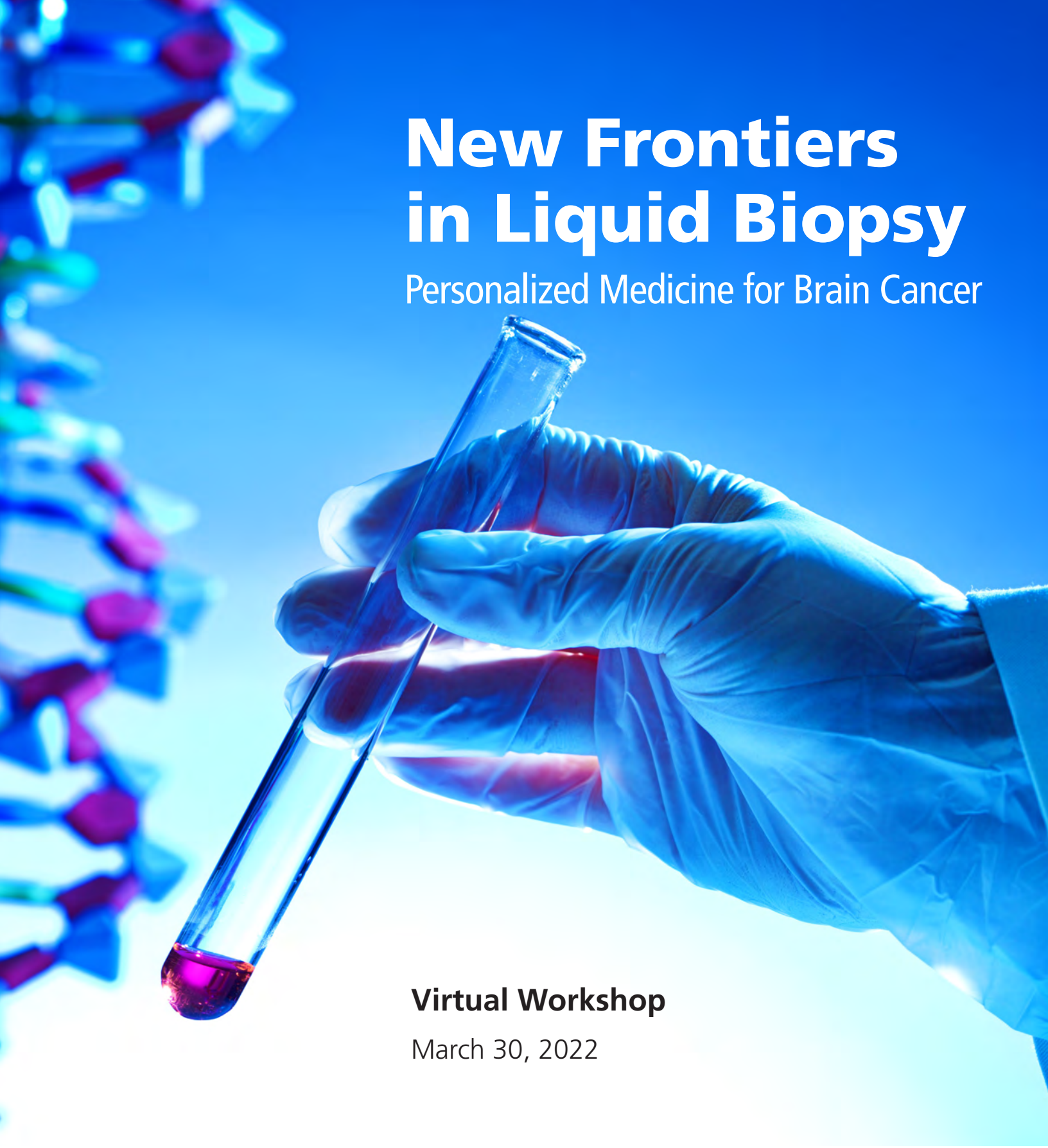

New Frontiers in Liquid Biopsy

Personalized Medicine for Brain Cancer

Virtual Workshop

March 30, 2022

Hosted by

BLOODPAC

In partnership with

Focused Ultrasound Foundation

C2i Genomics

Contents

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Executive Summary

On March 30, 2022, BLOODPAC hosted a virtual webinar on liquid biopsy (LB) for brain tumors in the adult and pediatric populations. The Focused Ultrasound Foundation and C2i Genomics, both members of BLOODPAC, contributed to creating the agenda for the event. The meeting brought together critical stakeholders including researchers, clinicians, medical device and pharmaceutical companies, and others to share and combine knowledge to advance the field.

The goals of the virtual workshop were to educate BLOODPAC's scientific committee and other invited guests on the state of the field of LB for brain tumors, identify gaps in knowledge and technology, discuss the barriers to clinical adoption, and suggest a roadmap to move forward.

