Unmet need for liquid biomarkers and the Brain-Liquid Biopsy Consortium

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Despite advances in surgical techniques and clinical regimens, malignant gliomas usually progress or recur after treatment. It is widely acknowledged that significant challenges remain in assessing response to treatment of glioma in the time frame in which response and progression occur. Currently, visual inspection of imaging data is the mainstay to monitor glioma progression; however, this approach may not be accurate or refined enough to monitor treatment response or evolving prognostic subtypes. Imaging data have limited ability to distinguish (1) gliomas from other tumors (eg, primary central nervous system [CNS] lymphoma), (2) progression from pseudoprogression resulting from therapy, or (3) minimal or remnant tumoral burden.[1] In addition, imaging is limited by its availability and feasibility for some patients. This hampers efforts to assess the response to standard treatment as well as to novel agents and therefore to manage patients appropriately. More importantly, it leaves patients with significant uncertainty, sometimes for many weeks. Developments in imaging technology and application of more advanced assessment of standard data through application of Artificial Intelligence (AI) and machine learning are moving rapidly but there remains no validated imaging-based approach to read out response to standard treatment that outperforms standard MRI.[2] In addition, among nonenhancing tumors, imaging cannot discriminate between tumor, edema, and postradiation effect or scarring on T2/FLAIR.

Earlier diagnosis is also a significant unmet need in gliomas, which to date have not proven amenable to any screening approach, which is hampered by the relative rarity of the diagnosis as well as the usually nonspecific presenting symptoms.[3] The assumption that earlier diagnoses can be made and that this would lead to better outcomes has therefore not been addressed in the field. An ideal screening blood-based biomarker would identify early stage disease, and/or select patients for specific, personalized therapies and/or monitor response thereby contributing to development of new treatment approaches.

Accurate diagnosis through a simple blood test will allow clinicians to detect the evolution of the disease in real time, thus identifying high-risk patients who may benefit from more aggressive therapy at an earlier point when intervention could be more effective. Blood-based biomarkers have been used routinely for many years in some cancers including prostate and ovarian cancer and are applied routinely in hematological malignancies to monitor disease burden and detect recurrence. In the era of precision medicine, predictive blood-based biomarkers have also been applied successfully in several tumor types, most notably nonsmall cell lung cancer, where sequential sampling of circulating tumor DNA allows detection of relevant targetable mutations as well as appearance of resistance mechanisms, for example to epidermal growth factor receptor (EFGR) targeting agents.[4] Across solid tumors, sampling tumor biology from blood or other relevant body fluid has been achieved using a wide range of assays and technologies. Some of these approaches, including isolation of circulating tumor cells (CTCs), have the additional advantage of permitting read out of RNA and protein levels and application of functional assays as well as monitoring mutational profile. Other approaches include isolation of tumor-derived exosomes, reflecting cancer-derived trafficking of genetic material, proteins and lipid and which have the advantage of relatively long half-life compared to ctDNA.[5]

The promise of all of these approaches is a real-time read out of tumor status or response without the need to access tumor directly which is particularly appealing in brain tumor patients. An ideal biomarker for response assessment has been recently described by Jones et al., showing a rapid sustained nadir that reflects response to treatment and a sensitive rise that signals relapse early.[6] This would permit treatment when disease is at lowest possible volume, very likely ahead of clinical symptoms an approach that is currently being validated in early breast cancer based on measurement of circulating tumor DNA to monitor relapse.[7] An additional advantage in the setting of novel agent treatments is that response as well as early relapse could also be measured. This is exemplified by the approach being tested in the ongoing CACTUS study in BRAFmut melanoma where

repeated testing of a small gene panel is being used to assess response to BRAF inhibition and support treatment switching to immunotherapy agents (https://clinicaltrials.gov/ct2/show/NCT03808441). Liquid biopsies applied in this way could also have a major impact on study design since it would significantly enhance our ability to compare approaches rapidly in platform studies with many parallel arms and potentially select patients for specific treatments, as is being done in the early phase TARGET study in metastatic and recurrent solid tumors.[8]

