Exploring the Feasibility of Cerebrospinal fluid (CSF) Liquid Biopsy in Patients with Metastatic Solid Tumours and Leptomeningeal Disease (Cohort A), Parenchymal Brain Metastases (Cohort B), or No Evidence of Central Nervous System (CNS) Metastases (Cohort C): A Pilot Study

STUDY SYNOPSIS

Research Questions:

1. Among patients with metastatic solid tumours in each of three pre-defined cohorts (A, B, C), what proportion of patients have a positive cytology, a positive circulating tumour DNA (ctDNA), and/or positive circulating tumour cells (CTC) in cerebrospinal fluid (CSF) samples?
2. Among the patients enrolled in the study, how do ctDNA and CTC results obtained from cerebral spinal fluid (CSF) and plasma compare in each of three cohorts (A, B, C)?
3. Is it feasible to perform proteomic analysis of CSF among patients with metastatic solid tumours who have at least one positive CSF biomarker (cytology and/or ctDNA and/or CTCs) in each of three cohorts (A, B, C)?

Population:

Inclusion Criteria:

1. Age > 18.
2. Diagnosed with a metastatic solid tumour in one of the following scenarios:
    a. Patients in Cohort A will have leptomeningeal metastatic disease (LMD) with or without parenchymal brain metastases.
    b. Patients in Cohort B will have parenchymal brain metastases but no evidence of LMD.
    c. Patients in Cohort C will have metastatic solid tumours no CNS metastases (i.e. no LMD nor brain metastases).
3. Patient is suitable for lumbar puncture and/or has an Ommaya reservoir that is accessible for CSF collection.
4. Patient is eligible at any time point in their treatment whether they have started treatment for LMD
5. Patients who previously enrolled in the study but for whom CSF biomarkers were negative can re-enroll later (e.g. upon progression of disease in the CNS).

Exclusion Criteria:

1. Inability to understand and not willing to sign a written informed consent document (language barrier not excluded; patients can have translator).

Intervention: One-time CSF collective via lumbar puncture or Ommaya reservoir and collection of concurrent plasma of peripheral blood (liquid biopsy).

Comparison: Not applicable.

End points:

1. Primary endpoint: Proportion of eligible patients with positive CSF cytology, positive ctDNA, and positive CTCs.
2. Secondary endpoint: Concordance of mutations identified on CSF ctDNA with plasma ctDNA.
3. 3. Exploratory endpoint: Feasibility of conducting proteomic analysis of CSF.

Study Design:

Prospective single centre feasibility trial conducted at the Sunnybrook Odette Cancer Centre (SOCC).

- Cohort A: 20 patients with any malignant solid tumour with LMD either suspected on imaging and/or confirmed via CSF cytology will be recruited.
- Cohort B: 20 patients with any malignant solid tumour with parenchymal brain metastases will be recruited.
- Cohort C: 20 patients with metastatic solid tumours and no evidence of LMD or parenchymal brain metastases (control group) will be recruited.

Time Target:

Patients will be recruited over a 12-month time period.

1. OBJECTIVES

Primary Objectives

To estimate the proportion of patients with solid tumours and CNS metastases (either meeting criteria for Cohort A or B), or no CNS metastases (Cohort C) who have positive CSF cytology, positive ctDNA, and/or positive CTCs

Secondary Objectives

1. The concordance of mutations identified based on ctDNA profiling in the CSF and blood plasma in each of three cohorts (A, B, C).
2. To explore the feasibility of proteomic analysis using CSF samples in each of three cohorts (A, B, C).

2. BACKGROUND

Leptomeningeal metastatic disease (LMD) is the spread of malignant cells into the pia and arachnoid mater of the brain and spinal cord, affecting approximately 5-8% of all solid tumour types[1]. It is a rare complication that is associated with a high symptom burden and poor prognosis, with median survival ranging from 6-8 weeks without treatment and 3-6 months with treatment[1,2]. Common solid tumours that result in LMD include breast, lung, gastro-intestinal (GI), melanoma, and primary central nervous system (CNS) cancers[1,3]. Resulting from the infiltration of cancer cells into the CNS, LMD can present with various symptoms such as headache, seizures, visual disturbances, weakness, and confusion[1]. The diagnosis and monitoring of LMD remain challenging due to the nonspecific clinical symptoms and false negative rates for MRI imaging and cerebrospinal fluid (CSF) analysis[1].