There were four presentations summarizing the state of the field of LB for brain tumors, current challenges with LB in the pediatric brain tumor space, the use of focused ultrasound (FUS) to enhance the yield of LB for brain tumors, and suggested strategies on how BLOODPAC can lead the field and improve the medical care of adults and children with brain tumors.

During the panel discussions, additional experts joined to discuss burning questions such as:

- How can LB advance clinical trials for drugs?
- What are the challenges to clinical adoption for LB and brain tumors?
- What are challenges and opportunities of using imaging alongside LB?
- How can BLOODPAC help incorporate LB for brain cancers?

After the panel discussion, attendees asked provocative questions during a live Q & A session. The entire group was thoroughly engaged. Going forward, BLOODPAC's scientific committee was asked to continue thinking and collaborating on these issues and to share any additional thoughts with their colleagues.

The link to view the workshop and the recorded presentations can be found on the Foundation's **YouTube channel:**
https://tinyurl.com/FUSF-Bloodpac

Welcome and Introduction

Watch the recording
5 minutes
https://youtu.be/9zuNPo723yo

BLOODPAC Executive Director **Lauren Leiman** welcomed attendees and recognized the webinar as a new effort on behalf of BLOODPAC to address underserved populations. To date, the use of LB for brain tumors has lagged behind its use in solid tumors outside the central nervous system (CNS). Due to recent conversations with **Suzanne LeBlang, MD**, a neuroradiologist from the Focused Ultrasound Foundation, regarding the potential of FUS to enhance the yield of LB for brain tumors, and **Asaf Zviran, PhD**, from C2i Genomics regarding technological advances in LB and pediatric brain tumors, BLOODPAC wanted to dedicate resources to explore the state of the field for LB of brain tumors in the adult and pediatric population.

By convening experts in the field to provide four educational presentations, a panel discussion, and live Q&A, the goal was to determine whether BLOODPAC's scientific committee deemed that BLOODPAC should take on the challenge of identifying if brain tumors is a topic BLOODPAC should further explore to help adult and pediatric patients suffering from brain tumors. Ms. Leiman stressed that this was an important topic and pivotal moment. She welcomed the workshop's moderators, Dr. LeBlang and Dr. Zviran, who are both members of BLOODPAC.

Dr. Zviran thanked the speakers and panelists for their contributions and time and described how his company C2i Genomics, provides a global platform for cancer treatment monitoring. He shared his personal journey of undergoing radiation therapy many years ago and sitting next to a young 6-year-old child with brain cancer whowas also undergoing radiation. These experiences inspired him to improve the treatment of pediatric cancers with LB. He wanted to convey this message to the team at BLOODPAC and the opportunity to make a difference in lives of adult and pediatric patients.

Dr. LeBlang shared the challenges and frustrations of diagnosing and following patients and friends with brain tumors on imaging studies. Due to her work as the Director of Clinical Relationships at the Focused Ultrasound Foundation, she has been intimately involved with the clinical trials using FUS to open the blood-brain barrier (BBB) and enhance drug delivery to brain tumors. Recent preclinical and clinical research has also shown the potential of FUS to allow bidirectional flow across the BBB, creating a "window" into the brain so that analytes from brain tumors can flow back onto the peripheral circulation and be detected by LB. In researching LB for brain tumors, she came across BLOODPAC, engaged in multiple discussions with Ms. Leiman, joined BLOODPAC, volunteered to serve on a working group, and is eager to collaborate to help patients with brain tumors.

Presentations

LB for Malignant Primary Brain Tumors
Current SOTF

Stephen Bagley, MD, MSCE
Medical Oncologist, Assistant Professor of Medicine and Neurosurgery, Penn Medicine

Watch the recording
19 minutes
https://youtu.be/rlLe1dQteN8

LB for Brain Cancer
Urgent and Unmet Clinical Need

Approximately 16,000 new gliomas are diagnosed in the U.S. each year, and 75% are Grade IV glioblastomas (GBMs). GBM is a diffuse, infiltrative tumor with microscopic spread throughout the brain along white matter tracts. It is nearly uniformly fatal despite standard of care therapy—the core components of which are unchanged since 2005—with a median overall survival (OS) of 12 to 15 months. Clinicians strive for more effective therapies, more accurate and precise disease monitoring tools, and less invasive diagnostics.

To spare more invasive and repeated biopsies, there are two areas of specific clinical need:

- Differentiate progression from pseudo progression—transient worsening of contrast enhancement on MRI from chemoradiation or novel immunotherapies is difficult to distinguish from true disease progression. LB could help.
- Noninvasive diagnosis and next-generation sequencing (NGS) is needed for patients with brainstem lesions that cannot be biopsied or removed or those with CNS metastatic foci distant from primary site, where the molecular profile of recurrence differs from that of the primary tumor.

