nature portfolio

Peer Review File

Targeted therapies plus radiotherapy for diffuse intrinsic pontine glioma: the randomized phase 2 BIOMEDE trial platform

Corresponding Author: Professor Marie-Anne Debily

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Attachments originally included by the reviewers as part of their assessment can be found at the end of this file.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
review is attached.

Reviewer #2

(Remarks to the Author)
The authors eloquently describe the findings of the BIOMEDE randomized phase 2 trial, and although the trial may not have reached its primary endpoint, the results described in the manuscript provide novel and invaluable insights about the molecular profiles of DIPGS that could influence response to treatment and patient outcomes.

A few questions/suggestions:

One of the major strengths of the paper is the in-depth molecular characterization of the tumors and the association of these molecular profiles with outcomes. The data collated in this paper would greatly add to the existing literature. The authors may consider highlighting this aspect of the paper in the introduction as it appears to be the primary focus of the results/discussion sections.

Could the authors explain how the misplacement of a gastrostomy that led to a patient death was a treatment-related complication?

Could the authors please expand upon the neurological adverse events seen with treatment?

Could the authors include whether patients had clinical improvement in their symptoms with treatment in each of the cohorts? Duration of improvement? Any differences between long-term and short and intermediate survivors?

Were the patient imaging responses measured by RANO 2.0 or another imaging criteria? What were the imaging responses to treatment (i.e.-PD, PR, SD, CR?)

Are the MRI images in FigS5 supposed to represent the patient with the complete response? If so, there still appears to be hyperintense signal in the pons on MR that could be indicative of tumor. Please clarify.

Given the recent FDA approval of dordaviprone, it may be of interest to the audience if the authors could potentially provider more information on how the two drugs may interact with one another.

Could the authors expand on how everolimus could potentially modulate a positive T-cell response with CAR T cell therapy? Would the authors suggest everolimus be utilized as part of a multimodal treatment regimen then?

Reviewer #3

(Remarks to the Author)
In this manuscript, Debily, Le Teuff, Kergrohen, Grill, and colleagues document the results of the BIOMEDE trial, a phase 2 platform trial of targeted therapies with radiation for DIPG. Overall, this is an important study and well written paper. Although there was no survival benefit shown for any of the arms compared to each other or a historical control, the group documents important findings about potential benefit and biomarkers for MTOR inhibitors in DIPG, as well as a series of validations and novel findings on molecular alterations in this disease. This is an original study of significance to the field. This is a rich dataset of high quality, and the methodology overall is sound. The figures are well presented overall. The statistical analysis and treatment of uncertainty is appropriate. The conclusions are valid overall and based on robust findings. My specific comments on suggested improvements are below by section.

There are significant language issues that merit editing by a science writer.

Abstract:
- Please state briefly how treatment was decided for each subject.
- Please state the nature of the control cohort (historical), and please explain the planned randomization more completely or not at all; I would not say that drugs are randomized but instead that patients are randomized to a drug or treatment arm.

Introduction
- Please briefly discuss the evolution of more recent nomenclature of DMG in the last two WHO editions, the difference between DIPG and DMG, and why the diagnosis and terminology of DIPG is used in this trial and manuscript.
- Please clarify that this oncogenic mechanism is not universal in posterior fossa ependymoma.

Results
- In Figure 1, please clarify in the caption or figure how the randomizations led to the comparisons; the color coding and distribution in 1a is confusing. In 1b, where are the patients with neither marker who received dasatinib in this diagram? I understand they were not included in the randomization, but this diagram is for the entire trial.
- The two-sided alpha level of 20% seems unusual and should be explained further.
- How would 250 patients in the trial be enough to ensure 90 patients in the smallest comparison if there are three comparisons?
- Please define/cite pseudoprogression.
- Please give more information on eligibility criteria: age, localized vs metastatic disease, performance status, etc.
- Why would patients without information on biomarkers be included in the randomization, i.e. how are they different from patients who were negative for the two biomarkers?
- It would be helpful to have a table or figure showing the data on biopsy complications in lines 194-5.
- The caption for Figure 2 repeats the caption for 2d and confusingly has “f” there twice.
- It would be helpful to have more details on the toxicities observed, and these statistics are confusing, e.g. the manuscript states that eye toxicities were less frequent with everolimus but does not say what type of eye toxicities were observed, and the rate of eye toxicity in everolimus and dasatinib appears nearly equal.
- What tumors are included in the comparison COSMIC database? Also, what does 100% refer to in this SNV section – 100% of what?
- BRAF mutations have been associated with better survival in DMG, but I don’t see these reported in this cohort – were these observed?
- It would be helpful to have a panel demonstrating the findings in lines 402-7, which are interesting and important.
- In line 466, please clarify what is meant by “genes involved in Pi3K … signaling were significantly decreased” – expression? Alterations?

Discussion
- I think it would be better to state the survival in months for the comparisons in lines 526-8 instead of making these qualitative statements with no statistical analysis.
- Please use either the term dordaviprone (preferably) or ONC201 after equating them in line 545, not both, for clarity.

Methods
- What were the cutoffs used for EGFR and PTEN expression used?
- Starting in line 786, the future tense is used, suggesting these paragraphs were taken perhaps from a grant proposal or the trial protocol.

References
- These are appropriate and complete.

[redacted]

Version 1:

Reviewer comments:

Reviewer #2

(Remarks to the Author)
The authors have done a thorough and comprehensive job of addressing the reviewers' comments, and the changes have strengthened the manuscript.

Reviewer #3

(Remarks to the Author)

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Dear Reviewers,

Reviewer #1 (Remarks to the Author):

We thank Reviewer #1 for his positive comments about our manuscript.

With respect to specific questions raised by Reviewer #1,

R1Q1

Minor comment: "Fisher exact test" was wrongly typed as "fischer exact test" on page 34.

Reply: We have corrected the typo in all the occurrence of the term (lines 190, 225, 228, 848, 855, 961, 968, 1424 and 1478.

R1Q2

Major comment: Given the nature this trial is an exploratory trial and not a confirmatory trial, the analysis plan presented in this article is appropriate. However, the descriptions of "statistical analysis plan"on page 33 could use more details to boost up its statistical rigor.

