeks, yr = years, exp = expectancy, NF = neurofibromatosis, N = number of patients, Gr = glioma grade, BT = brain tumors other than ependymoma, GBM = glioblastoma multiforme HGG = high-grade glioma, refr = refractory, Ref = reference, HDC = high-dose chemotherapy with autologous stem cell reinfusion. BCNU = carmustine, HD = high-dose, MTX = methotrexate, VCR = vincristine, TMZ = temozolomide, VP-16 = etoposide.

^a Cranial nerve deficits, long tract signs, or ataxia.

^b Malignant glioma no grade reported.

^c No tumor grade reported.

^d 1 Oligodendroglioma grade II and 1 oligoastrocytoma grade II.

^e 3 Oligoastrocytoma grade III.

Table 2
Response and survival rates of published studies including newly diagnosed DIPG patients from 2005.

Therapy	CR	PR	SD	PD	MOS (mths)	CI	1-year OS	2-year OS	3-year OS	PFS (mths)	1-year PFS	Ref. No.
<i>Pre-irradiation therapy</i>												
HDC (and adj VCR-lomustine)	-	-	-	-	13	-	70%	10%	-	10	40%	22
Cisplatin, VP-16 (and adj isotretinoin)	-	-	-	-	9	-	29%	12%	-	5	12%	22
Vinorelbine	-	-	-	-	9	-	43%	21%	-	7	21%	22
Carmustine, cisplatin, tamoxifen, HD-MTX	-	-	-	-	17	11–20	65%	22%	4%	-	-	12
Carboplatin, VCR, MTX, cyclophosphamide, cisplatin	-	-	-	-	3.6	-	14%	0%	0%	2.5	0%	15
<i>Radiotherapy</i>												
Hypofractionation	-	-	-	-	8.6	-	-	-	-	5	-	18
Hypofractionation	-	-	-	-	7.6	-	-	-	-	5.7	-	24
<i>Chemo-radiotherapy</i>												
TMZ	-	-	-	-	9.2	-	35%	15%	10%	6.9	-	17
TMZ	-	-	-	-	9.8	-	20%	7%	-	5.1	7%	26
TMZ	0%	31%	34%	14%	9.6	-	40%	3.6%	0%	6.1	14%	11
TMZ and cis-retinoic acid	0%	58%	33%	9%	13.5	6–22	58%	-	-	10.2	-	27
TMZ and thalidomide	0%	83%	8%	8%	12.7	10–15	58%	25%	-	7.2	17%	19
Cisplatin, VP-16, VCR, ifosfamide	3%	22%	46%	30%	13.6	-	-	-	-	4.8	0%	30
Tamoxifen	-	-	-	-	6.3	-	16%	6%	6%	3.9	3%	23
VCR and oral VP-16	-	-	-	-	12	-	45%	18%	-	7	30%	21
VP-16, cytarabine, ifosfamide, cisplatin, dactinomycin	0%	26%	67%	7%	9	-	27%	3%	0%	-	-	22
<i>Radiosensitizers</i>												
Carbogen	-	-	-	-	9.6	-	-	-	-	8	-	8
Motexafin gadolinium	-	-	-	-	7	8–3	-	-	-	-	-	9
<i>Adjuvant chemotherapy</i>												
Interferon γ and cyclofosfamide	-	-	-	-	9.6	-	-	0%	0%	-	-	29
<i>Anti-angiogenesis therapy</i>												
Thalidomide	0%	54%	15%	23%	9	-	-	0%	0%	5	-	28
Topotecan, adjuvant thalidomide, celecoxib, etoposide	-	50% ^a	-	-	12.5	-	63%	-	-	11	-	20
<i>Targeted therapy</i>												
Tipifarnib	-	-	-	-	-	-	36%	-	-	-	-	16
Imatinib	0%	6%	-	-	11	-	45%	-	-	-	24%	25
Gefitinib	-	-	-	-	-	-	48%	-	-	-	16%	14
Vandetanib	-	-	-	-	-	-	38%	21%	-	-	-	10
Erlotinib	0	17%	50%	33%	12	-	50%	19%	-	8	-	13

mths = months, CR = complete response, PR = partial response: >50% decrease, SD = stable disease: <50% decrease and <25% increase, PD = progressive disease: >25% increase, MOS = median overall survival, PFS = progression-free survival, OS = overall survival, Ref. = reference, HDC = high-dose chemotherapy with autologous stem cell transfusion, MTX = methotrexate, VCR = vincristine, TMZ = temozolomide, VP-16 = etoposide.