Current State of the Field of LB for Brain Tumors

Most studies are researching cell-free deoxyribonucleic acid (cfDNA) levels rather than extracellular vesicles or circulating tumor cells. Due to the BBB, traditional LB methods (collecting plasma for NGS) have limited clinical utility for brain tumors,[1] reporting essentially no match between mutations in plasma and those from tissue in newly diagnosed GBM.

There are varying approaches for using LB for brain tumors. A current approach uses tumor-guided whole exome sequencing (WES) for potential disease monitoring and response assessment. Furthermore, a technique called personalized, hybrid-based capture can evaluate multiple mutations, whereas droplet digital polymerase chain reaction (PCR) can detect one specific mutation.

A new approach, highlighted in a pilot study of plasma cfDNA WGS by Bagley et. al., is based on the hypothesis that WGS of tumor tissue may produce a personalized tumor signature that can be subsequently detected in the plasma [given the scarcity of circulating

tumor deoxyribonucleic acid (ctDNA) in patients with GBM]. Bagley et. al. reported that the breadth of sequencing supplants the depth of sequencing to overcome the barrier of low ctDNA levels.[2] Bagley's group also found a high correlation of copy number variation (CNV) profiles between tumor tissue and plasma samples and that the tumor fraction (TF) at the first postsurgical timepoint on day one correlated with OS. A high TF was associated with an OS of 8 months whereas a low TF was associated with 19 months of OS. The researchers wondered whether this schema had the potential for following minimal residual disease.

Another recent report showed that plasma cfDNA methylation profiles revealed specific signatures to accurately discriminate primary intracranial tumors.[3] Other centers are studying whether cerebrospinal fluid (CSF) can be a reliable source for LB. Because it may have more intimate contact with tumor, CSF may yield 10-fold higher ctDNA levels compared with plasma. Other benefits of CSF include that it is CSF paucicellular with lower background levels of cfDNA. Reports have detected a variance of shared mutations between CSF and tissue. However, CSF content may vary depending on tumor location, whether the sample is obtained from lumbar puncture versus intraoperative collection, and whether it is fresh or frozen.

In a recent review of brain tumor clinical trials, it was noted that increasing immunotherapy trials need better ability to discriminate tumor progression versus pseudo progression.[4] In addition, a number of molecular-targeted therapies require NGS for eligibility (to test for epidermal growth factor receptor [EGFR]). The momentum is accelerating toward the need for multicenter trials, as described in a recent article by the Response Assessment in Neuro-Oncology (RANO) LB working group.[5]

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Summary/Take Home messages

1. LB technologies hold great promise to improve the care of patients with brain tumors.

a. Establish a diagnosis when surgery is impossible
b. Detect potentially targetable genetic alterations missed by tissue sequencing (tumor heterogeneity)
c. Improve disease monitoring, leading to earlier and more accurate detection of tumor progression and distinguish pseudo progression
d. Avoid repeat surgeries to obtain up-to-date tumor genetics at time of recurrence

2. Need to identify

a. Standards of biofluid collection, processing, and storage
b. Choose highest priority analytes and their optimal detection strategy
c. Adequately powered, prospective multicenter studies are needed
    i. Pre-specified LB biomarkers should accompany therapeutic clinical trials
    ii. Identify optimal population, disease setting, and more for each biomarker

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Current Challenges in Using LB for Diagnosis and Follow Up in Pediatric Neuro-Oncology

Matija Snuderl, MD
Neuropathologist and Director of Molecular Pathology and Diagnostics, NYU Langone Health

Watch the recording
17 minutes
https://youtu.be/pl5dHNrxFg8

Background Information on Pediatric Brain Tumors

The World Health Organization now defines brain tumors molecularly and not just histologically. Single histologic subtypes may have numerous molecular subtypes. Whole genome methylation is used to subclassify brain tumors. The molecular signature impacts disease monitoring and OS, so assays that are sensitive, noninvasive, and easily repeatable are needed.

Pediatric brain tumors are the most common solid tumor in children (representing 25% of all cancers in patients less than 25 years old) and the leading cause of cancer-related deaths in this population. There has been a 35% rise in incidence from the mid 1970s to mid 1990s. Diagnostic delay is common, with a median time frame of 6 to 14 weeks. Survival rates improved from less than 50% to 70% 5-year OS in first world countries. The diagnosis, management, and long-term follow up currently uses MRI but needs to improve.