Reply: The paragraph on the statistical methodology for the biomarker study has been rewritten in the online Methods section (lines 1466 to 1486). We describe how we have separated the population of the study; population was stratified to perform the exploratory analyses: three groups were defined for overall survival (less than 1 year, between 1 and 2 years and above 2 years). Additional details for each analysis are also provided in the figure legends.

Reviewer #2 (Remarks to the Author):

The authors eloquently describe the findings of the BIOMEDE randomized phase 2 trial, and although the trial may not have reached its primary endpoint, the results described in the manuscript provide novel and invaluable insights about the molecular profiles of DIPGS that could influence response to treatment and patient outcomes.

A few questions/suggestions:

We thank Reviewer #2 for comments that helped improve the manuscript. Below, we address the specific questions and suggestions raised.

R2Q1

One of the major strengths of the paper is the in-depth molecular characterization of the tumors and the association of these molecular profiles with outcomes. The data collated in this paper would greatly add to the existing literature. The authors may consider highlighting this aspect of the paper in the introduction as it appears to be the primary focus of the results/discussion sections.

Reply: We thank the Reviewer 2# for recognizing the value of the molecular information integrated with the clinical outcomes of the trial. We have revised the last paragraph of the Introduction to emphasize the in-depth molecular characterization of tumors and the association between molecular profiles, treatment response, and outcomes. We also clarify the different exploratory objectives of the biomarker analyses with respect to treatment outcome (lines 111 to 115).

R2Q2

Could the authors explain how the misplacement of a gastrostomy that led to a patient death was a treatment-related complication?

Reply: We thank the reviewer for this comment. This sentence was indeed erroneous. This patient's death was classified by both the treating physician and by the pharmacovigilance unit as a complication of the gastrostomy and not related to the study drug. The text was revised as follows: "*No patient died from study treatment related complications."* (line 217)

We also added a sentence describing treatment discontinuation due to toxicity: *"Treatment was stopped because of toxicity in 20%, 3% and 14% for erlotinib, everolimus and dasatinib respectively (Fisher Exact test, p=0.004)."* (lines 226 to 228)

R2Q3

Could the authors please expand upon the neurological adverse events seen with treatment?

Reply: Adverse events are detailed in Supplementary Table S3 *"Distribution of the maximum grade of adverse events (0-2 vs. >=3), by System Organ Class, according to the treatment arm, regardless of causality, over the whole treatment duration"* and summarized in Table Extended data 6.

A sentence has been added to detail the neurological side effects: "*Irrespective to the imputability to the treatment or the disease, grade 3 or above neurological side effect reported did not differ in their frequency among treatments groups (Fisher Exact test, p=0.65) (TABLE S3); the three most frequent were headaches, hydrocephalus and dizziness*." (lines 224 to 226)

R2Q4

Could the authors include whether patients had clinical improvement in their symptoms with treatment in each of the cohorts? Duration of improvement? Any differences between long-term and short and intermediate survivors?

Reply: Among the 226 patients who started study treatment (4 patients did not start study treatment) and for whom clinical evaluation was available (3 had no clinical evaluation reported despite prolonged treatment duration), clinical improvement during treatment was reported in 169 patients (75%), while clinical status was stable in 44 (19%) and worsened in 13 (6%). As detailed in the table below, the distribution of best clinical response did not differ significantly between treatment groups (Fisher exact test, p=0.315).

Best clinical response Treatment group	**Improved**	**Stable**	**Worse**	**Total**
Erlotinib	**26 (74%)**	**8 (23%)**	**1 (3%)**	**35**
Everolimus	**72 (79%)**	**12 (13%)**	**7 (8%)**	**91**
Dasatinib	**71 (71%)**	**24 (24%)**	**5 (5%)**	**100**

Total	**169 (75%)**	**44 (19%)**	**13 (6%)**	**226**

Among the 169 patients with reported clinical improvement, the mean duration of improvement was 3.4 months and the median was 2.1 months. This duration did not differ significantly between treatment groups (Kruskall Wallis test, p=0.744).

Treatment	N obs	Mean	Median	Q1	Q3	Maximum
Erlotinib	**26**	**3.5**	**2.1**	**1.0**	**3.7**	**26.2**
Everolimus	**72**	**4.1**	**2.1**	**1.1**	**3.8**	**53.4**
Dasatinib	**71**	**2.7**	**1.6**	**1.0**	**3.8**	**11.6**

We compared the overall survival curves between the 169 patients who experienced clinical improvement and the 44 patients whose best clinical response was stable disease, excluding patients with worsening symptoms only. Survival curves did not differ significantly between the two groups (log-rank test, p=). Median OS was 11.2 months (95%CI, 10.3-12.3) in patients with clinical improvement *versus* 9.2 months (95%CI, 6.4-10.9) in patients with stable disease.
These findings are summarized in the first paragraph of a new Results section entitled **Response to therapy**: "*Clinical improvement anytime during first line treatment was reported in75% of the patients, whereas the clinical status was stable in19% and deteriorated in 6%. Clinical response did not differ among treatment arms (TableS4). Mean duration of improvement was 3.4 months with no difference between arms. The OS of the patients with clinical improvement (11.2 months;95%CI 10.3-12.3) did not differ from the one of the 44 patients remaining clinically stable (9.2 months; 95%CI 6.4-10.9) (log-rank test).*" (Lines 233 to 238). The first table included in this response to Reviewer #2 has been added to the manuscript as Table S4.

R2Q5
Were the patient imaging responses measured by RANO 2.0 or another imaging criteria? What were the imaging responses to treatment (i.e.-PD, PR, SD, CR?)
Reply: A specific imaging protocol (see details in the protocol provided as an annex) was implemented at pre-specified time-points: at diagnosis, 48 hours post-biopsy, early after completion of irradiation (*i.e.,* during the first week after the last day of radiotherapy), then every two months during treatment, and every three to four months after treatment completion. The imaging protocol accounted for RANO recommendations. To date, imaging data have not yet undergone central review and will be the subject of a separate publication. Here, we report only local radiologist assessments using a simplified reporting scheme (improved, stable or progressive disease).
The best radiological response, as evaluated by local radiologists, was available for 223 patients who started treatment (4 patients did not start study treatment) and had at least one radiological assessment during treatment (6 patients with no radiological evaluation during treatment): radiological improvement was reported in 121 patients (54%), stable disease in 70 (31%) and progression in 32 (14%). As detailed in the table below, the distribution of the best clinical response did not differ significantly between treatment groups (Chi2 test, p=0.402).