In the context of glioma, a large range of assays and technologies have been investigated to date. It is clear that compared to other solid tumors, CNS tumors release less tumor material into the systemic circulation, presumably due to the presence of the blood–brain barrier. Whilst cerebrospinal fluid (CSF) appears to be a better source of tumor-derived material, repeated sampling is less practical if the ultimate goal is to fine-tune treatment in real time.[9] One interesting approach to address this may be to make use of blood–brain barrier opening approaches including focused ultrasound to increase shedding and sensitivity,[10] but then again it does not offer the best utility if the aim is to access recurrence in real time. The well-described issue of tumor heterogeneity in many CNS tumors, particularly low- and high-grade gliomas also adds complexity to any tumor-derived sampling including liquid biopsy.[11] Nevertheless, although the rarity of tumor-derived material, especially CTC in glioma seems to limit their applicability in this context, early data suggest that several other liquid biopsy approaches may hold promise including tumor educated platelets, ctDNA, and exosomes,[12–14] as well as serum spectroscopy.[15] We encourage the reader to review the companion papers in this edition to learn more about tumor educated platelets, ctDNA, and exosomes. Recent data also suggest that methylome analysis of cell-free DNA may be particularly powerful as it can identify cancer-specific large-scale epigenetic aberrations as well as cancer-specific immune signatures, which increases sensitivity of detection and may lend itself to a screening approach.[16,17] A comprehensive review of noninvasive methylation markers in gliomas is discussed in the article by Noushmehr et al. within the same issue.[18] Other approaches to enhance sensitivity include application of fragmentomics as an initial screen of blood or CSF-derived tumor DNA.[19]

Whilst we are entering an era in which one or several of these promising approaches may soon be clinically applicable, significant challenges still lie ahead. There is a pressing need for data from prospective clinical studies, requiring a close interaction with appropriate clinical study teams. Once the assay technology is optimized the pathway from discovery science to clinical implementation is also complex, including standardization, analytical statistical methods, and validation between labs and across diagnoses as well as time and resource intensive authorization steps. The field therefore needs to prioritize the most promising approaches to focus efforts in the short and medium term and to work with relevant stakeholders including industry, where diagnostic companies and drug developers have been quick to enter this space. It also seems unlikely that one assay will fulfill all the requirements and unmet needs for early diagnosis, disease

monitoring, and surveillance and new agent evaluation, so different approaches likely need to be combined. This will require new, collaborative approaches to study design and assessment.

It also needs to be recognized that some of the most promising assays may rely on technology that is developing rapidly and may not yet be widely available, meaning that roll out and timing can be problematic.[20] This may also raise issues about equitable access across regions, jurisdictions, and funding models.

Finally, it should be appreciated that blood-based testing for disease evaluation will be unfamiliar to our patient groups and that we therefore need to engage with them as a community to ensure literacy and buy-in to test these new approaches within clinical studies and beyond.

To address some of these challenges, the "Brain-Liquid Biopsy consortium" was established in 2020 as a group of researchers and clinicians with a shared interest in translating the advances in biomarker technology development into clinical advances in the field of neuro oncology. The mission of the consortium is to "*accelerate research and translation of liquid biopsy approaches for brain tumour patients and to support sharing of data and of relevant tissue resources with the ultimate aim to identify liquid biopsy techniques that are relevant in real world clinical settings to improve diagnosis and monitoring for brain tumour patients*." Participants with a shared interest are encouraged to join. Plans for the consortium include (1) linking investigators with relevant expertise to those with relevant tissue, (2) sharing of standard operating procedures across a variety of different approaches as described above, (3) encouraging collaborations and connecting members to industry to expedite bringing technology to consumers, and (4) providing support for researchers applying for federal and philanthropic support. Since inception the consortium has grown to include 45 members from 10 countries. Activities to date include a series of webinars, workshops, and meetings to encourage expertise and resource sharing and to link discovery science to relevant clinical study teams. Consortium members worked with the Society for Neuro Oncology administration to run consecutive education days during the 2020 and 2021 annual meeting on the topic of tissue and liquid biomarker research. The consortium also has links to industry and other relevant academic and translational groups including the Focused Ultrasound Foundation and Blood Pac. Inclusion of statistical and clinical expertise with links to national efforts to collect relevant samples routinely from glioma patients, for example through the UK Brain Matrix study is an important component of the consortium's strengths (https://clinicaltrials.gov/NCT04274283), alongside involvement of partners with experience of development of liquid biomarkers through to approval in other settings. We believe this consortium is well suited to support and encourage collaborations with principal investigators involved in clinical trials by sharing data and findings with consortium members. A liquid biopsy approach has the ability to monitor and survey tumor progression after initial treatment. Those patients on specific clinical trials could have their liquid biopsy taken pre- and post-treatment to determine the effect of the trial compared to standard treatment. An example of how liquid biopsy can detect differences between standard

vs trial treatment was recently described by Sabedot et al.[17] They provided three such examples each highlighting dramatic changes in their GeLB score as a response to a specific trial treatment. Investigators of clinical trials or those planning trials should reach out to the consortium for further guidance on how we may synergize efforts.

