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Phase 2 trial of dasatinib in target-selected patients with recurrent glioblastoma (RTOG 0627)

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See the editorial by Schiff and Sarkaria, on pages 910–911.

Background. We conducted a phase II trial to evaluate the efficacy of dasatinib, a multitargeted tyrosine kinase inhibitor, for adults with recurrent glioblastoma (GBM).

Methods. Eligibility requirements were Karnofsky performance status ≥60%; no concurrent hepatic enzyme-inducing anticonvulsants; prior treatment with surgery, radiotherapy, and temozolomide exclusively; and activation or overexpression of ≥2 putative dasatinib targets in GBM (ie, SRC, c-KIT, EPHA2, and PDGFR). Using a 2-stage design, 77 eligible participants (27 in stage 1, if favorable, and then 50 in stage 2) were needed to detect an absolute improvement in the proportion of patients either alive and progression-free patients at 6 months (6mPFS) or responding (any duration) from a historical 11% to 25%.

Results. A high rate of ineligibility (27%) to stage 1 precluded a powered assessment of efficacy, but there was also infrequent treatment-related toxicity at 100 mg twice daily. Therefore, the study was redesigned to allow intrapatient escalation by 50 mg daily every cycle as tolerated (stage 1B) before determining whether to proceed to stage 2. Escalation was tolerable in 10 of 17 (59%) participants evaluable for that endpoint; however, among all eligible patients (stages 1 and 1B, $n = 50$), there were no radiographic responses, median overall survival was 7.9 months, median PFS was 1.7 months, and the 6mPFS rate was 6%. The clinical benefit was insufficient to correlate tested biomarkers with efficacy. The trial was closed without proceeding to stage 2.

Conclusions. Intraparticipant dose escalation was feasible, but dasatinib was ineffective in recurrent GBM. Clinical trials.gov identified. NCT00423735 (available at http://clinicaltrials.gov/ct2/show/NCT00423735).

Keywords: chemotherapy, dasatinib, glioblastoma, phase II, tyrosine kinase inhibitor.

Glioblastoma (GBM) has a poor prognosis after recurrence, and median overall survival (OS) is 4–6 months. Cytotoxic chemotherapy such as carmustine controls growth for ≥6 months in only 10%–20% of patients.[1] Small molecule inhibitors are also generally ineffective.[2–4] Bevacizumab, either alone or with irinotecan, is associated with 6-month progression-free survival (6mPFS) and response rates of ∼40%.[5,6] However, there are concerns that bevacizumab may induce more invasive and treatment-refractory tumor biology.[7,8] Recent results have also demonstrated that bevacizumab does not prolong OS when added to radiotherapy and temozolomide as part of first-line therapy.[9,10] Therefore, an opportunity remains

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for testing novel agents at recurrence in the bevacizumab-naïve setting.

GBM is a molecularly complex disease. Potential explanations for lack of efficacy include inadequate target inhibition or improper patient selection. In addition, treatment failure with single agents that target one signaling abnormality may result from the uninhibited actions of other "bypass" molecular abnormalities or from the need to target more than one oncogenic signal simultaneously. Therefore, treatment with a single agent that could target several key signaling pathways, especially in the appropriate patient population, represents an attractive therapeutic approach. Dasatinib (Sprycel, Bristol-Myers Squibb, previously BMS-354825) is such an agent.

Dasatinib has inhibitory effects on at least 5 kinase families involved in human malignancies: SRC, KIT, PDGFR, and EPHA2, and *BCR-ABL* fusion.[11–13] It is also FDA approved for *BCR-ABL* mutant hematological malignancies. Although *BCR-ABL* is not implicated in GBM, the other 4 targets may contribute to GBM progression or therapeutic resistance. For example, the majority of GBMs exhibit amplification/overexpression of SRC (~60%), PDGFR (~75%), and ephrin (~90%); ~50% exhibit c-KIT amplification.[14–17] Mouse modeling by transgenic and somatic-cell gene transfer methods have further confirmed the importance of both SRC and PDGFR signaling in gliomagenesis as reviewed elsewhere.[18] In addition, although imatinib is ineffective in GBM,[19,20] dasatinib inhibits PDGFR more potently than imatinib.[12,21] Preclinical data also suggest that dasatinib may be effective in glioma.[22] Therefore, we hypothesized that dasatinib might be more effective for recurrent GBMs than other receptor tyrosine kinase inhibitors, including imatinib, because of its broader spectrum of molecular targets and increased potency against key targets.