Best radiological response Treatment group	Improved	Stable	Progression	Total
Erlotinib	**18 (51%)**	**15 (43%)**	**2 (6%)**	**35**
Everolimus	**49 (54%)**	**27 (30%)**	**15 (16%)**	**91**
Dasatinib	**54 (56%)**	**28 (29%)**	**15 (15%)**	**97**

Total	**121 (54%)**	**70 (31%)**	**32 (14%)**	**223**

We have summarized these findings in the second paragraph of the new Results section entitled **Response to therapy**: "*Radiologic improvement as reported by the treating physician during first line treatment was observed in 121 patients (54%) while the disease remained stable as best response in 70 (31%) or progressed in 32 (14%) with no difference between treatment arms (Chi2 test, p=0.402).*" (lines 239-241).
A sentence was added to this paragraph about the pseudoprogression to address a request from another reviewer: "*Pseudoprogression was reported in 110/233 patients (49%) with no significant difference between arms (Chi2 test, p=0.870).*" (Lines 242-243).

R2Q6
Are the MRI images in FigS5 supposed to represent the patient with the complete response? If so, there still appears to be hyperintense signal in the pons on MR that could be indicative of tumor. Please clarify.
Reply: The imaging of the four long-term survivors is presented here; however, only one patient (BIOMEDE 143) with concomitant H3K27M and IDH1 R132C mutations, achieved complete radiological remission: the circular signal observed on follow-up T2-Flair imaging corresponds to the hole left after the biopsy. Imaging findings are now more thoroughly described for each case in the legend of panel A of ExtendedData9 (lines 1040 to 1051).

R2Q7
Given the recent FDA approval of dordaviprone, it may be of interest to the audience if the authors could potentially provider more information on how the two drugs may interact with one another.
Reply: We have added a sentence in the Discussion regarding the combination of ONC201 with other mTOR inhibitors: "*Although preclinical studies suggest combining mTOR inhibition with ONC201, ONC201 induces cytochrome P450 3A4 and may increase the clearance of CYP3A4 substrates such as everolimus*." Lines 478-480
Most of the available literature indicates that cyt.P450 3A4 inducers should be avoided when using ONC201 (Jaiswal et al, CPT Pharmacometrics Syst Phramacol 2025). While PK modeling suggested no change in cytP450 3A4 midazolam, studies using human liver microsomes have shown that ONC201 is a weak cytP450 3A4 inducer and may therefore interfere with cyt.P450 3A4 substrates.

R2Q8
Could the authors expand on how everolimus could potentially modulate a positive T-cell response with CAR T cell therapy? Would the authors suggest everolimus be utilized as part of a multimodal treatment regimen then?
Reply: Everolimus can modulate T-cell function, as mTOR inhibitors are used to prevent graft *versus* host reaction. It has been discussed in the literature that "*The combination of everolimus with CART cells could modulate positively the T-cell response limiting their exhaustion due to mTOR metabolic activation70.*" However, this hypothesis would require experimental studies.

Reviewer #3 (Remarks to the Author):
We thank Reviewer #3 for their useful comments, which helped us improve the manuscript.
With respect to the specific questions raised:

R3Q1
There are significant language issues that merit editing by a science writer.

Reply: the manuscript was reviewed by two native English-speaking researchers (Chris Jones and David Ziegler).

Abstract:

R3Q2

- Please state briefly how treatment was decided for each subject.

Reply: We thank the reviewer for this suggestion, as the information was not clearly stated. We have added the following sentence: "*Treatment allocation was performed by randomization, designed so that a drug could not be allocated if the corresponding biomarker was absent in the tumor*" (Line 62-63).

R3Q3

- Please state the nature of the control cohort (historical), and please explain the planned randomization more completely or not at all; I would not say that drugs are randomized but instead that patients are randomized to a drug or treatment arm.

Reply: The control cohort is now formally described in the abstract with the following sentence: "*An historical cohort of 66 children with the same inclusion criteria and treated previously with temozolomide-based regimen was used as control*". (Lines 60 to 62). We also revised the wording to remove ambiguity. The sentence now reads : "*Treatment allocation was performed by randomization, designed so that a drug could not be allocated if the corresponding biomarker was absent in the tumor*". (line 62-63).

Introduction

R3Q4

- Please briefly discuss the evolution of more recent nomenclature of DMG in the last two WHO editions, the difference between DIPG and DMG, and why the diagnosis and terminology of DIPG is used in this trial and manuscript.

Reply: We have described in the Introduction recent changes in the definition of theses neoplasms (Lines 79 to 88). DIPG is now considered a DMG, H3K27-altered, located primarily in the pons (Line 87-88). Recent literature indicates that response to therapy in DMG H3K27-altered may differ according to tumor location (see for example the recent paper of Di Carlo et al, Eur J Cancer 2024: PMID: 39700833 on response to ONC201). For clarity, we therefore present only results from patients with radiologically-defined DIPG harboring an H3K27 alteration who were randomized in the trial. Outcomes of DMG located outside the pons will be reported separately.

R3Q5

- Please clarify that this oncogenic mechanism is not universal in posterior fossa ependymoma.

Reply: We clarified that H3K27-alteration is also observed in ependymoma of the PFA subtype (Line 83-84). Notably, EZHIP overexpression is found in approximatively 90% of PFA ependymomas, whereas H3K27M mutations are present in about 10%, with the reverse proportions observed in DIPG (see Mariet et al, Acta Neuropathol Commun 2022; PMID: 36104744). As this is not the focus of the present manuscript, this entity was not described in further detail in the Introduction.

Results

R3Q6

- In Figure 1, please clarify in the caption or figure how the randomizations led to the comparisons; the color coding and distribution in 1a is confusing.

