Prediction of lung brain metastases recurrence through an epigenomic biomarker

Letter of Intent

Patients with stage IV lung cancer frequently present with brain metastases, a devastating complication that historically carried a dismal prognosis. For decades, these patients were often excluded from clinical trials, and research efforts understandably focused on earlier-stage disease where the prospects for cure were greater. Yet the landscape has changed dramatically. Advances in neurosurgery, stereotactic radiosurgery, and systemic targeted and immunotherapies have extended survival, and we now see a growing population of patients with brain metastases living longer than ever before. Despite these encouraging improvements, recurrence of brain metastases after surgery and radiation remains a major clinical challenge, occurring in up to 60% of patients. For survivors, this recurrence not only drives mortality, but also represents a profound source of anxiety and reduced quality of life.

Unfortunately, research has not kept pace with this reality. Prognostic tools for patients with lung brain metastases remain rudimentary, based largely on tumor size, number of lesions, and clinical staging. These factors fail to capture the complex biology that drives recurrence. What is urgently needed are molecular biomarkers that can stratify recurrence risk, guiding both surveillance intensity and therapeutic decision-making in this vulnerable patient group.

Our proposed project seeks to address this gap by leveraging DNA methylation profiling, a powerful epigenomic technology that captures how genes are regulated in cancer. Methylation-based classifiers have already proven transformative in the classification and prognostication of primary brain tumors. We seek to apply this approach to a cohort of lung brain metastasis patients, comparing those who experience recurrence after surgery and radiation to those who remain recurrence-free. From these analyses, we aim to develop a predictive model capable of identifying patients at highest risk of recurrence.

The ultimate goal is to provide clinicians and patients with a practical biomarker-driven tool that can better inform treatment planning and follow-up care. By focusing on patients with stage IV lung cancer—a group long underrepresented in research—this project directly addresses an urgent and growing clinical need. With support from the *Give a Breath* initiative, we hope to generate findings that can improve both survival and quality of life for patients navigating the challenges of brain metastases.

Background

The development of metastatic brain tumors from systemic cancers is a common and devastating event in lung cancer progression. A quarter of all cancer patients will have a brain metastasis (LBM), a diagnosis which carries a median overall survival of 10-16 months despite standard of care treatment with surgical resection and stereotactic radiosurgery.[1] Advancements in radiotherapy, neurosurgery, and medical oncology have improved the traditionally dismal outcome for patients suffering from BM, leading to an increased number of longer-term survivors.[2,3] However despite these advancements, LBMs tend to recur in up to 60% of patients after surgery and adjuvant radiotherapy, which is a major source of anxiety amongst cancer survivors, and a leading cause of disease mortality.[4,5] Currently, there are very few prognostic tools available to clinicians aimed at predicting disease recurrence, beyond merely extent of resection and tumor burden.[6–8]

The identification of genetic alterations within systemic cancers has resulted in an explosion of targetable molecular drivers of disease, such as *EGFR* and *ALK* in lung adenocarcinoma. Their discoveries have led to targeted therapies, which now play a major role in improving survival in LBM patients.[3,9] The identification of genetic markers has led to intense research for additional biomarkers to help make more accurate prognostic predictions, and to identify more targetable molecular pathways; however, somatic mutations may lack the granularity required to fulfill this goal.[10] As such, the field has turned toward decoding the epigenetic landscape of cancers, particularly through the identification of DNA methylation patterns.[11,12] DNA methylation profiling has proven to be a powerful tool in both the identification and classification of central nervous system tumors.[5,13]

We have established that methylation signatures can predict outcome and recurrence in meningiomas, which are the most common brain tumor type.[14] On the success of this modelling of tumor methylation we have focused on exploring whether DNA methylation signatures from primary lung cancer can be utilizes to accurately predict BM development. We have built a composite nomogram combining methylation based predictor scores with existing TNM cancer staging (Figure 1).

In this proposal we aim to employ a similar approach to uncover epigenomic markers that are associated with recurrent/progressive metastatic lung brain tumors.