Current research states that CSF analysis is the gold standard for diagnosing LMD, however circulating tumour cells (CTCs) and circulating tumour DNA (ctDNA) are new alternative diagnostic methods that have not been thoroughly evaluated to-date[1]. In this study, CSF samples collected via lumbar puncture (LP) or an Ommaya reservoir, along with blood plasma, will be obtained from patients with solid tumours who have diagnosed or suspected LMD. These samples will be analyzed for biomarkers to explore their association with clinical outcomes and potential therapeutic targets, ultimately guiding more effective treatment strategies.

In addition, there is an unmet need to identify biomarkers in the CSF among patients with and without parenchymal brain metastases, even with the absences of LMD. If our exploratory study is successful, we may identify a novel mechanism to understand targetable mutations of CNS metastases through relatively less invasive mechanisms than craniotomy to surgically resect brain metastases.

3. STUDY DESIGN

Cohort A
20 patients with any malignant solid tumour with LMD either suspected on imaging and/or confirmed via CSF cytology will be recruited with or without brain metastases.

Cohort B
20 patients with any malignant solid tumour and parenchymal brain metastases without evidence of LMD will be recruited.

Cohort C
20 patients with any malignant solid tumour and no evidence of CNS involvement (no LMD or parenchymal brain metastases) will be recruited as a control group.