Reply: We agree that the definition of subtrials and the construction of pairwise comparison based on biomarkers was complex and required clarification. We therefore modified the caption of this Figure (formerly figure 1a and now ExtendedData1) as follows:

"Description of the 3 subtrials and the construction of the pairwise comparisons. Patients with EGFR immunopositive tumors but not PTEN loss of expression were randomized in R1 to receive either erlotinib or dasatinib. Patients with tumors showing PTEN loss only without EGFR immunopositivity were randomized in R2 to receive either everolimus or dasatinib. Patients with tumors showing both EGFR immunopositivity and PTEN loss were randomized in R3 to receive one of the three drugs tested in the trial. Patients with an uninformative result for EGFR and/or an uninformative result for PTEN were also randomized in R3 to receive one of the three drugs. Patients with tumors showing no EGFR immunopositivity and no PTEN loss of expression were not randomized and received dasatinib. By combining the subtrials, pairwise comparisons were constructed to maximize patient numbers in each comparison: all patients included in the R1 trial were included the Erlotinib versus Dasatinib comparison, as well as patients included in the R3 trial, allocated to Erlotinib or Dasatinib. Similarly, all patients included in the R2 trial were included the Everolimus versus Dasatinib comparison, as well as patients included in the R3 trial, allocated to Everolimus or Dasatinib. Lastly, patients included in the R3 trial, allocated to Erlotinib or Everolimus were included in the Erlotinib versus Everolimus comparison."

R3Q7

In 1b, where are the patients with neither marker who received dasatinib in this diagram? I understand they were not included in the randomization, but this diagram is for the entire trial.

Reply: There was only 1 patient with neither marker *(i.e*. EGFR-positive and no PT-Loss). We have modified the information in the participant flow diagram of former figure 1b (now Figure 1 in the revised version) as this was not clearly stated. The label has been changed from "biomarker profile" to "EGFR-negative, no PTEN loss".

R3Q8

- The two-sided alpha level of 20% seems unusual and should be explained further.

Reply: The randomized trial was designed as an exploratory Phase II trial in a rare disease. In this context, a two-sided alpha of 20% (equivalent to a one-sided alpha of 10% for single-arm trial) was considered an acceptable compromise to ensure sufficient statistical power for the comparisons. A sentence has been added to the second section of the Results about the trial design and endpoints: "*Considering the smallest pairwise comparison (erlotinib versus everolimus), a total of 79 events and 90 patients were required to have an 80%-power in the comparison of the whole OS curves by a logrank test at a 2-sided alpha=20%, if the Hazard Ratio of death was 0.62, equivalent to a 10.6% increase in the two-year OS (5% vs. 15.6%) assuming an exponential survival distribution, proportional hazards and an expected accrual of 22.5 patients per year (East software)*." (Lines 164 to 169).

R3Q9

- How would 250 patients in the trial be enough to ensure 90 patients in the smallest comparison if there are three comparisons?

Reply: The allocation rule was based on EGFR overexpression and PTEN loss of expression assessed by immunohistochemistry.

At the design stage of the trial, it was anticipated that :

The results would be uninformative in 15% of cases,

And, when the tumor material was informative (85% of cases),

90% of cases show PTEN-loss,

50% of cases show EGFR overexpression.

These two biomarkers would be independent.

Based on these assumptions, the expected distribution across subtrials was as follows:

-R1-trial, erlotinib *versus* dasatinib: 4.25%;

-R2-trial, everolimus *versus* dasatinib: 38.25%;

-R3-trial, erlotinib *versus* everolimus *versus* dasatinib: 38.25%+15%=63.25%;
Cohort dasatinib (non-randomized): 4.25%

As each patient from the R3 subtrial contributes to two of the three pairwise comparisons, the expected distribution of patients per pairwise randomized comparison was:
-"Erlotinib *versus* Dasatinib" comparison: 100% of R1 + 2/3 of R3 = 40%
-"Everolimus *versus* Dasatinib" comparison: 100% of R2 + 2/3 of R3 = 74%
-"Erlotinib *versus* Everolimus" comparison: 2/3 of R3 = 36%

	Subtrial				**Pairwise comparisons**	
Pairwise comparisons	**R1 (4.25%)**	**R2 (38.25%)**	**R3 (53.25%)**	**Not randomized (4.25%)**	**Expected %**	**Expected sample size if total N=250**
Erlotinib *versus* Dasatinib (100% of R1 + 2/3 of R3)	**4.25%**		**35.50%**		**40%**	**~100**
Everolimus *versus* Dasatinib (100% of R2 + 2/3 of R3)		**38.25%**	**35.50%**		**74%**	**~185**
Erlotinib *versus* Everolimus (2/3 of R3)			**35.50%**		**36%**	**~90**

Accordingly, the smallest pairwise comparison was the "Erlotinib *versus* Everolimus", representing 36% of the total randomized population, corresponding approximately 90 patients if 250 patients were recruited in the randomized subtrials.
The expected distributions across subtrials and pairwise comparisons are shown in ExtendedData1.
Additional explanatory details have been added to the figure caption of ExtendedData1 to clarify these assumptions.

R3Q10
- Please define/cite pseudoprogression.
Reply: Situations in which pseudoprogression should be considered were defined in the annex of the protocol. These criteria are summarized in the Methods section (Response Assessment): "*Pseudoprogression was considered when MRI changes occurred within the radiation field, typically presenting as new ring contrast enhancement, during the first six months following radiotherapy. The main characteristics supporting this diagnosis were increased perfusion early after radiotherapy*[6,7] *without increased infiltration outside the initially fields involved*[6]*. A confirmatory MRI demonstrating a decreased in enhancement on follow-up imaging was required. All doubtful cases were reviewed centrally in real time, blinded to treatment allocation. When true progression was identified on subsequent MRI, the date of progression was backdated to the date of the previous MRI*. » (line 1349-1357).
An expedited central review involving the expert radiologists (NB, VDR and RC) was implemented in cases of uncertainty to avoid premature treatment discontinuation due to pseudoprogression.

Overall, "*pseudo-progression was reported in 110/233 patients (49%), with no significant difference between treatment groups (Chi2, p=0.870).*"(Line 242 to 244). Although not explicitly requested by the reviewer, this information was included because such data have not previously been reported in DIPG trials and highlight the complexity of response assessment in this type of neoplasm.