Research Aim

Rationale: Currently there exist no clear biomarkers that can be used to predict recurrence or disease progression after surgery and radiotherapy for LBMs. An epigenetic approach to characterizing brain metastases may result in establishment of a set of DNA methylation features that can be used to predict BM recurrence/progression following surgical resection and radiotherapy.

Methodology: At our institution (UHN), we have a robust clinically annotated surgical brain tumor biobank directed by Dr. Zadeh (PD/PI). Our biobank has matched patient tumor samples from over 5500 patients that are stored from the time of surgery and afterwards through longitudinal clinical follow-up in our multidisciplinary Brain Metastasis Clinic. We continue to gather new prospective patients, completing approximately 800 new surgical cases per year.

From this cohort, we have identified a total of N=60 patients who have undergone surgical resection of their LBM and adjuvant SRS, and have experienced a recurrence requiring repeat intervention within 2 year will be identified at our institution. These will be matched with a cohort of N=60 samples from patients with no evidence of recurrence for 2 years. These samples have been identified and are currently available in our Biobank. Clinical data will be collected on patients including demographics, imaging features (T1, T2, etc on MRI), molecular data, pathology records, treatment details and BM recurrence details with related timepoints. DNA methylation profiling of the collected BM tissue will be performed on the Illumina Infinium EPIC array to determine their methylation status at 850,000 CpG sites. Cox-based time to event analyses will be used to determine which CpG site methylation statuses significantly predict recurrence in 70% of patients (training subcohort). Generalized boosted regression classification modeling will be built using these variably methylated CpG sites in the training subcohort and the resulting model will then be applied to the independent 30% testing subcohort to determine patient-specific BM recurrence risk scores. High and low-risk groups will be identified based on median risk scores. A Kaplan-Meier curve of time to BM recurrence will be plotted along with associated log-rank statistic to determine prognostic utility. Area under receiver operating characteristic curves will determine accuracy of the model in classifying patients who will and will not experience recurrence. Methylation based prediction outputs will be compared to clinical factors in multivariate cox analyses in the testing cohort to identify independently prognostic variables.

Impact

This work will uncover unique methylation signatures involved in brain metastasis recurrence. We anticipate that our project will identify signatures that will help predict which patients are at highest risk of disease progression. Furthermore, this information can then be leveraged to create a method of determining the risk of disease recurrence, which can be applied clinically through the use of a nomogram. Finally, the epigenetic features we uncover may be used to isolate targetable pathways for future study. Should we be successful, our work has the potential to identify patience at risk of recurrence prior to even beginning therapy. The results will help improve early detection of recurrence, allowing for earlier intervention with better symptom management and growth control. Moreover, patients at lower risk may benefit from earlier de-escalation or reduced surveillance, decreasing the burden of these interventions on themselves and caregivers.