We conducted a single-arm phase II trial of dasatinib as monotherapy, initially at 100 mg twice daily, for patients with bevacizumab-naïve recurrent GBM harboring overexpression/activity of SRC, PDGFR, EPHA2, and/or c-KIT, following radiotherapy and temozolomide.[23]

Materials and Methods

Eligibility Criteria

Major eligibility criteria included GBM (or subtype) histology confirmed centrally (by K.D.A.); prior treatment with surgery, radiotherapy, and temozolomide only; worsening disease by imaging or histological confirmation; Karnofsky performance status $\geq$ 60%[24]; age $\geq$18 years; and normal end-organ function (hepatic, renal, bone marrow; Supplementary material, Table S1). Concurrent use of H2 blockers or proton pump inhibitors was prohibited because of the potential effects on stomach pH and drug absorption. Patients taking antiplatelet agents, anticoagulants, or nonsteroidal anti-inflammatory drugs were excluded because of concerns about increased bleeding risk. Use of hepatic CYP450 enzyme-inducing antiepileptic drugs (EIAEDs) was also prohibited for $\geq$ 2 weeks before registration because of potential effects on dasatinib metabolism. Participants of child-bearing potential agreed to use contraception, and women were neither pregnant nor nursing mothers. Availability and testing of pretreatment tumor tissue was also required (described below). All participants (or appropriate representatives) signed a study-specific informed consent form approved by the local Institutional Review Board (or equivalent body) at the participating institution. The protocol was also approved by the American College of Radiology Institutional Review Board. This protocol is registered with clinicaltrials.gov (identifier NCT00423735).

Treatment

Dasatinib was initiated at 100 mg twice daily until disease progression or intolerable toxicity. A cycle was defined as 28 days, although treatment was continuous. Baseline evaluations included physical examination, blood chemistries, complete blood count, electrocardiogram, and brain imaging with contrast-enhanced MRI (or CT for patients unable to tolerate MRI). Evaluations during treatment included physical examinations every other week, complete blood counts and serum chemistries weekly, electrocardiograms as clinically indicated every cycle, and follow-up brain imaging every other cycle. The primary endpoint was a hybrid of 6mPFS rate and radiographic response rate (either 6mPFS or response of any duration). Objective responses were assessed by the Macdonald criteria.[25] Partial response was defined as $\geq$ 50% decrease in size of enhancing tumor on consecutive brain MRI (or CT for those unable to tolerate MRI) scans at least 1 month apart, stable or reduced corticosteroid dosing, and no neurological deterioration. Complete response required total disappearance of all enhancing tumor on consecutive MRI (or CT) scans at least 1 month apart, discontinuation of corticosteroids, and no neurological deterioration. Progressive disease was defined as $\geq$25% increase in the size of enhancing tumor or any new tumor; or clinical progression not attributable to another cause. Other responses were classified as stable (eg, tumors between 50% smaller and 25% larger).

Toxicity was initially assessed by the National Cancer Institute (NCI) Common Toxicity Criteria for Adverse Events (CTCAE) version 3.0. For agent-related (possibly, probably, or definitely) intolerable or severe toxicities, dose reductions were permitted to a minimum of 70 mg once daily. Dasatinib was supplied by the Pharmaceutical Management Branch of the NCI under a collaborative agreement with Bristol-Myers Squibb. Chemotherapy reviews were performed on all cases (A.B.L.).

Statistical Design

The trial was initially conceived as a single-arm phase 2 trial using a 2-stage design[26] with a combined 6mPFS and response (either was sufficient to reduce delay in proceeding to stage 2 if responses were observed in stage 1) rate of 25% considered promising and requiring 77 eligible patients to achieve 95% power for detecting an increase over the estimated historical combined 6mPFS and response rate of 11% associated with ineffective therapy. Kaplan-Meier methodology was used to estimate median PFS and OS.[27] Stage 1 planned to accrue 27 eligible patients. If $\geq$3 of 27 eligible patients in stage 1 achieved either 6mPFS or a radiographic response, then stage 2 would accrue 50 additional eligible participants.