Limitations of LB for Pediatric Brain Tumors

A comprehensive review of LB for pediatric brain tumors[6] highlighted the following:

- **Overall challenges for CNS tumors**
  - Low number of mutations in coding regions when compared to non-CNS tumors
  - Brain tumors lack known somatic/coding drivers despite distinct RNA and DNA methylation signatures
  - Brain tumors are molecularly highly heterogeneous, and there are more than 100 distinct molecular subclasses
  - Brain tumors are rare and thus difficult to study many patients
  - Presumed low ctDNA load in peripheral blood due to BBB
- **Additional challenges in the pediatric population**
  - Fewer mutations and even fewer coding mutations
  - No recurrent hotspot mutation drivers
  - Large volumes of blood required for testing not practical
  - Rare tumors
  - Multiple molecular subtypes with different clinical prognosis
  - Requires collaborative multi-institutional efforts

Several articles have provided additional information to help advance the field. These are summarized below.

1. LB Detection of Genomic Alterations in Pediatric Brain Tumors from cfDNA in Peripheral Blood, CSF, and Urine.[7] Analysis of 564 specimens from 258 patients using ultra low pass WGS for CNV and pediatric tumor hybrid-capture panel for deep sequencing of mutations and fusions showed:

- Very few CNV and mutations in all samples
- Most samples had insufficient somatic mutations to provide sufficient power to detect TF greater than 0.1%
- Positive results only in high-grade tumors

2. Next-Generation Sequencing of CSF for Clinical Molecular Diagnosis in Pediatric, Adolescent, and Young Adult Brain Tumor Patients.[8] Analysis of 64 samples from 45 patients using aliquots ranging from 0.4 to 12 mL for MSK-IMPACT assay showed somatic alterations in 30 of 64 samples (46.9%) but positively correlated with disseminated disease at the time of collection (84.6% of samples with disseminated disease were positive) and thus not good for detecting minimal residual disease (MRD).

3. Pilot study using patient-specific tumor signature WGS applied to plasma at diagnosis and for repeat monitoring.[9] This study used only 2 to 3 mL of blood. It found that mutations in coding regions were much lower than non-coding regions. The technique is valuable across different brain tumor types and can be used in different clinical scenarios.

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Summary/Take Home messages

1. **Because brain cancers are rare and molecularly diverse, collaborative multi-institutional studies are needed.**
2. **The amount of blood needed from pediatric patients could be a limiting preanalytical factor, but new ctDNA WGS can be performed with only 2 to 4mL of blood.**
3. **CSF LB shows higher yield but is more invasive, higher risk, and not suitable for repeat monitoring.**

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FUS for Brain Tumors
An Emerging Tool for Liquid Biopsy and Therapy

Nir Lipsman, MD, PhD
Neurosurgeon, Sunnybrook Health Sciences Centre

Watch the recording
13 minutes
https://youtu.be/ED5Qvu9eswQ

Background of FUS

LB is a rapidly growing field driven by advances in oncology. The low-frequency FUS system used for BBB opening can deliver therapeutics into the brain and can also allow for analytes to flow out of the brain. Other approaches to bypass the BBB have not been universally effective. FUS uses intravenously injected microbubbles (contrast agents) that absorb energy when exposed to FUS. This causes the microbubbles to oscillate, which physically opens the BBB in a transient, targeted, and safe fashion under MR guidance.

The successful detection of rare events, such as cf/ctDNA, is highly dependent on sufficient blood sampling and shedding of biomarkers from tumors. Is there a less invasive, targeted approach to enhance peripheral biomarkers for CNS diseases that is safe and clinically effective? There is compelling evidence from more than 300 preclinical studies for temporary, repeated, and safe BBB opening with FUS from rodents to non-human primates.[10] Numerous ongoing clinical studies with FUS-enhanced targeted drug delivery confirm BBB opening with gadolinium enhancement. Phase 2 trials in patients with GBM are performed with the patient awake. It is a noninvasive, day procedure that lasts two to three hours with greater than 30 cc of BBB opening for a 2-cm margin around the resection cavity without any anatomic restrictions. There is an ongoing Phase 1 trial with metastatic Her2+ breast cancer delivering Herceptin to eloquent areas, including the brainstem and posterior fossa. This study is unique because Herceptin is tagged with Indium-111 to visualize definitive evidence of delivering large antibodies (145KDa) across the BBB.

FUS and LB – Bidirectional Flow Across the BBB

A paper titled "MR-guided Focused Ultrasound (MRgFUS) Liquid Biopsy Enriches Circulating Biomarkers in Patients with Brain Tumors,"[11] provided the first evidence of safe, noninvasive, targeted BBB opening in brain cancer leading to enriched signal of circulating brain-derived biomarkers. A prospective, single-arm, open-label pilot trial evaluated nine patients with GBM who underwent 38 FUS BBB opening procedures and two patients with Alzheimer's disease who also obtained a FUS BBB opening treatment. Opening the BBB leads to increased detection of cfDNA, by at least two-fold. cfDNA increases correlated with BBB opening volume. Methylation profiling differentiated patient populations.