Pseudo-progression Treatment group	**No**	**Yes**	**Total**

Erlotinib	**19 (53%)**	**17 (47%)**	**36**
Everolimus	**52 (55%)**	**43 (45%)**	**95**
Dasatinib	**52 (51%)**	**50 (49%)**	**102**
Total	**123 (55%)**	**110 (49%)**	**233**

R3Q11

- Please give more information on eligibility criteria: age, localized vs metastatic disease, performance status, etc.

Reply: additional details on eligibility criteria are summarized in the main text (paragraph **Trial design and endpoints**, Lines 142 to 148) and described in details in the third paragraph of the online Methods section (Lines 1171 to 1259). The key eligibility criteria reported in the main text are: "*Previously untreated DIPG i.e. DMG H3K27-altered with an epicenter in the pons (metastatic disease allowed), age over 6 months, absence of active intratumoral hemorrhage, ability to undergo radiotherapy, Lansky Play Scale > 50% and life expectancy greater than three months.*" (line145-148).

R3Q12

- Why would patients without information on biomarkers be included in the randomization, i.e. how are they different from patients who were negative for the two biomarkers?

Reply: At the design stage of the trial, it was anticipated that among informative tumors, 90% would show a PTEN-loss and 50% EGFR overexpression, with independence between biomarkers. Accordingly, only 5% of tumors were expected to be EGFR-negative without PTEN-loss, and thus ineligible for Erlotinib or Everolimus.

Based on these assumptions, patients with uninformative results due to technical issues were elligible for randomization across the three treatment arms. Due to space constraints, this rationale was not included in the Results section.

R3Q13

- It would be helpful to have a table or figure showing the data on biopsy complications in lines 194-5.

Reply: A detailed analysis performed after accrual of the first 195 patients was reviewed. Among these patients recruited in the study, 2 did not undergo biopsy or initial surgery. Of the remaining 193 patients, information on the surgical technique and complications was available, at least partially, for 176 patients:

5 underwent partial resection,

171 underwent biopsy

157 underwent stereotactic surgery

12 underwent open surgery

Information on technique was missing for 2 patients.

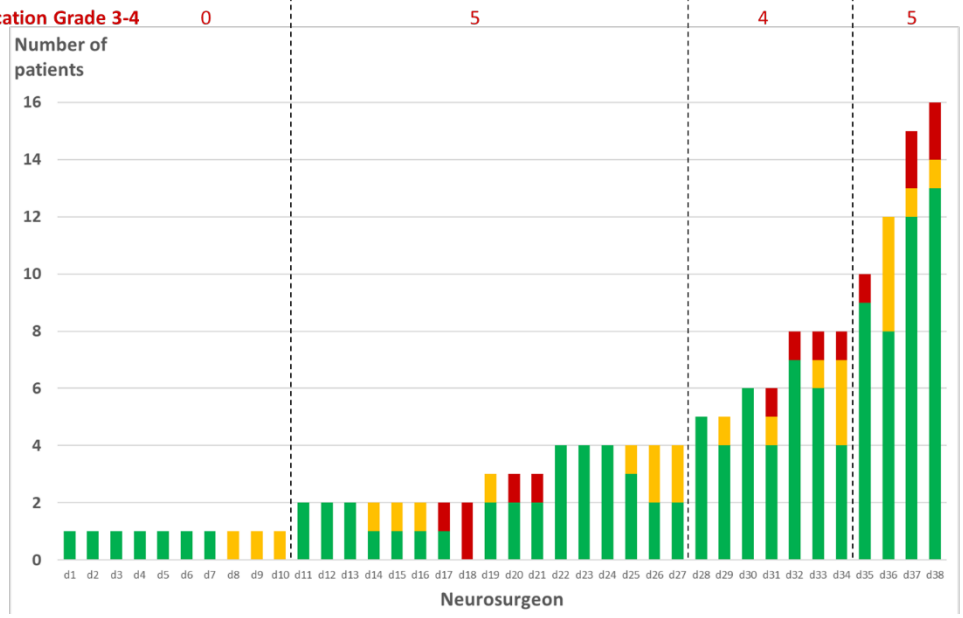
Among the 157 stereotactic biopsies performed as first or second biopsy with information on the name of the neurosurgeon, 38 different neurosurgeons performed the procedures: 10 neurosurgeons performed 1 biopsy in the study, 17 performed 2 to 4 biopsies (total: 48 biopsies), 7 performed 5 to 9 biopsies (total: 46 biopsies), and 4 neurosurgeons performed 10 to 16 biopsies (total: 53).

Among the 157 patients who underwent a stereotactic biopsy, 38 (24%) experienced an adverse event classified as related (N=28, 18%) or possibly related (N=10, 6%) to the biopsy, including 14 grade 3 or 4 events (9%). The type of complications is detailed in the table below.

Type of event	Grade					Total
	1	2	3	4	MD	
Event related to the biopsy	**2**	**13**	**10**	**2**	**1**	**28**
- Symptomatic hemorrhage		2		1		3
- CSF leakage			1	1		2
- Infection			1		1	2
- CSF leakage & infection			1			1
- Neurological symptoms without other complication (new symptoms or worsening of existing symptoms) without CSF leakage, infection or hemorrhage	2	11	7			20
Event possibly related to the biopsy	2	6	1	1		10
- Worsening of preexisting neurological symptoms without other complication	2	6	1	1		10
Total	**4**	**19**	**11**	**3**	**1**	**38**

The distribution of biopsy-related adverse events according to neurosurgeon experience is shown in the figure below.

N. Biopsies / neurosurgeon	1	2 to 4	5 to 9	10 to 16
Total number of biopsies	10	48	46	53
No complication	7	34	36	42
Complication Grade 1-2	3	9	6	6
Complication Grade 3-4	0	5	4	5



No clear association was observed between complication rate and neurosurgeon experience (based on the number of biopsies performed in the study). The proportion of patients with any grade complication was 30%, 29%, 22% and 21% for the neuro-surgeons performing 1, 2-4, 5-9 and 10-16 biopsies, respectively (p=0.72). The proportion with grade 3 or higher was: 0%, 10%, 9% and 9%, respectively (p=0.77).