^a Partial response was defined as >20% decrease in this study.

tients cohort reported by Cohen et al.¹¹ The combination of radiotherapy, temozolomide and either thalidomide or retinoic acid, resulted in a median OS of 13 (95% CI, 10–15) months and 14 (95% CI, 6–21) months, respectively.^{19,27} Temozolomide was dosed at 75–90 mg/m² daily during radiotherapy and at 200 mg/m², 5 days per 28-day cycle of adjuvant temozolomide^{17,19,27} in all but one study.²⁶ This latter study had a metronomic schedule: 85 mg/m² daily concurrent with radiotherapy and adjuvant in 6-week cycles with a 1-week break.²⁶ Main temozolomide related toxicity was bone marrow suppression, especially grade III lymphopenia up to 87%.²⁶

Other chemoradiotherapy

Studies on concurrent chemotherapy other than temozolomide (including a vincristine-etoposide, a tamoxifen and an intensified chemotherapy protocol) reported median OS rates ranging from 6 to 14 months, with the best outcome for the intensified chemotherapy trial by Wolff et al.^{21,23,30} However, with regard to the latter protocol (consisting of cisplatin, etoposide, vincristine and ifosfamide) the authors concluded that there was no improvement compared to their historical Hirntumor-glioblastoma multiforme DIPG cohorts (a prospective German language multicenter study cohort).³⁰

Non-cytotoxic radiosensitizers

Two non-cytotoxic radiosensitizers have been investigated: inhaled carbogen, a potential radiosensitizer of hypoxic tumors, and motexafin gadolinium, an expanded metalloporphyrin with radiosensitizing activity based on depletion of DNA repair enzymes.^{8,9}

The carbogen trial was discontinued after 10 patients because of lack of efficacy; for motexafin gadolinium, a phase II trial has been initiated.

Anti-angiogenic therapy

Thalidomide, a drug with antiangiogenic activity, was studied as a single agent in a phase II trial.²⁸ None of the 12 patients completed the 12 months of treatment, mainly due to progressive disease or toxicity (23%). Prolonged steroid usage was noted in patients enrolled on this study: 11 patients required steroids for at least 6 weeks and 8 patients for more than 12 weeks. The median OS was 9 months (C.I. not reported). Two trials in which thalidomide was studied in combination with other drugs (either temozolomide or celecoxib-etoposide) both had a median OS of 13 months.^{19,20}

Targeted therapy

In the past 5 years several tyrosine kinase inhibitors and other targeted agents have been introduced in DIPG, mostly in phase I trials; therefore, survival rates should be interpreted with caution. A phase I trial on the farnesyltransferase inhibitor tipifarnib (inhibiting rat sarcoma; Ras) reported a maximum tolerated dose of 125 mg/m²/dose twice daily concurrent with radiotherapy and 200 mg/m²/dose as adjuvant treatment¹⁶; the 1-year OS rate was 36% (SE 17%). In addition, the platelet-derived growth factor receptor (PDGFR) and C-KIT inhibitor imatinib was studied in a phase I trial²⁵; the 1-year OS rate was 46% (SD 9%). Importantly, the 6-month estimate of cumulative incidence of intratumoral hemorrhage (ITH) was high (33%) and led to a study amendment. How-