Next steps for evaluating brain cancer and LB involve continued sampling of blood during trials for drug delivery, including primary brain tumors (GBM, malignant astrocytoma, subependymal giant cell astrocytoma), secondary brain tumors (Her2+, lung, and melanoma metastases), and pediatric tumors (diffuse intrinsic pontine glioma).

Summary/Take Home messages

1. **FUS-enhanced LB has the potential to increase analytes from brain tumors in the peripheral blood in a safe, targeted, and easily repeatable fashion.**
2. **Various questions need to be considered by topic:**
   - **Patients/Diseases**
     - Which primary?
     - What stage of treatment?
     - What is the role in standard of care?
     - Role in leptomeningeal disease?
   - **Treatment and therapeutic**
     - Which analytes?
     - How to optimize FUS parameters?
     - Therapeutic vs BBB opening alone?
   - **Follow up**
     - How to define outcomes?
     - What are meaningful outcomes?
     - Go/no-go rules?
     - How to optimize imaging?
     - Differentiate progression from pseudoprogression?

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Liquid Biopsies in Brain Tumors
Strategies for BLOODPAC to Lead the Field and Improve the Medical Care of this Underserved Population

Manmeet Singh Ahluwalia, MD, MBA
Chief of Medical Oncology, CSO, and Deputy Director, Miami Cancer Institute,
Professor of Medicine, Herbert Wertheim College of Medicine

Watch the recording
12 minutes
https://youtu.be/RtaiaZw0NHQ

Current Challenges and Limitations of LB for Brain Tumors

Outside the CNS, LB predicts response to immunotherapy, helps identify progression from pseudo progression, and can predict OS. CNS tumors are challenging with difficulties in drug delivery and detecting LB analytes. The sensitivity for ctDNA in brain tumors less than 50% of patient's versus systemic cancers, which can be used in more than 95% of patients.[12] Gliomas have the least amount of ctDNA in the blood.[13] The BBB limits the shedding of ctDNA into the blood.

There is an unmet need in brain tumors and personalized medicine that requires routine invasive biopsies. This is addressed in several publications:

1. **Tracking Tumor Evolution in Glioma through Liquid Biopsies of CSF.**[14]
   This study reported that lumbar puncture does not produce a higher success rate of LB than plasma sampling (49.4% detection rate):

   "The ability to monitor evolution of the glioma genome through a minimally invasive technique could advance the clinical development of genotype directed therapies for glioma, one of the most aggressive human cancers."

2. **Blood-Based Biomarkers for the Diagnosis and Monitoring of Gliomas.**[15]
   This article states that there is an:

   "…incredible need for a minimally invasive 'liquid biopsy' to assist in molecularly characterizing the tumors while also aiding in the identification of true progression in GBM," and "the ability to obtain serial 'liquid biopsies' will provide unique opportunities to study the evolution of tumors and mechanisms of treatment resistance and monitor for mutational changes in response to therapy."

3. **Re-Evaluating Biopsy for Recurrent GBM; A position Statement by the Christopher Davidson Forum Investigators.**[16]
   This article says:

   "we propose a dramatic but necessary change to routine management of patients with GBM to advance the field: to routinely biopsy…."

FUS-enhanced LB holds promise for providing a noninvasive method to improve the care of patients with brain tumors. Meng, et al. demonstrated that MRgFUS can safely and transiently open the BBB to allow passage of cfDNA for analysis, providing an opportunity for less invasive access into the brain.[11] These data were also confirmed by unpublished results from the University of Maryland and Dr. Graeme Woodworth.

A 2022 position statement was published in *Neuro-Oncology:* "Liquid Biopsy in Gliomas: A RANO Review and Proposals for Clinical Applications.[5] The RANO team summarized that ctDNA has a higher sensitivity and capacity to represent the spatial and temporal heterogeneity in comparison to circulating tumor cells. In addition, clinical applications of LB for brain tumors may include the ability to establish a diagnosis when tissue is not available, monitor residual disease after surgery, distinguish progression from pseudo progression, and predict outcomes. The authors stressed the need for standardization with rigorous testing in future clinical trials.

Insightec (Haifa, Israel) is starting the LIBERATE clinical trial: Liquid Biopsy with Low Intensity FUS in Brain Tumors to evaluate the safety and effectiveness of opening the BBB for LB in patients with GBM. This study is recruiting patients with suspected GBM on preoperative scans who have been scheduled for surgical resection or biopsy within four weeks of the study procedure. It is an unblinded, prospective multicenter self-controlled, pivotal clinical trial using a central pathology laboratory. There will be 50 subjects enrolled across up to 10 treatment sites. Pre- and post-FUS BBB opening blood draws at 30 minutes, 1 hour, 2 hours, and 3 hours. The primary endpoint is the ratio between cfDNA one hour after FUS BBB opening compared with pre—BBB opening. The secondary endpoint will evaluate the correlation between patterns in biomarkers of resected tissue/ biopsy and blood samples drawn one hour after BBB opening.