Tissue Analyses

Among the multiple targets inhibited by dasatinib, there are at least 4 known targets of major importance in GBM biology: SRC,

PDGFR-alpha, EPHA2, and KIT. We hypothesized that absence of expression or activity of these targets in tumor tissue would reduce the likelihood of benefit. Therefore, expression/activation of at least 2 of these potential targets was required for eligibility in an attempt to exclude those participants least likely to benefit. Using commercially available antibodies (eg, anti-PDGFR and antiphospho-PDGFR), immunohistochemistry (IHC) was performed and immunostaining was scored on a 4-point scale (0–3) analogous to that developed by others.[28] Conditions for IHC of paraffin-embedded sections were applied as described previously.[29] Antibodies and dilutions were as follows: Kit (Lab Vision # RB- 9038-R7, 1:500); EphA2 (Santa Cruz # sc 924, 1:200); p-Src (Tyr527, Cell Signaling # 2105, 1:100); and PDGFR-apha (Cell Signaling # 3164, 1:200). Tumors were separated into upper and lower halves based on target expression with the lower half having no or mild expression (ie, score 0–1) and the upper half having strong expression (ie, score 2–3). For each target, an IHC staining score of 2–3 was considered positive. Overall, staining was dichotomized as positive and negative based on an overall view of the tumor tissue relative to controls and established staining patterns specific to each protein. A score of positive required, at a minimum, focal robust and clear positivity of a substantial proportion of the tumor available for study. If the pattern was restricted to weak and diffuse staining only, this was not considered positive for this study. Appropriate positive and negative controls were included in each batch of immunohistochemical staining.

Pharmacokinetic Analyses

Blood samples for dasatinib pharmacokinetics (stage 1B only, described below) were collected in EDTA-anticoagulated vacutainers on cycle 1 day 1, cycle 3 day 1, and cycle 5 day 1. Samples were taken before, and 1, 2, 4, and 6–8 hours after the morning dose of dasatinib. Plasma was prepared by centrifugation and immediately frozen at −20°C. Plasma concentrations of dasatinib were quantitated with a validated LC-MS/MS assay as previously described.[30]

Plasma pharmacokinetic parameters, including area under the concentration versus time curve (AUC), of dasatinib were extracted from the data by noncompartmental methods with PK Solutions 2.0 (Summit Research Services).

Results

Stage 1

There were 21 patients (10 men, 11 women) who met both the clinical and molecular eligibility criteria. Median age was 51 years (range, 33y–81y, Table 1). Molecular analyses revealed that among the eligible patients, 8 (38%) had tumors that harbored 2 putative dasatinib targets, 10 (48%) had 3 targets, and 3 (14%) had all 4 targets (Table 2). However, among 29 participants registered, 8 were ineligible because of exclusionary concurrent medications, prior therapy, or laboratory results. Therefore, underaccrual of eligible patients (21 rather than the 27 planned) precluded the preplanned, appropriately powered assessment of efficacy. Moreover, toxicity was also much milder than anticipated with neither pleural effusions (reported as a concerning agent-related toxicity in other cancers) nor grade 4–5 agent-related toxicities (Table 3 and Supplementary material, Table S2). This led to the concern that participants were underdosed. Therefore, the protocol was amended to allow intrapatient dose escalation through stage 1B rather than reopening stage 1 to complete accrual of 27 eligible patients using the same dosing schedule that was potentially inadequate and before opening stage 2 with 50 additional participants with insufficient evidence of efficacy. In this design, participants would escalate dosing by 50 mg per day per cycle up to a maximum of 400 mg total per day, absent intolerable toxicity. Therefore, cycle 1 consisted of 100 mg twice daily; cycle 2 was 100 mg in the morning and 150 mg at night, cycle 3 was 150 mg twice daily; etc. Pharmacokinetic