ever, three patients not receiving imatinib also developed ITH, which suggests that ITH is a spontaneously occurring phenomenon in the course of this disease. ITH was also observed in a phase I trial on gefitinib, an inhibitor of the epidermal growth factor receptor (EGFR).¹⁴ Because ITH occurred in 25% of the patients at a dose of 375 mg/m², the recommended dose for phase II studies is 250 mg/m². In the gefitinib study the 1-year OS for patients with DIPG was 48% (SE 11%).¹⁴ Additionally, erlotinib (another EGFR inhibitor), was studied in a phase I trial in newly diagnosed DIPG and recurrent HGG.¹³ Most frequently reported toxicity was skin toxicity, diarrhea and asthenia, with ITH occurring only in non-pontine gliomas. The maximum tolerated dose was established at 125 mg/m² with and without radiotherapy. The median OS was 12 months in the DIPG subgroup of this study, being the only study in which histological confirmation was required for inclusion. The availability of tumor samples enabled EGFR expression and amplification analysis. EGFR expression in patients with DIPG showed a trend to correlation with PFS (median PFS of 10 months in EGFR + patients; $n = 6$) versus 6 months in EGFR patients ($n = 11$) (HR: 0.35; $p = 0.06$). Recently, a phase I trial reported on vandetanib, a tyrosine kinase inhibitor of the EGFR and vascular endothelial growth factor (VEGFR).¹⁰ The maximum tolerated dose was 145 mg/m², with a caveat that blood pressure should be monitored; two patients developed hypertension with reversible posterior encephalopathic syndrome, both in combination with steroid use. The 1-year OS was 38% (SD 11%), with two long-term survivors.

Non-published completed and ongoing trials

Several trials in newly diagnosed DIPG are ongoing and/or have not yet been published; these are summarized in Table 3.

Results of the HEAD-start protocols, neo-adjuvant high-dose chemotherapy followed by autologous stem cell transplantation, have not yet been published (Patel et al., *Neuro Oncol* 2008; 10:427: abstract). Temozolomide is still being studied in metronomic and traditional dose schedules in multiple trials (Chassot, 2010, Kramm, 2010, Bailey, 2007; *clinicaltrials.gov*). Studies with motexafin and capecitabine are ongoing in DIPG. Capecitabine is an oral family member of the fluoropyrimidine (5-FU) group, a widely used cytostatic drug which appears to have radiosensitizing capacity *in vitro*.^{32,33}

Several trials with targeted agents are currently ongoing in DIPG. EGFR inhibition has been mainly studied with the humanized monoclonal antibody nimotuzumab. Preliminary results of the first nimotuzumab trial in progressive DIPG were encouraging (150 mg/m² once weekly *i.v.*), since 10/22 DIPG patients experienced stable disease or partial responses. (Fleischhack et al., *Pediatr Blood Cancer* 45:444, 2005: abstract). However, preliminary results of the subsequent phase II/III trial in newly diagnosed patients showed a median OS of 10 (± 1) months which provided no benefit compared to historical controls (Fleischhack et al., *Neuro Oncol* 12(6):9, 2010: abstract). VEGF inhibition with the monoclonal antibody bevacizumab is currently being studied in newly diagnosed DIPG, either with irinotecan or with valproic acid (Fouladi, Blaney, 2009; *clinicaltrials.gov*). One trial is investigating a multi-targeted therapy approach with nimotuzumab, bevacizumab and valproic acid combined with radiotherapy, following a neo-adjuvant window phase with cisplatin-irinotecan (Cruz et al., *Neuro Oncol* 12(6):10, 2010: abstract). Additionally, PDGFR inhibition by dasatinib is being studied in combination with vandetanib (Broniscer, 2009; *clinicaltrials.gov*). Finally, the first molecular target-based trial is planned in which patients are treated with radiotherapy with concomitant bevacizumab, followed by bevacizumab maintenance therapy with either temozolomide and/or erlotinib. Maintenance therapy stratification will be based on MGMT pro-

motor methylation status and EGFR expression in tumor biopsy samples taken at diagnosis (Kieran, 2011; *clinicaltrials.gov*).