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Summary/Take Home messages

1. **MRgFUS may increase the yield of LB for patients with brain tumors.**
2. **Coordinated efforts are needed.**
3. **The time is now for BLOODPAC to help lead these efforts.**

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Panel Discusson

Moderators
Dr. Zviran and **Dr. LeBlang**

Panelists
Patrick Wen, MD, Dana Farber Cancer Institute; **Lauren Abrey, MD**, Novartis;
Uri Weinberg, MD, PhD, Novocure; and **Kirk Tanner, PhD**, National Brain Tumor Society

Watch the recording
50 minutes
https://youtu.be/y7OmXkYA4M4

Take-Home Messages

- LB has the potential to assist in brain tumor clinical trials for drug and technology development.
- There are numerous challenges for the clinical adoption of LB, including the need to collaborate across institutions given the rarity of tumors and incorporate industry, nonprofits, and government into the discussions.
- LB could fill a much-needed gap in diagnosing and following brain tumor patients. Current imaging with MRI is not sensitive or specific enough to characterize various types of brain tumors, distinguish tumors from other pathologies such as multiple sclerosis, or reliably differentiate progression from pseudo progression.
- Further research using CSF as a source of LB is warranted, and potentially important for leptomeningeal disease as well.
- BLOODPAC has the potential to form critical collaborations in the brain tumor space to help set standards, design and support multicenter clinical trials, and house and interpret data.

Q&A Session

Can we improve clinical trials for drug/device development for brain cancers by using LB?

Dr. Abrey. My wish is to use ctDNA or other analytes to develop drugs intentionally for brain tumors. Practically, we need to standardize efforts because interpretation of imaging studies is vague. More confidence with LB can help differentiate progression from pseudo progression and rely also on OS. Thus far, the barrier is that numerous trials have been negative in using LB for these purposes.

Dr. Weinberg. Tumor-treating fields use alternating electric fields to act as anti-mitotic therapy and the device that creates them is U.S. Food and Drug Administration (FDA)—approved for newly diagnosed GBM. Many critical elements to further develop this modality and fine tune or adjust the properties/parameters of the electric fields are needed to personalize each patient's treatment. Feedback from LB could potentially increase the success of tumor-treating field therapies and help identify patients that could most benefit from Novacure via identifying/measuring a biomarker.

What are the challenges for adoption of LB?

Dr. Tanner. We need clear hypotheses how diagnostics can work in brain cancer. Motivated patient and caregiver communities are committed to research that will cooperate with academic centers. We need to partner with the FDA and other organizations. There is a critical need for capital investment and to foster partnerships between big pharma, venture capital, venture philanthropy, family offices, foundations, and government funding agencies. The bar is set low for clinical improvement, but we do need generate a lot of data by sharing, because brain tumors are rare. Priority review vouchers for pediatric studies and breakthrough designations can help expedite the process. There is a real opportunity with the present Biden Administration to focus on brain tumors.

What are challenges/opportunities of using imaging alongside LB?

Dr. Wen. It is hard to imagine a cancer that needs LB more than GBM, but there are also more challenges. In addition to sensitivity and specificity needing improvement, because tumors are rapidly growing, we need to consider the timing for getting LB results back to impact treatment. LB can aide in differentiating tumors, such as lymphoma, GBM, and metastases, diagnosing tumors from active multiple sclerosis plaques, and diagnosing progression from pseudo progression (which occurs in 30% to 50% of patients after radiation). Pseudo progression is also problematic after immunotherapies because MRI is not specific. If CSF LB is effective, consider it easier than a bone marrow biopsy. Temporal heterogeneity is a challenge, and EGFR mutation is lost after recurrence. Yet recruitment into trials is based on original diagnosis, so we need updated information with LB to guide targeted therapies.

Dr. Abrey. I agree with Dr. Wen, and this is an important topic as we potentially discard a therapy that may be good and thus need updated information on recurrence with unstable genetic alterations. We can have positive effects with drugs with stable mutations that won't change over the course of therapy. Also, the transformation of low-grade gliomas into high grade is difficult on imaging but LB could be helpful.

What is the risk of lumbar puncture vs liquid biopsy?

Dr. Ahluwahlia. Lumbar puncture without large mass effect is safe. We perform it in patients with metastases, but not necessarily those with primary brain tumors. As we get more drugs, we do not know how to use them. If we use EGFR-directed therapy, there is a more than 50% chance of change in this status. CSF is an exciting prospect.

Dr. Lipsman. Lumbar punctures are quite safe compared to spectrum of invasiveness with brain surgery, which is the ultimate risk. Lumbar puncture is safe but cumbersome, and it is hard to justify multiple procedures over time, so LB could be helpful. We are comparing pre- and post-FUS BBB opening with plasma and tissue and comparing it to imaging studies at various timepoints to help distinguish progression from pseudo progression.