Table 1. Pretreatment characteristics

	Stage 1 ($n = 21$)	Stage 1B ($n = 29$)
Age (years)		
Median	51	54
Min–Max	33–81	26–75
<50	9 (43%)	8 (28%)
≥50	12 (57%)	21 (74%)
Sex		
Male	10 (48%)	17 (59%)
Female	11 (52%)	12 (41%)
Race		
Asian	0 (0%)	1 (3%)
Black or African American	1 (5%)	2 (7%)
White	20 (95%)	26 (90%)
Ethnicity		
Hispanic or Latino	3 (14%)	1 (3%)
Not Hispanic or Latino	18 (86%)	23 (79%)
Not reported	0 (0%)	5 (17%)
Karnofsky performance status		
60%–80%	15 (71%)	11 (38%)
90%–100%	6 (29%)	18 (62%)
Neurological symptoms		
None	3 (14%)	9 (31%)
Minor	9 (43%)	14 (48%)
Moderate but fully active	6 (29%)	1 (3%)
Moderate but required assistance	3 (14%)	5 (17%)
Initial extent of resection		
Biopsy	2 (10%)	1 (3%)
Subtotal resection	10 (48%)	9 (31%)
Gross total resection	9 (43%)	18 (62%)
Other	0 (0%)	1 (3%)
Additional Surgery		
None	15 (71%)	22 (76%)
Subtotal resection	2 (10%)	3 (10%)
Gross total resection	4 (19%)	4 (14%)
Corticosteroids		
No	5 (24%)	15 (52%)
Yes	16 (76%)	14 (48%)
Anticonvulsants (non-enzyme inducing)		
No	9 (43%)	8 (28%)
Yes	12 (57%)	21 (72%)

Table 2. Pretreatment molecular analysis among eligible patients

	Stage 1 ($n = 21$)	Stage1B ($n = 29$)
p-SRC		
No staining - negative	12 (57%)	8 (28%)
Strongly positive	9 (43%)	21 (72%)
PDGFR		
No staining - negative	10 (48%)	14 (48%)
Mild/moderate - positive	1 (5%)	0 (0%)
Strongly positive	10 (48%)	15 (52%)
EPHA2		
No staining - negative	2 (10%)	5 (17%)
Strongly positive	19 (91%)	24 (83%)
c-KIT		
No staining - negative	2 (10%)	7 (24%)
Strongly positive	19 (91%)	22 (76%)
Number of positive molecular markers		
2	8 (38%)	10 (35%)
3	10 (48%)	14 (48%)
4	3 (14%)	5 (17%)

Table 3. Summary of worst dasatinib-related adverse event per participant

Adverse Event	CTCAE Grade	Stage 1 ($n = 21$)	Stage 1B ($n = 29$)
Worst nonhematological	1	4 (19%)	6 (21%)
	2	8 (38%)	12 (41%)
	3	8 (38%)	5 (17%)
	4	0 (0%)	1 (3%)
	5	0 (0%)	0 (0%)
Worst overall	1	0 (0%)	5 (17%)
	2	8 (38%)	11 (38%)
	3	12 (57%)	8 (28%)
	4	0 (0%)	1 (3%)
	5	0 (0%)	0 (0%)

Includes adverse events in which relationship to protocol treatment is missing.

analyses were conducted. Accrual would continue to stage 2 (with 50 additional participants) if both escalation could be achieved safely (defined as a majority experiencing no dose-limiting toxicity during escalation) and if ≥ 3 of 27 eligible participants achieved 6mPFS or radiographical response. Because escalation would first occur during cycle 2, participants considered evaluable for escalation were those who completed ≥ 2 cycles without progression (or death) or those who discontinued after ≤ 2 cycles because of toxicity.

Stage 1B

Twenty-nine eligible participants were accrued. Pretreatment characteristics were similar to those in stage 1 (Table 1). Intrapatient dose escalation was feasible: 10 (59%) participants escalated among 17 evaluable for that endpoint. Toxicity was also limited, although toxicities were more common than in stage 1 (Table 3 and Supplementary material, Table S2). The highest dose achieved was 350 mg per day (150 mg in the morning and 200 mg evening). However, efficacy was limited (Fig. 1, Table 5). Only 2 participants achieved 6mPFS, and there were no partial or complete responses. Therefore, accrual was terminated without proceeding to Stage 2.

All 29 eligible participants in Stage 1B were studied for pharmacokinetics and had sufficient data to estimate all pharmacokinetic parameters on cycle 1day 1; parameters of 4 participants could be estimated on cycle 3 day 1, and one participant could be estimated on cycle 5 day 1 (Supplementary material, Fig. S1). The portion of the AUC extrapolated beyond the last time point was 17% on average (range, 6%–42%). Pharmacokinetic parameters on cycle 1 day 1 are presented in Table 4. The low number of participants with repeated pharmacokinetic sampling precluded a formal comparison of pharmacokinetic behavior over time.