Currently, a study is recruiting patients in which IL-13 pseudomonas exotoxin is infused by convection enhanced delivery in children with recurrent DIPG and high-grade gliomas (NIHCC, 2009; *clinicaltrials.gov*). Convection enhanced delivery is a method in which drugs are administered directly into the tumor under a continuous high-pressure gradient. It has thus far been applied in the brainstem in two patients, of which one had progressive DIPG and the other Gaucher disease.³⁴ Reported morbidity was mild and transient in both procedures. The patient with DIPG received IL-13 bound to pseudomonas exotoxin and survived 4 months after administration.³⁴

Biology

Knowledge on DIPG biology was limited until recently, mainly due to a lack of available tumor tissue. Historically, biopsies were not regularly performed on DIPG, since diagnosis on MRI was found to be reliable and a histological diagnosis would not change therapy for the individual patient. In addition, a stereotactic biopsy was considered to be a dangerous procedure.² However, the question regarding tumor tissue has been raised again; an FDA meeting (2009) and a European meeting (2011) were organised to discuss biopsies in DIPG (Pena et al. www.fda.gov/AdvisoryCommittees). In France (Paris) and the UK (Nottingham), where patients with DIPG have been biopsied regularly in the past few years, only transient morbidity and no mortality have occurred.^{3–5} Furthermore, biopsies have been performed as part of a clinical trial to enable correlation of specific kinase expression to response to molecular targeted therapy.¹³

Autopsies are another important source of DIPG tissue. Two recent studies evaluated the feasibility of DIPG sample collection from autopsy.^{35,36} In both studies, about 50% of the informed parents consented to autopsy. In the study by Broniscer et al. DNA and RNA, suitable for genome-wide analysis could be obtained from 100% to 63% of the tumor samples, respectively.³⁶ Several research groups are working on culturing DIPG tumor cells obtained via biopsy or even post-mortem material.³⁷ This will enable multiple drug screening and xenografting of DIPG cell lines, which may give direction to future trials. Although brainstem glioma animal models exist, they are thus far based on adult glioma cell lines, with a different biological signature or genetically engineered (=PMID 20197468).^{38,39} Our group has developed a human DIPG mouse model that closely resembles the clinical pathomorphology of human DIPG, with diffusely infiltrating tumor cells, whereas previous models have shown a more focal pattern of tumor growth.⁴⁰

This recent paradigm shift to obtain tissue from DIPG, has resulted in improved knowledge on DIPG biology. One of the first studies on DIPG biology was by Gilbertson et al. who examined EGFR expression in 28 brainstem glioma samples (18 biopsy and 10 post-mortem specimens). The samples showed significant increase in EGFR expression with increasing tumor grade: of the glioblastomas, almost half overexpressed EGFR extensively and 28% also had high-level gene amplification of EGFR.⁴¹ Other studies showed high EGFR expression in 27–40% of the DIPG samples. However, these studies did not report EGFR amplification in DIPG, although chromosome 7 polysomy, harbouring the EGFR gene, was reported in 25% of the DIPG samples in one study.^{13,42–44} However, PDGFR α amplification in DIPG has been reported in multiple studies, in up to 36% of the patient samples.^{42,44,45} In general, PDGFR α was found to be amplified much more frequently in childhood HGG than in adult HGG.⁴⁵ In one study, high PDGFR α protein expression was present in 63% of the samples.⁴⁴ In that study, the phosphomammalian target of rapamycin, a protein downstream of the PDGFR and EGFR pathway, was immunopositive in all 11 samples

Table 3
Non-published completed and ongoing studies in DIPG.