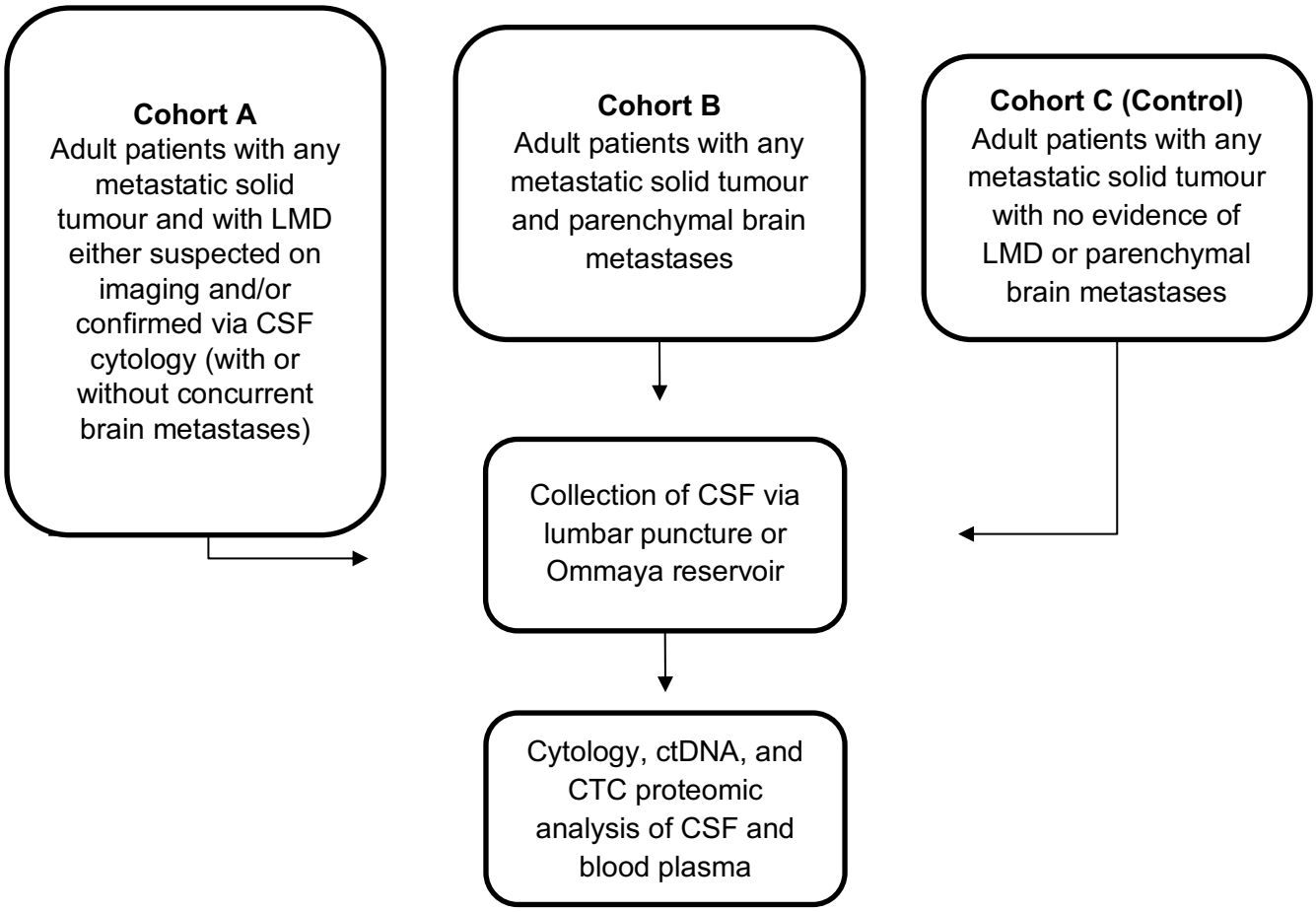

Figure 1: Study Schema

4. ENROLLMENT PROCEDURES

Patient Enrollment

Patients will be screened for eligibility to participate in the study. The study team will inform the treating oncologist regarding their eligibility prior to their medical appointment. After the study is introduced to eligible patients by their oncologist, the study will be discussed in more detail by the study team to obtain informed consent.

5. PLANNED DATA COLLECTION

Planned CSF and Blood Plasma Collection:

CSF collection: Using standard techniques, CSF samples of 15-20 cc will be collected via lumbar puncture (LP) or an Ommaya reservoir. For LP's, the use of a 20-gauge spinal needle and 2% lidocaine anaesthesia at L3/L4 or L4/L5 intervertebral disc space, with the patient in the lateral decubitus position[4]. For ventricular reservoirs such as the Ommaya, a 25-gauge butterfly needle will be used without anaesthesia, and the first 2 mL of CSF collected will be discarded[4]. Due to the fast clearance of tumour cells after sampling, analysis must be performed rapidly by a cytologist[4,5].

Blood plasma collection: Blood samples will be drawn into Streck Tubes. Two 10 mL vials of blood will be drawn per participant, gently mixed, and started upright at 4°C until processing[6]. Processing should occur within four hours of collection[6]. Samples will be centrifuged at 1500 g for 10 minutes at 4°C using a sealed swinging bucket rotor to separate the plasma[6]. Plasma will then be carefully extracted with a pipette set to 900 µL into 1.8 mL aliquots in 2 mL labelled microcentrifuge tubes and placed on ice[6]. A second high-speed centrifugation at 10,000 rpm will be used to remove residual cellular debris[6]. The buffy coat layer will be isolated and stored separately, and any remaining plasma will be used to resuspend the cellular pellet[6]. Compatible plasma samples will be pooled, aliquoted, and stored at -80°C, alongside the buffy coat and pellet[6]. All samples will be clearly labelled and handled under appropriate biosafety and waste disposal protocols[6].

6. BIOMARKER

Biomarker Plan

This study involves the assessment of several biomarkers (cytology, ctDNA, CTCs, proteomics) in the blood and CSF of participants. These analyses will be performed at Sunnybrook and through collaborations with external centres/companies once appropriate material and data transfer agreements are obtained.

7. STATISTICAL ANALYSIS

Planned Cytological Analysis:

CSF and blood plasma samples will undergo proteomic and cytological analysis using the genomic equipment at the Sunnybrook Research Institute (SRI). While the specific platform has yet to be finalized, technologies such as Next Generation Sequencing (NGS), the QX200 Droplet Digital PCR System, and ANGLE plc's Parsortix® system are under consideration for their suitability in cytological and proteomic applications[6].

Planned Statistical Analysis:

For the first objective, binary data will be analyzed to determine the proportion of positive CSF samples from the total number of samples collected. These proportions will then be stratified by tumour type for comparison. The second objective is comparing ctDNA detection in CSF and blood plasma samples. This will involve observing the unique and shared mutations across sample types, and ultimately summarizing findings in a concordance table. From the table, test performance metrics such as sensitivity and specificity will also be derived. The third objective evaluates the feasibility of proteomic analysis and will be measured by the proportion of CSF samples that yield sufficient protein concentrations. The mean number of proteins detected per samples and any commonalities will be reported. All statistical analysis will be conducted using Excel and R. Given the exploratory nature of the study, graphical illustrations and plots will be emphasized over hypothesis testing with p-value.

References

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