Combined Results

Molecular analyses were performed on 94 potentially eligible patients. Immunostaining demonstrated that 2 (2%) participants harbored none of the putative dasatinib targets, 10 (11%) harbored 1, 22 (23%) harbored 2, 45 (48%) harbored 3 (including one not able to be tested for c-KIT), and 15 (16%) harbored all 4. Therefore almost all (83 participants, 88%) met the molecular criteria.

Best response among the 50 eligible participants (combined stages 1 and 1B) was stable disease in 12 (24%) and progression in 36 (72%). There were no responses. Median OS (Table 5, Fig. 1) was 7.9 months (95% CI, 5.6–10.2 months), median PFS was 1.7 months (95% CI, 1.3–1.9 months), and the 6mPFS rate was 6.0% (95%, 0%–12.8%).

Discussion

Dasatinib failed to demonstrate efficacy as monotherapy for recurrent GBM despite attempts to enrich the population and increase the dose. Two phase I trials tested dasatinib in combination with lomustine (which was excessively toxic)[31] or erlotinib (well tolerated),[32] and neither demonstrated any therapeutic benefit. A retrospective study showed limited toxicity but no activity when dasatinib was combined with bevacizumab following bevacizumab failure. The Alliance for Clinical Trials in Oncology recently completed accrual to a trial (NCCTG N0877) adding dasatinib to radiotherapy and temozolomide for newly diagnosed GBM, and results are pending.

It is possible that we observed both limited efficacy and toxicity because of inadequate dosing despite the relatively high starting dose of 100 mg twice daily (FDA approved dose is 100–140 mg once daily). However, pharmacokinetic results do not support this conclusion. For example, the dasatinib AUC and Cmax values observed were ~400 ng·h/mL and 120 ng/mL, respectively, which is higher than published data from other studies.[13] Dasatinib exposure is known to be quite variable within and between patients, with coefficients of variation of up to 100% for both AUC and Cmax.[13,33] It is therefore difficult to assess the pharmacokinetic effects observed in this

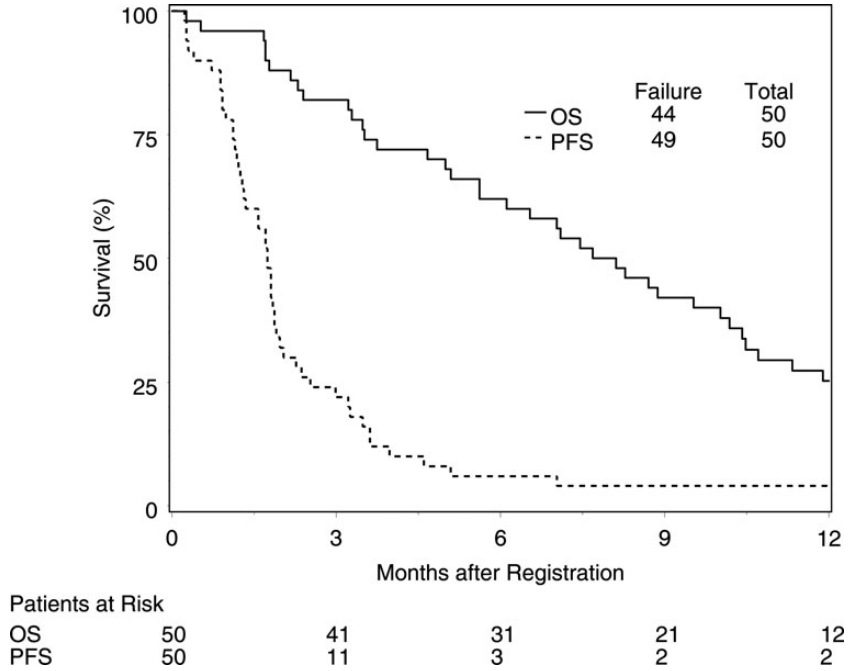

Fig. 1. Kaplan-Meier survival curves of all eligible patients (stage 1 + 1B combined). OS, overall survival; PFS, progression-free survival.