Ongoing studies	Therapy	Patient group	Current status	N	Age (yr)	P.I.	Phase	Start year	
Neo-adjuvant Chemoradiotherapy	HEAD-protocols ^a Temozolomide	DIPG	Completed	15	<7	Finlay	II	2001	
		DIPG	Not recruiting	18	3–18	Chassot	II	2005	
		Temozolomide Temozolomide	DIPG and HGG	Recruiting	135	3–18	Kramm	II	2009
			DIPG	Not recruiting	41	2–21	Bailey	II	2007
		Paclitaxel	DIPG	Not recruiting	12	3–21	Belasco	I	2002
		Irinotecan, adj. irinotecan & BCNU	DIPG	Not reported	9	3–21	Larrier	II	–
		Thalidomide and Carboplatin	DIPG	Completed	47	3–21	Goldman	II	1999
		ACNU and VCR, followed by etoposide	DIPG	Completed	12	5–20	Sugiyama	II	2003
		Lenalidomide	DIPG and HGG	Recruiting	30	<18	Warren	I	2010
		Capecitabine	DIPG and HGG	Not recruiting	18	3–21	Blaney	I	2006
	Capecitabine	DIPG	Recruiting	44	3–21	Hoffman-La Roche	II	2007	
Radiosensitizers	Motexafin Gadolinium	DIPG	Completed	60	<21	Bradley	II	2006	
	Topotecan, G-CSF	DIPG	Suspended	72	3–21	Robertson	II	2005	
	Arsenic trioxide	DIPG and HGG	Suspended	36	3–21	Cohen	I	2004	
Histone deacetylase inhibitor	Vorinostat and RT	DIPG	Recruiting	80	3–21	Su	I–II	2011	
EGFR monoclonal antibodies	Nimotuzumab	DIPG	Not recruiting	41	3–20	Bode	III	2007	
	Nimotuzumab	DIPG	Not recruiting	44	3–18	Bouffet, Bartels	II	2008	
	Nimotuzumab	DIPG	Recruiting	–	3–18	Cabanas	II	2007	
	Neo-adj. cisplatin and irinotecan, RT and temozolomide, adj. nimotuzumab, bevacizumab and valproic acid	DIPG	Recruiting	15	0.5–18	Cruz	II	2006	
	Nimotuzumab	DIPG	Not open yet	40	3–18	Epelman	III	2010	
	Nimotuzumab and vinorelbine	DIPG	Recruiting	–	3–18	Massimino	II	2009	
	Cetuximab and irinotecan	DIPG	Recruiting	51	3–21	Dunkel	II	2010	
VEGF-monoclonal antibodies	RT and valproic acid, adjuvant bevacizumab and valproic acid	DIPG	Recruiting	56	3–21	Blaney	II	2009	
	RT and bevacizumab and TMZ, adj bevacizumab, TMZ and irinotecan	DIPG and HGG	Recruiting	35	3–30	Fouladi	I–II	2009	
VEGFR and PDGFR-TKI	Vandetanib and dasatinib	DIPG	Recruiting	28	1.5–21	Broniscer	I	2009	
	Molecular based therapy	Temozolomide/erlotinib/bevacizumab	DIPG	Not open yet	100	3–18	Kieran	II	2011
$\alpha_v\beta_3$ & $\alpha_v\beta_5$ integrins inhibitor	Cilengitide	DIPG	Recruiting	40	0.5–21	Le Blond	I	2010	
Immunotherapy	HLA-A2-restricted synthetic glioma antigen peptides vaccine PI EGFRvIII Peptide vaccination	DIPG and HGG	Recruiting	36	1.5–20	Jakacki	I	2009	
		DIPG	Not open yet	15	≤18	Fisher	I	2010	
	Interferon α 2b	DIPG	Not recruiting	32	<21	Warren	II	2002	
Convection enhanced delivery	Interleukin-13-PE38QQR	Progressive DIPG and HGG	Recruiting	20	≤17	NIHCC	I	2009	

N = estimated inclusion number, yr = years, P.I. = principal investigator, EGFR = epidermal growth factor receptor, VEGF = vascular endothelial growth factor, VEGFR = vascular endothelial growth factor receptor, TKI = tyrosine kinase inhibitor, PDGFR = platelet-derived growth factor receptor, TMZ = temozolomide, VCR = vincristine, RT = radiotherapy, HGG = high-grade glioma, LGG = low-grade glioma, PE = pseudomonas exotoxin.

^a 3–5 cycles of; vincristine, etoposide, cisplatin, and cyclophosphamide (regimen A); or prednisone, lomustine, vincristine, and carboplatin (regimen B); or vincristine, carboplatin, and temozolomide (regimen C).

Table 4
Drugable targets in DIPG.