Reply: The paragraph on the safety of the biopsy was therefore rewritten as follow: "*All neurosurgeons performing the biopsies were either authorized based on their previous track records for this procedure or trained in two specific sessions held in Strasbourg (Pr Stéphanie Puget) and in London (Pr Kristian Aquilina). No death secondary to the biopsy was reported. The median duration of hospital stay for the biopsy was 2 days (range: 1 to 5 days). In the 157 biopsy procedures centrally reviewed, 38 patients (24%) experienced neurological worsening of which 27 occurred within the 48 hours of the biopsy and were therefore considered related to the procedure. Fourteen severe (grade 3 and 4) complications*

were observed, without any significant frequency associated with the number of biopsies performed." (Lines 201 to 208)

R3Q14

- The caption for Figure 2 repeats the caption for 2d and confusingly has "f" there twice.

Reply: We have revised both the panels and the captions of Figure 2.

R3Q15

- It would be helpful to have more details on the toxicities observed, and these statistics are confusing, e.g. the manuscript states that eye toxicities were less frequent with everolimus but does not say what type of eye toxicities were observed, and the rate of eye toxicity in everolimus and dasatinib appears nearly equal.

Reply: Adverse events are summarized in ExtendedData6 and detailed in Supplementary Table S3 "*Distribution of the maximum grade of adverse events (0-2 vs. >=3), by System Organ Class, according to the treatment arm, regardless of causality, over the whole treatment duration*". Regarding Eye Toxicity, the details are as follows:

System Organ Class / Prefered Term	Erlotinib (N= 35)				Everolimus (N= 91)				Dasatinib (N= 101)				Comparison between the 3 arms	
	Gr >=1		Gr >=3		Gr >=1		Gr >=3		Gr >=1		Gr >=3		Gr >=1	Gr >=3
	N	%	N	%	N	%	N	%	N	%	N	%	p-value	p-value
Eye disorders	**28**	***80***	**2**	***6***	**32**	***35***	**1**	***1***	**39**	***39***	**1**	***1***	**<0.0001**	**0.22**
Blepharitis	1	*3*	0	*0*	0	*0*	0	*0*	0	*0*	0	*0*		
Blurred vision	11	*31*	0	*0*	11	*12*	0	*0*	11	*11*	0	*0*		
Conjunctivitis	16	*46*	0	*0*	15	*16*	0	*0*	20	*20*	0	*0*		
Diplopia	1	*3*	0	*0*	0	*0*	0	*0*	1	*1*	0	*0*		
Dry eye	8	*23*	0	*0*	10	*11*	0	*0*	9	*9*	0	*0*		
Eye pain	2	*6*	0	*0*	1	*1*	0	*0*	2	*2*	0	*0*		
Growth of eyelashes	3	*9*	0	*0*	0	*0*	0	*0*	0	*0*	0	*0*		
Keratitis/Corneal ulcer	6	*17*	1	*3*	6	*7*	1	*1*	8	*8*	1	*1*		
Papilledema	1	*3*	1	*3*	0	*0*	0	*0*	0	*0*	0	*0*		
Photophobia	1	*3*	0	*0*	1	*1*	0	*0*	1	*1*	0	*0*		
Visual acuity reduced	1	*3*	1	*3*	0	*0*	0	*0*	0	*0*	0	*0*		
Eye disorders - Other	0	*0*	0	*0*	0	*0*	0	*0*	1	*1*	0	*0*		

You are right: Eye toxicity was more frequent in the erlotinib group, but did not differ between the everolimus and dasatinib groups. The manuscript has been modified accordingly. The Results section now states "*When considering AEs of any grade, eye (p<0.0001), skin (p=0.004) and infectious (p=0.042) AEs were more frequent with erlotinib while the metabolic AEs were more frequent with everolimus (p=0.0003) (ExtendedData5F-G, ExtendedData6, TableS3). Severe (grade >or = 3) skin AEs were significantly more frequent with erlotinib (p<0.0001) and severe renal AE (p=0.0054) and gastro-intestinal AE (p=0.038) were significantly more frequent with dasatinib*." (Lines 219 to 223).

R3Q16

- What tumors are included in the comparison COSMIC database? Also, what does 100% refer to in this SNV section – 100% of what?

Reply: due to space constraints, we decided to remove the paragraph comparing the type of alterations observed in DIPG with the spectrum of mutations reported for the same genes in the COSMIC database

considering all tumor types. This comparison was potentially confusing and did not substantially add to the interpretation of the results.

R3Q17

- BRAF mutations have been associated with better survival in DMG, but I don't see these reported in this cohort – were these observed?

Reply: As a matter of fact, no patient with a BRAF V600E mutation in addition to H3K27M was included in the randomized trial. One such patient was excluded because central pathological review concluded that the diagnosis was ganglioglioma rather than diffuse glioma.

R3Q18

- It would be helpful to have a panel demonstrating the findings in lines 402-7, which are interesting and important.

Reply: These data are already presented in the panel showing the volcano plot of differentially expressed genes, currently panel K of Extended Data 8.

R3Q19

- In line 466, please clarify what is meant by "genes involved in Pi3K … signaling were significantly decreased" – expression? Alterations?

Reply: The sentence has been revised and now reads: "Overexpression of the PI3K/AKT/MTOR signature showed a trend toward improved response to *everolimus (interaction p=0,1085)*." (Lines 434 to 437)

Discussion

R3Q20

- I think it would be better to state the survival in months for the comparisons in lines 526-8 instead of making these qualitative statements with no statistical analysis.

Reply: We have added median OS estimates to the Discussion section. The text now states: *"No significant survival differences were observed among treatment arms, although median OS was slightly longer with everolimus compared with dasatinib, median OS was 11.3 months (95%CI, 10.3-13.4) versus 9.4 months (8.2-10.8), respectively."* (Lines 457 to 459)

R3Q21

- Please use either the term dordaviprone (preferably) or ONC201 after equating them in line 545, not both, for clarity.

Reply: we have consistently used the term ONC201 throughout the manuscript, as this is currently the designation most widely recognized by the scientific community.

Methods

R3Q22

- What were the cutoffs used for EGFR and PTEN expression used?