In summary, the detection of a validated blood based-specific markers through a noninvasive approach, such as liquid biopsy, will not only enable clinical neuro-oncologists the opportunity to assess treatment response in real time and monitor impending disease progression and recurrence, but also identify actionable molecular targets to better stratify patients to the appropriate clinical trials. The prospective assessment of liquid biopsy will also aid in determining the best treatment course during clinical follow-up of one of the most aggressive human cancers to date. At the moment, we cannot recommend the best approach to detect and monitor progression, however, we believe that the approaches with the highest potential would be the ones that are cost-effective, where the liquid biopsy sample is easily attainable (eg, simple blood draw, urine, or saliva), and for which the approach can demonstrate high accuracy (ie, at or near 100%). Coupled with advances in tissue biomarkers and advances in large-scale integrative "omics" data as well as novel machine learning methods emerge, it is clear that noninvasive detection and monitoring of gliomas and other CNS diseases offers exciting new treatment opportunities that can lead to improved quality of life for our patients.

References

1. Thust SC, van den Bent MJ, Smits M. Pseudoprogression of brain tumors. *J Magn Reson Imaging*. 2018;48(3):571–589.
2. Booth TC, Williams M, Luis A, et al. Machine learning and glioma imaging biomarkers. *Clin Radiol*. 2020;75(1):20–32.
3. Rasmussen BK, Hansen S, Laursen RJ, et al. Epidemiology of glioma: clinical characteristics, symptoms, and predictors of glioma patients grade I–IV in the Danish Neuro-Oncology Registry. *J Neurooncol*. 2017;135(3):571–579.
4. Kwapisz D. The first liquid biopsy test approved. Is it a new era of mutation testing for non-small cell lung cancer? *Ann Transl Med*. 2017;5(3):46.
5. Kok VC, Yu C. Cancer-derived exosomes: their role in cancer biology and biomarker development. *Int J Nanomedicine*. 2020;15:8019–8036.
6. Jones JN, Nguyen H, Drummond K, Morokoff A. Circulating biomarkers for glioma: a review. *Neurosurgery*. 2021;88(3):E221–E230.
7. Garcia-Murillas IC, Chopra N, Comino-Mendez I, et al. Assessment of molecular relapse detection in early stage breast cancer. *JAMA Oncol*. 2019;5(10):1473–1478.
8. Rothwell DG, Ayub M, Cook N, et al. Utility of ctDNA to support patient selection for early phase clinical trials: the TARGET study. *Nat Med*. 2019;25(5):738–743.
9. Miller AM, Shah RH, Pentsova EI, et al. Tracking tumour evolution in glioma through liquid biopsies of cerebrospinal fluid. *Nature*. 2019;565(7741):654–658.
10. Zhu L, Nazeri A, Pacia CP, Yue Y, Chen H. Focused ultrasound for safe and effective release of brain tumor biomarkers into the peripheral circulation. *PLoS One*. 2020;15(6):e0234182.
11. Tirosh I, Suva ML. Dissecting human gliomas by single-cell RNA sequencing. *Neuro Oncol*. 2018;20(1):37–43.
12. Muller Bark J, Kulasinghe A, Chua B, Day BW, Punyadeera C. Circulating biomarkers in patients with glioblastoma. *Br J Cancer*. 2020;122(3):295–305.
13. Sol N, In 't Veld S, Vancura A, et al. Tumor-educated platelet RNA for the detection and (pseudo)progression monitoring of glioblastoma. *Cell Rep Med*. 2020;1(7):100101.
14. Ricklefs FL, Maire CL, Matschke J, et al. FASN is a biomarker enriched in malignant glioma-derived extracellular vesicles. *Int J Mol Sci*. 2020;21(6):1931.
15. Theakstone AG, Brennan PM, Jenkinson MD, et al. Rapid spectroscopic liquid biopsy for the universal detection of brain tumours. *Cancers*. 2021;13(15):3851.
16. Nassiri F, Chakravarthy A, Feng S, et al. Detection and discrimination of intracranial tumors using plasma cell-free DNA methylomes. *Nat Med*. 2020;26(7):1044–1047.
17. Sabedot TS, Malta TM, Snyder J, et al. A serum-based DNA methylation assay provides accurate detection of glioma. *Neuro Oncol*. 2021;23(9):1494–1508.
18. Noushmehr H, Herrgott G, Morosini NS, Castro AV. Noninvasive approaches to detect methylation-based markers to monitor gliomas. *Neuro Oncol Adv*. 2022;4(S2):ii22–ii32.
19. Mouliere F, Chandrananda D, Piskorz AM, et al. Enhanced detection of circulating tumor DNA by fragment size analysis. *Sci Transl Med*. 2018;10(466):eaat4921.
20. Mair R, Mouliere F. Cell-free DNA technologies for the analysis of brain cancer. *Br J Cancer*. 2022;126(3):371–378.