How can BLOODPAC help incorporate LB for brain cancers?

Ms. Leiman. There are three ways we can easily move forward, following past BLOODPAC models of collaboration: 1) Set standards for biofluid collection under a recommended data elements working group; 2) Simulate projects like EXHALE and aggregate data for LB brain; and 3) Perform a prospective pilot study like the ones funded by BCRF, PCF, FDA (lung) as academic centers receive funding from these organizations and BLOODPAC corporate partners donate services in kind.

Dr. Jim Godsey, PhD. Congratulations to the speakers on their research efforts. I loved hearing about C2i WGS breadth versus depth allowing for specific testing and learning how FUS can reach all areas of brain for greater than two-fold increase in analytes with BBB opening, which can increase diagnostic yield and allow large molecules into the brain for precision medicine.

Dr. Kelli Bramlett, PhD. Consider gene expression with RNA. BLOODPAC can help define some experiments.

Does FUS have negative side effects from opening the BBB? Does it promote metastases?

Dr. Lipsman. So far, safety data for MRgFUS BBB opening is compelling but under active investigation. Most patients are on standard-of-care chemotherapy, so this may decrease metastases. Well-controlled, systemic disease has been followed for months and years with no development of new intracranial disease thus far. Clinically, it is a well-tolerated procedure and patients go home the same day with no serious adverse events. There are minor adverse events related to the use of the stereotactic frame or from lying in the MRI scanner. These are improving with time. Radiographically, no significant adverse event bleeding or swelling has been observed, so the procedure appears to be safe and well-tolerated.

Dr. Bramlett. Thanks for showing us what the MRgFUS device looks like.

Dr. Lipsman. Using image guidance while the patient is inside the MRI scanner is very valuable. The helmet coupled to MRI can develop point of care noninvasive biopsy and better tools.

Dr. Howard Scher, MD. I suggest baby steps. We need early clinical validation and standardized validation of MRI. We could dramatically change patient management.

Dr. Phil Febbo, MD. The presentations were outstanding. It is disappointing that gliomas had only a small amount of cfDNA from Bettegowda's paper, but signal is improving, and MRgFUS can add value. For BLOODPAC, the MRD space is critical. If we want to make meaningful progress in therapies, we must get more facile on how we sample tissue, and this has been a limitation based on location. Apply the combination of sampling the blood, perhaps intervening with MRgFUS to amplify signal in the blood, and create an opportunity to find a path for new therapies and assess them more rapidly, especially where imaging is not helpful. I can now appreciate the need to go broad to assess signature. This is promising and very exciting!

Dr. Don Johann, MD, MSc. BLOODPAC data commons can harmonize data and compare solid tissue biopsy and tumor analysis. Bob Grossman designed NCI data commons, and correlation with clinical data and modalities with high thruput NGS is critical to arrive at answers quicker especially with the use of novel techniques.

Dr. Achal Achrol, MD, PhD. Understanding the clearance of cfDNA is interesting for MRD and calling for response to therapies and distinguishing progression from pseudo progression could be clinically quite useful. If we can predict response using LB, it is promising, but yield is still a factor. Researchers reported a seven-fold boost in signal in Dr. Lipsman's paper with MRgFUS, and the pivotal trial will note if this technique can help. MRgFUS may act synergistically by opening the BBB to deliver therapeutics, amplifying the signal from analytes, and releasing neo antigens with their potential immune priming effect turning a cold tumor hot. MRgFUS could be layered in a monthly treatment regimen. More than 100 patients with 400 cycles of treatment have now been treated with MRgFUS BBB opening with no serious adverse events, so now studies can focus on the clinical benefit of treatments.

Is there a biobank for CSF?

Dr. Snuderl. NYU has program for banking CSF, but it is a precious commodity.

Jonathan Beer, MBA – Novartis. BLOODPAC helped standardize blood collection parameters using commercially acquired material. This will be difficult for CSF.

Dr. Alexandra Miller, MD PhD. We have 300 samples in the adult space and 200 pediatric samples of CSF in brain tumors. We could build a bank across institutions. Laboratories need 3 cc for LB and typically take 20 cc, so this would be possible.

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Acknowledgements

Dr. LeBlang thanked everyone for attending and participating in the workshop. She appreciated the collaboration across industry, academia, and nonprofits in hopes of working together to expedite research and development. She thanked Ms. Leiman for leading BLOODPAC and creating this event for underserved population and Dr. Zviran for his vision in creating the agenda.

Dr. Zviran summarized that we are on the verge of exciting advances in cancer management. Data show that using low collection volumes and assessing coding and noncoding regions allows us to evaluate low mutation loads, especially in pediatrics. He looks forward to working with BLOODPAC. He thanked the speakers and panelists as well as the audience for their participation.