Target	Expression/amplification	% of samples	Targeted by drugs ^a
EGFR	Protein expression	27–50%	Erlotinib, gefitinib, nimotuzumab, cetuximab, vandetanib (also VEGFR)
	Amplification	0%	
PDGFR	Protein expression	63–100%	Imatinib, dasatinib
	Amplification	36%	
VEGF(R)	NA	NA	Vandetanib (also EGFR), bevacizumab
MTOR	Protein expression	100%	
PARP	Amplification	NA	Everolimus, sirolimus
	Expression	36%	
MGMT	Amplification	27%	ABT-888 (study ongoing)
	Protein expression	0%	
RAS	NA	NA	O6-benzylguanine
avβ3 and avβ5	NA	NA	Lonofarnib, tipifarnib
IL-13	NA	NA	Cilengitide (EMD121974)
	NA	NA	IL13-PE38QQR

NA = not analyzed, EGFR = epidermal growth factor receptor, VEGF(R) = vascular endothelial growth factor (receptor), PDGFR = platelet-derived growth factor receptor, MTOR = mammalian target of rapamycin, PARP = poly (ADP-ribose) polymerase, MGMT = methylguanine methyltransferase, RAS = RAT Sarcoma, PE = pseudomonas exotoxin.

^a The enumeration of drugs is not exhaustive.

(strong in two, moderate in five and weak in four samples).⁴⁴ PARP-1 gene amplification was reported in 27% of the samples. Furthermore, genes involved in double-strand break repair were frequently deleted or subjected to loss of heterozygosity. Additionally, PTEN loss (rare in supratentorial childhood HGG) was found to be common in DIPG, which is associated with a worse prognosis in gliomas.^{13,46} Drugable targets are listed in Table 4.

Multiple studies have shown that DIPG differ from non-brain-stem HGG. Barrow et al. showed 17p loss (associated with the loss of p53 in adult HGG) and 14q loss to be much more frequent, and 10q loss to be much less frequent, in DIPG compared to non-brain-stem HGG.⁴² Zarghooni et al. using single nucleotide polymorphism arrays, observed clear differences in the regions of copy number alterations between both groups, indicating a different genetic profile.⁴⁴ An abstract from Puget et al. reports on comparative genomic hybridization and gene expression profiling of 35 frozen DIPG samples. (Puget et al., *Neuro Oncol* 12(6):8-9, 2010 abstract). Gains of chromosome 1q (34%), Xq (25%), 2p and 7p (22%), losses of chromosomes 14q (31%), 10q (28%) and 17p (25%), and amplifications for numerous genes including PDGFR α locus, RNNH1, LRP1 and MET, were found. A molecular subgroup characterized by the appearance of gains or amplifications in PDGFR α showed a shorter survival compared to a subgroup characterized by angiogenic and adhesion genes.

Discussion

The prognosis of DIPG has not improved during the past six years. Only one study, by Frappaz et al. clearly showed an improvement in survival duration: pre-irradiation therapy consisting of high-dose methotrexate, BCNU, cisplatin and tamoxifen until progressive disease occurred, then followed by radiotherapy.¹² In that study, the improved survival may have been influenced by the relatively long duration of symptoms (60 days), which may indicate a less aggressive tumor type. The drug combination may be potential therapy; however, high-dose MTX and tamoxifen separately did not improve survival in previous studies.^{23,47,48} Another explanation is that radiotherapy retains its activity in patients developing progressive disease under chemotherapy. It should be noted that Frappaz et al. reported a prolonged hospitalization duration compared to historical controls (57 versus 25 days).¹² In addition, it is less likely that this approach of consecutive treatment elements will eventually lead to cure: there was no increase of long-term survivors in their study.¹² However, the strategy of combining drugs deserves further investigation in future trials.

The high expectations of temozolomide could not be realized. It has been hypothesized that temozolomide resistance is due to unmethylated O6-methylguanine DNA methyltransferase (MGMT), but this does not seem to apply to DIPG, since Zarghooni et al. did not find MGMT expression in any of their eleven DIPG samples.⁴⁴