Reply: EGFR positivity was defined as a Hirsch index above 150 (the Hirsch index combines the percentage of positive tumor cells multiplied by the staining intensity (graded 1 to 3), with a maximum score of 300 and 0 indicating no expression).

This definition has been explicitly included in the Methods section (Immunohistochemistry and FISH analysis): *"Accumulation of TP53, EGFR Hirsch score, and loss of PTEN were assessed as previously described8,9. Briefly, an Hirsch index equal or above 150 was considered indicative of positive*

overexpression and PTEN loss in tumor cells was considered only in the internal control (vessels) was positive." (Lines 1370 to 1373).

R3Q23

- Starting in line 786, the future tense is used, suggesting these paragraphs were taken perhaps from a grant proposal or the trial protocol.

Reply: the tense inconsistencies in the Statistical analyses section have been corrected in the (Lines 1466 to 1486).

Old - Nom Figure-table	Old - Nom panel	Description	New- 6 **main display items** (inclusive of figures and tables)	New 10 **Extended Data** display items (inclusive of figures and tables)	New **Supplement al** data
Figure 1	A	CONSORT diagram, patient baseline characteristics and primary endpoint data		Extended Data 1	
Figure 1	B	consort diagram	Figure 1		
Figure 2	A	OS curves - erlotinib vs. Dasatinib	Figure 2A		
Figure 2	B	OS curves - everolimus vs. Erlotinib	Figure 2B		
Figure 2	C	OS curves - everolimus vs. Dasatinib	Figure 2C		
Figure 2	D	Progression free survival in patients treated with everolimus or dasatinib	Figure 2D		
Figure 2	E	Forest plot for OS		Extended Data 10A	
Figure 2	F	Forest plot for PFS		Extended Data 10B	
Figure 2	G	OS since the biopsy in the three treatment arms compared with the historical control	Figure 2E		
Figure 2	H	Radar plot for all grade AE by system organ class		Extended Data 5F	
Figure 2	I	Radar plot for all severe AE (grade > or = 3)		Extended Data 5G	
Figure 3	A	Oncoplot depicting the top somatic mutated	Figure 3A		
Figure 3	B	Distribution of the VAF	Figure 3B		
Figure 3	C	Distribution of H3-K27M, TP53 and PIK3CA alterations, MYC gain	Figure 3C		
Figure 3	D	Violin plot of the SBS significantly enriched in short versus long survival		Extended Data 8A	
Figure 4	A	Heatmap representing the copy number genomic alterations		Extended Data 8b	
Figure 4	B	Box plot reflecting the distribution of percentage of altered genome	Figure 3D		
Figure 4	C	Box plot reflecting the distribution of percentage of number of segments	Figure 3E		
Figure 4	D	Violin plot comparing the percentage of CNA belonging to CN signature		Extended Data 8c	
Figure 4	E	Distribution of clinicopathological and molecular information presented on the panel A in *TP53* -mutated and *TP53* -wild-type subgroups	Figure 3F		
Figure 4	F	Violin plot of the mutated genes for which the VAF		Extended Data 8J	
Figure 4	G	OS since the biopsy in patients with TP53MUT and TP53WT DIPG	Figure 3G		
Figure 5	A	Pathways with a significant modulation of Gene set variation analysis (GSVA)	Figure 4A		
Figure 5	B	Cell type composition of DIPG tumors from deconvolution of bulk RNAseq	Figure 4B		
Figure 5	C	Violin plot reflecting the tumor composition in short and long overall	Figure 4C		
Figure 6	A	Forest plot comparing the differential effect on overall survival of everolimus and dasatinib	Figure 5A		
Figure 6	B	Forest plot comparing the differential effect on progression-free survival of everolimus and dasatinib	Figure 5B		
Figure 6	C	Overall survival in patients without PI3K/AKT/MTOR alterations	Figure 5C		
Figure 6	D	Overall survival in patients with PI3K/AKT/MTOR alterations	Figure 5D		
Figure 6	E	Progression-free survival in patients without PI3K/AKT/MTOR alterations	Figure 5E		
Figure 6	F	Progression-free survival in patients with PI3K/AKT/MTOR alterations	Figure 5F		
Figure 6	G	Oncoplot presenting SNV alteration of genes involved in PI3K/AKT/MTOR pathway	Figure 5G		
Figure S1	A	Representative MRI images		Extended Data 5A	
Figure S1	B	Representative MRI images		Extended Data 5B	
Figure S1	C	Representative MRI images		Extended Data 5C	
Figure S1	D	Variant Allele Frequency of the H3K27M mutation versus biopsy site		Extended Data 5D	
Figure S1	E	Variant Allele Frequency of the H3K27M mutation versus tumor load		Extended Data 5E	
Figure S2	A	PFS		Extended Data 4A	
Figure S2	B	OS curves - everolimus vs. Erlotinib		Extended Data 4B	
Figure S2	C	Os of the trial cohort according to the type of histone H3K27 alteration		Extended Data 4C	
Figure S2	D	OS of the trial cohort according to the age at diagnosis histone H3K27 alteration		Extended Data 4D	
Figure S3	A	oncoplot germinal alteration		Extended Data 7A	
Figure S3	B	CNV of the mutated genes presenting the highest median VAF		Extended Data 7B	
Figure S3	C	Mutual exclusivity analysis		Extended Data 7C	
Figure S3	D	Oncoplot depicting the top mutated genes sorted and ordered by decreasing frequency in DIPG		Extended Data 7D	
Figure S3	E	Distributions of H3-K27, TP53, PIK3CA mutations, MYC gain and EGFR overexpression according to the age at diagnosis are shown.		Extended Data 7E	
Figure S3	F	Oncoprint representation of an integrated annotation of somatic mutations and DNA copy number changes affecting specific pathways		Extended Data 7F	
Figure S3	G	Exhaustive view of the COSMIC Single Base Substitution signatures (SBS)		Extended Data 7G	
Figure S3	H	H-Barplot presenting the percentage of mutation per patient belonging to each of the 15 more recurrent COSMIC		Extended Data 7H	
Figure S4	A	Heatmap representing the copy number genomic alterations of the DIPG patients			Fig S1
Figure S4	B	Associations between the exposures of genome alteration signatures, or the difference of exposure, and molecular features.		Extended Data 8d	
Figure S4	C	Lolliplot of TP53 alterations		Extended Data 8E	
Figure S4	D	Box plots reflecting the percentage of altered genome and the distribution of number of segments detected by CNV analysis		Extended Data 8F	
Figure S4	E	Box plots reflecting the percentage of altered genome and the distribution of number of segments detected by CNV analysis		Extended Data 8G	
Figure S4	F	Box plot reflecting the distribution of the number of segments detected by chromosome associated with gains or losses		Extended Data 8H	
Figure S4	G	Dot blot presenting the chromothripsis score		Extended Data 8I	
Figure S4	H	Volcano plot of gene expression in TP53MUT versus TP53WT		Extended Data 8K	
Figure S4	I	GSEA plots showing GOBP ectoderm development		Extended Data 8L	
Figure S5	A	Circos plot presenting genome alterations in the 3 alive patients.		Extended Data 9A	
Figure S5	B	Volcano plot of differential analysis in patients with a long versus short overall survival. DEGs associated with adjusted pvalue <0.001 are color-coded in red or green			FigS2A
Figure S5	C	Gene ontology tree			FigS2B
Figure S5	D	*GSEA plots showing*		Extended Data 9b	
Figure S5	E	Box plot presenting the RNAseq expression levels (tpm) of the top modulated genes in patients with a long versus short overall survival (adj p-value < 5.2 e-09).		Extended Data 9C	
Figure S5	F	Expression level (tpm) of E2F7 and EZH2 in DMG patients		Extended Data 9D	
Figure S5	G	Oncoplot presenting SNV alteration of genes		Extended Data 10G	
Figure S6	A	Overall survival in patients with 1q gains:		Extended Data 10C	
Figure S6	B	Overall survival in patients without 1q gains:		Extended Data 10D	
Figure S6	C	PFS		Extended Data 10E	
Figure S6	D	PFS		Extended Data 10F	
Supplemental Table S1	A	Screen failures related to diagnosis			Table S1
Supplemental Table S1	B	Patient and disease characteristics, overall and by treatment group allocated by randomization (n=233)	Table 1		
Supplemental Table S1	C	Timing of the procedures			Table S2
Supplemental Table S1	D	Subtrial allocation according to biomarker profile among the 233 patients matching the general eligibility criteria		Extended Data Table 2	
Supplemental Table S2	A	Summary of overall survival analyses in the randomized trial		Extended Data Table 3	
Supplemental Table S2	B	Summary of prognostic factors analyses			Table S5
Supplemental Table S2	C	Association between Histone mutation and TP53 mutation			Table S6
Supplemental Table S2	D	Association between age and Histone mutation, TP53 mutation or the combination of both			Table S7
Supplemental Table S3		Summary of safety analyses			Table S3
Supplemental Table S4		WT description			Table S8
Supplemental Table S5		PI3K/AKTmTOR pathway			Table S9
Table		résume advert event/safety data		Extended Data Table 6	
supplementary table	NEW	clinical response according to subroup			Table S4

