Author's response to reviews

Title: Over-Representation of Specific Regions of Chromosome 22 in Cells from Human Glioma Correlate with Resistance to 1,3-bis(2-chloroethyl)-1-nitrosourea

Authors:
Nicole C Hank (nicole.hank@chw.edu)
Joan R Shapiro (joan.shapiro@chw.edu)
Adrienne C Scheck (adrienne.scheck@chw.edu)

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Author's response to reviews:

We thank the reviewers for their helpful comments and we hope the manuscript is now acceptable for publication.

Response to all reviewers:

We realize the number of cases is small, but we feel that the ability to analyze cells from the primary tumor and the same patients' recurrent tumors prior to, and following in vitro selection for therapy resistance in part overcomes this issue.

Reviewer Judith Jeuken:

1. All our GBM patients get radiation and chemotherapy so it is not possible to look at untreated recurrent tumor. In primary tumors (which are untreated), cells with over-representation of chromosome 22 are present as only a very minor subpopulation. These cells become a dominant population in the recurrent tumor following treatment. This is mentioned in the background and referenced.
2. We agree that since all patients get RT, it is possible that this induced the chromosome 22 aberrations. We feel this is not the case because one can find cells with over-representation of chromosome 22 (by karyotype) in the primary tumor, although in a very small percentage. Based on the presence of marker chromosomes, these same cells are present in increased numbers in the recurrent tumor. This point has been expanded in the background section.
3. Serial passage without chemotherapy is done on all cell lines (mock treatment). This has been emphasized in the text.

Reviewer Peter Warnke:

1. Were the tumors really resistant? Additional enhancement was seen on MRIs within 3 months of the surgery for the primary tumor and surgery for tumor recurrence was required within less than a year of the primary tumor. These were considered resistant due to their rapid recurrence. Furthermore, cells obtained from these tumors were treated with BCNU within 2-3 passages of removal and cells from the recurrent tumors were more resistant than cells from the primary tumor. The difference in the time required to select for resistance to 10μg/ml BCNU also indicates that the cells from the recurrent tumor, even at higher passages, were more resistant to BCNU than cells from the primary tumor. We added this information to the manuscript.
2. The patients whose tumors were used in this study ranged in age from 38 - 49. We agree this is younger than many GBM patients, but it is not that unusual to see GBM patients in this age range. Furthermore, these patients are more likely to survive longer and undergo surgery for recurrent tumor. A survival of 466 days is not considered exceptionally long. This has been commented on in the text.
3. Statistical analyses have been done. The differences we noted are statistically significant. This has been added to the text.

Reviewer Juergen Schlegel

1-3. The text has been reworded to clarify the issues brought up in questions 1-3.
4. Information regarding the level of intrinsic resistance such as the time required to select for cells resistant
to 10μg/ml BCNU has been added to the text.

5. Passage number data has been added to the text. It should be noted that the results obtained from multiple experiments were consistent across the passage numbers used.

6. The diagnoses were made by the same neuropathologist. This has been added to the table legend. Clinicians feel there is no real clinical (outcome, therapy response) difference between gliosarcomas and GBMs. In fact, gliosarcomas are considered a variant of glioblastoma multiforme. Furthermore, there have been a number of studies demonstrating that there are few, if any differences in the genetic profiles of these tumors. This has been added to the manuscript with appropriate references.

7. Statistical analyses have been done. The differences we noted are statistically significant. This has been added to the text.