Although biopsies have been reintroduced in some hospitals, this is not yet regular policy. The attitude towards collection of tumor tissue via biopsy and autopsy needs to change. Parents should become more involved with the pros and cons of these procedures, which raise challenging ethical and technical questions. By means of biopsies and autopsies, new potential treatment targets in DIPG have been identified, such as PDGFR α . Drugs targeting the downstream pathway phospho-mammalian target of rapamycin are also of interest. Single-agent therapy with the PDGFR inhibitor imatinib did not improve prognosis, but in that study tumor tissue was not obtained and no PDGFR α gene/protein expressing subgroup could be identified.²⁵ The high rate of ITH in the imatinib trial warrants investigating other PDGFR α inhibitors, such as dasatinib.²⁵ ITH was a commonly observed phenomenon in targeted therapy trials in DIPG, necessitating intensive monitoring in future trials. In addition, PARP-1 is an interesting target in DIPG. As was shown by Zarghooni et al., DIPGs harbour multiple deficits in double-strand break repair genes, either by loss of heterozygosity or deletion. As in other tumor types that have loss of these repair mechanisms, PARP inhibition could potentiate synthetic lethality in DIPG.⁴⁹ PTEN loss, in this respect, has also been related to deficient DNA double-strand breaks repair.⁵⁰ EGFR may still be a rational target since it is highly expressed in a subgroup of DIPG patients, and gene amplification is present in a subset of patients. However, single-agent EGFR-inhibition by nimotuzumab, erlotinib or gefitinib has not improved the prognosis. Expression of VEGFR, which is known to stimulate angiogenesis and is frequently overexpressed in adult gliomas, has not yet been investigated in DIPG.⁵¹ Also, Ras mutations have not yet been studied in DIPG. Adult gliomas rarely show Ras mutations, but aberrations in several tyrosine kinase receptors can activate Ras; therefore, Ras is attractive for targeting. Indeed, human glioma cell lines overexpressing EGFR showed enhanced response rates to farnesyl transferase inhibitors regardless of Ras mutational status.⁵²

In general, the complex biology and drug resistance of these tumors render an unselected single-agent approach less likely to be effective. Instead, a multi-targeted approach seems to be required to improve the prognosis. We believe that treatment stratification based on target expression is debatable. As in adult cancer, wild-type target expression often does not correlate with survival. Auto-phosphorylated or mutated proteins are considered to be better targets for therapy. Furthermore, the heterogeneity of gliomas with

target determination based on a single biopsy sample imposes the risk of decisions being made that either overestimate/underestimate the effect of a targeted therapy or wrongly include/exclude patients from treatment strata. Reduction of this potential sample error, by obtaining multiple samples, should be advocated. The small number of patients also hampers molecular target stratified trials, emphasizing the need for larger studies conducted by collaborative groups.

A major challenge in DIPG is drug distribution. Penetration of drugs in DIPG seems to be poor, likely due to an intact blood–brain barrier and/or high pressure in the pons. This is illustrated by the lack of gadolinium enhancement in at least 50% of the patients with DIPG.⁵³ The question of drug penetration may be answered by PET imaging of drug-labelled positron emitters. This technique enables to monitor drug distribution in multiple adult cancers.^{54–56} Moreover, labelling monoclonal antibodies and small molecules will give insight into expression of biological targets which may lead to effective personalized treatment and help prevent the administration of inactive drugs with their accompanying side effects.⁵⁷ From a therapeutic point of view, improving tumor drug distribution is crucial. This may be accomplished either by disrupting the blood–brain barrier or with permeability-increasing agents (e.g. mannitol, morphine or (met)amphetamine).^{58–60} Another opportunity is local delivery, including local tumor injection and convection enhanced delivery, enabling high drug concentrations in the pons, including drugs that normally do not pass the blood–brain barrier.^{34,61} Although not yet under study in DIPG, nanoparticles may play an important role in local drug delivery drugs in the future.^{62,63}

Finally, it is emphasized that international collaboration in pre-clinical and clinical research is essential in order to accelerate progress in the acquisition of knowledge and, ultimately, improvement of the prognosis in DIPG. Recent collaborative efforts have resulted in the establishment of a European DIPG Network, focusing on centralization of clinical data, standardization of imaging criteria and the collection of tumor tissue for European research projects.

Conclusion

No clear improvement in survival has been achieved in DIPG during recent years. Trials still show a wide variation in their inclusion criteria. However, with ever-increasing biological data from in vitro studies, genome-wide analyses and in vivo models, a better basis for future clinical trials has been established. Translation of this knowledge into clinical trials in combination with improved drug distribution and response prediction methods may lead to more effective treatment of this devastating disease.

Conflict of interest

None declared.

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