The workshop was hosted by BLOODPAC and planned by Ms. Leiman, Dr. LeBlang, and Dr. Zviran and This white paper was written by Suzanne LeBlang, MD, and edited by Jill W. Roberts, M.S. Graphic design was provided by Sara Myhre and Anne Chesnut. Presenters and panelists were provided the opportunity to review this document.

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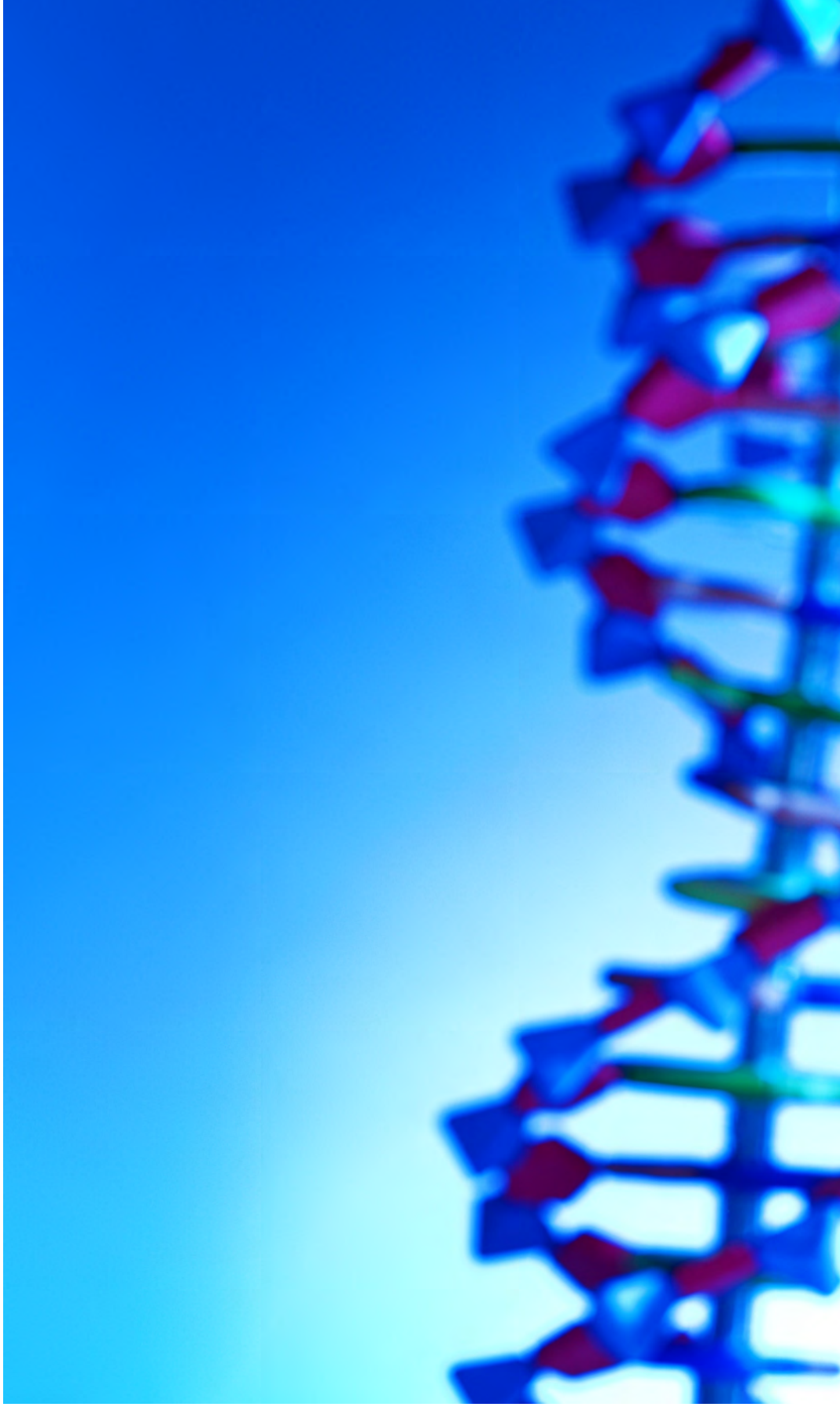
Abbreviations

BBB	blood-brain barrier
cfDNA	cell-free deoxyribonucleic acid
ctDNA	circulating tumor deoxyribonucleic acid
CNS	central nervous system
CNV	copy number variation
CSF	cerebrospinal fluid
FDA	U.S. Food and Drug Administration
FUS	focused ultrasound
GBM	glioblastoma
LB	liquid biopsy
MRD	minimal residual disease
NGS	next-generation sequencing
OS	overall survival
PCR	polymerase chain reaction
RANO	Response Assessment in Neuro-Oncology
TF	tumor fraction
WES	whole exome sequencing



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