Report on "Biological Medicines for Diffuse Intrinsic 1 Pontine Gliomas (DIPG) Eradication (BIOMEDE) An International Randomized Phase II Biomarker-driven Platform Trial Comparing Three Targeted Therapies in Combination with Radiotherapy"

Overview:

The current manuscript aims to provide results of the largest, randomized biology-driven phase II study (BIOMEDE) in DIPG evaluating the efficacy in terms of overall survival as primary endpoint of EGFR inhibitor erlotinib, mTOR inhibitor everolimus and the multitarget receptor tyrosine kinase inhibitor dasatinib in combination with radiation therapy in patients with a DIPG.

According to the protocol, this trial is an exploratory trial and not a confirmatory trial. The main analysis were direct pairwise comparisons (erlotinib versus dasatinib from R1 and R3 randomisations; everolimus versus dasatinib from R2 and R3 randomisations; and erlotinib versus everolimus from R3 randomisation).

The trial was ended for futility of the primary endpoint following the recommendations of the independent data monitoring committee without a significant difference of overall survival (OS) from diagnosis with the control cohort (10.8 months) in one of the three arms 67 (9.7 months for erlotinib, 9.9 months for dasatinib and 11.9 months for everolimus).

In the following, I will provide my detailed comments:

1. Regarding endpoints:

   The primary endpoints of the study were overall survival, while the secondary endpoints were 2-year overall survival, progression-free, survival and safety profile. Exploratory objectives were the study of the association between biomarkers and drug efficacy, and of radiographic changes after radiotherapy. The OS and PFS curves were estimated using Kaplan-Meier method.

   The endpoints reported were as specified in the clinical study protocol.

2. Regarding the appropriateness of statistical analysis and sutability of the descriptions of statistical analysis plan:

   The main presented statistical analysis is pairwise comparisons of overall survival (OS) between treatment groups allocated by randomization (intention to treat analysis) in R1, R2, R3. The overall survival is computed using the Kaplan-Meier method.

   Due to the fact that the randomization was stopped prematurely, the primary outcome measure of the trial (pairwise randomized comparisons) did not show any significant difference in OS.

For all the comparisons, median OS survival for each group is presented along with its 95% confidence interval. Hazard ratios in R1, R2, and R2 are reported along with 95% confidence intervals and corresponding P-values.

Statistical analysis for the secondary endpoints (2-year overall survival, progression-free survival and toxicity) are also appropriate.

The "Survival analysis" on page 28 is comprehensive and well written. The "statistical analysis" on page 33 is quite brief by simply mentioning paired t-test, Fisher exact test, and Wicoxon test.

Minor comment: "Fisher exact test" was wrongly typed as "fischer exact test" on page 34.

Major comment: Given the nature this trial is an exploratory trial and not a confirmatory trial, the analysis plan presented in this article is appropriate. However, the descriptions of "statistical analysis plan"on page 33 could use more details to boost up its statistical rigor.

3. Regarding whether the interpretation of the results is supported by the data and statistics:

   Overall, I have found the interpretation of results in all the analysis is supported by the data and statistics. While the planned analysis were not complicated, the results were thoughtfully presented for all the endpoints.