Table 4. Plasma dasatinib pharmacokinetics on cycle 1 day 1 after 100 mg of oral dasatinib

PK Parameter	Unit	Mean (CV%)	Median	Geometric Mean	Published Geometric Mean (CV%)[13]
C_{max}	ng/mL	151 (74)	144	120	56 (118)
T_{max}	h	1.5 (63)	1.0	1.3	1.5
$t_{1/2}$	h	2.2 (42)	1.7	2.0	4.3 (40)
$AUC_{0\text{-inf}}$	ng·h/mL	458 (53)	393	391	218 (102)
Cl/F	L/h	318 (80)	255	256	667 (81)
Vd/F	L	1030 (106)	655	753	4224 (84)

Abbreviation: CV%, coefficient of variation.

Table 5. Survival

	Stage 1	Stage 1B	Overall
Median PFS (95% CI)	1.7 months (1.0–2.0)	1.8 months (1.2–2.0)	1.7 months (1.3–1.9)
Median OS (95% CI)	6.5 months (3.5–9.5)	8.9 months (5.0–11.3)	7.9 months (5.6–10.2)
6mPFS rate (95%) CI)	4.8% (0–14.7)	6.9% (0–16.7)	6.0% (0–12.8)

Abbreviations: OS, overall survival; PFS, progression-free survival; 6mPFS, 6-month progression-free survival; CV%, coefficient of variation.

study in more than a semiquantitative fashion. The Cmax was approximately triple that of published levels (Table 4), yet the AUC was only double because the half-life was also shorter (2.0 vs 4.3 h geometric mean) than prior reports. Dasatinib discontinuation or dose reduction because of disease progression or toxicity precluded collection of sufficient samples following dose escalation above the starting dose to make meaningful conclusions about the effects on AUC or Cmax. Despite the high Cmax and AUC, it is possible that toxicities were masked by concurrent use of corticosteroids, which is more common in patients with gliomas (Table 1) than other cancers. This trial did not incorporate a surgical arm through which participants received treatment before tumor resection. Therefore, it is possible that tumor penetration into brain was insufficient for

antineoplastic effect despite increased daily dosing. Preclinical data suggested that p-glycoprotein and related molecules limit accumulation of dasatinib into the brain, and brain tumors, through active efflux.[34,35]

We required activation or overexpression of 2, 3, or 4 of 4 putative dasatinib targets in an attempt to enrich the population for those most likely to benefit. Based on literature estimates of expression of each marker, we hypothesized that only 50% of participants who were clinically eligible would also meet this molecular eligibility criterion. However, 2, 3, or 4 of 4 putative dasatinib targets were detected in nearly all (88%) tumors. Therefore, it was not an effective strategy to preselect enrollment. A more effective approach may have been to require expression of all 4 of 4 targets in archival tissue. It is also possible that restricting accrual to cases with only a single, presumably driver target with significant signaling activity would have proven more effective. Finally, the molecular profile of dasatinib targets in archival tumor resected at GBM diagnosis may differ from the profile at disease recurrence. Absence of any responses precluded correlation with the tumor molecular profile, although there was no suggestion of correlation between the number of positive markers observed in those with stable disease versus those with progressive disease (Supplementary material, Table S3).

It is also possible, if not likely, that wild-type target expression (regardless of number or level) may be insufficient for tumor response. For example, *EGFR* amplification does not correlate with response to EGFR tyrosine kinase inhibitors.[36] Instead, response to dasatinib in GBM may require as yet unidentified driver mutations in dasatinib targets, analogous to *BCR-ABL* fusion in leukemia or kinase mutations in other cancers as reviewed elsewhere.[37] For example, mutations in *discoidin domain receptor-2* (*DDR2*)[38,39] and *BRAF*[40] were recently reported to predict response to dasatinib in lung cancer but were unknown when this trial accrued. A phase 2 trial is currently in progress (NCT01514864) for patients with non-small cell lung cancer harboring these specific mutations. If any future glioma studies of dasatinib are conducted, it would be prudent to consider prescreening for *DDR2* or *BRAF* mutations in addition to increased daily or intermittent dosing[41] to increase brain penetration.

Despite these limitations, we were able to escalate the dasatinib dose and perform centralized molecular pre-screening in all patients in real time without delaying registration in a multicenter cooperative group setting.

Supplementary Material

Supplementary material is available at *Neuro-Oncology Journal* online (http://neuro-oncology.oxfordjournals.org/).

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