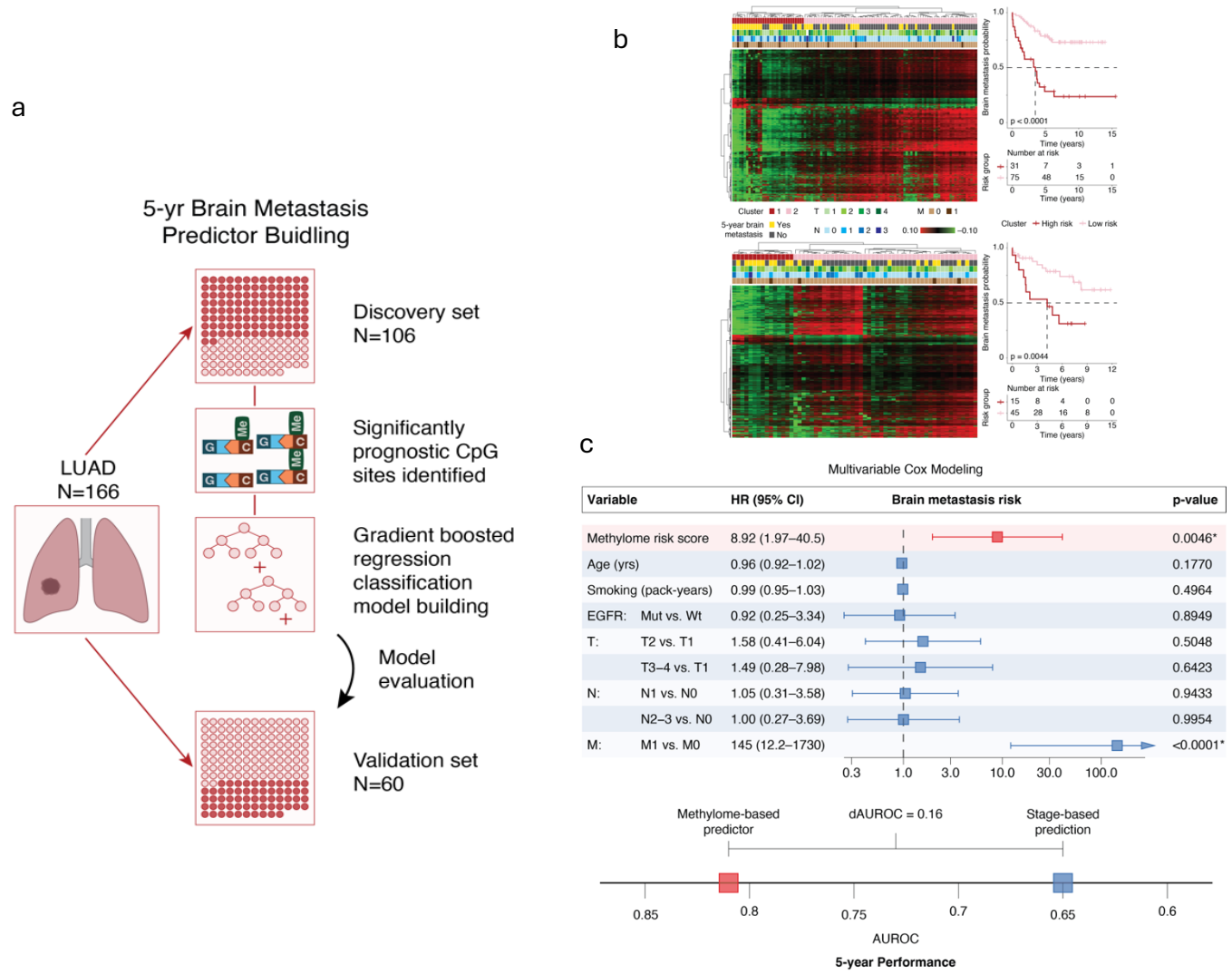

Figure 1. Methylation-based predictor of brain metastasis recurrence in lung adenocarcinoma.
a. DNA methylation profiles from LUAD patients with brain metastases ($N = 166$) were split into discovery ($N = 106$) and validation ($N = 60$) cohorts. Prognostic CpG sites were identified and used to build a gradient boosted regression model. **b.** Heatmaps depicting clustering of prognostic CpGs with corresponding Kaplan–Meier curves stratifying high- vs. low-risk groups. **c.** Multivariable Cox analysis confirmed the methylome risk score as an independent predictor of recurrence (HR 8.92, $p = 0.0046$). Model performance outperformed stage-based prediction

Budget Justification (Total: $25,000)

This project aims to establish DNA methylation biomarkers predictive of recurrence in lung cancer brain metastases. Resources will be dedicated exclusively to essential laboratory consumables for sample processing and methylation profiling. Personnel and infrastructure costs will be covered by institutional support.

1. DNA Extraction (QIAGEN DNeasy Blood & Tissue Kits)

- Required for extraction of high-quality DNA from formalin-fixed paraffin-embedded (FFPE) brain metastasis tissues.
- Estimated cost per sample: **$8–10**.
- For 120 samples (60 recurrence, 60 non-recurrence): **$1,200**.

2. DNA Bisulfite Conversion (Zymo EZ DNA Methylation Kits)

- Required for conversion of unmethylated cytosines prior to methylation array profiling.
- Estimated cost per sample: **$450** (per 96-sample kit).
- For 2 × 96-sample kits: **$900**.

3. Illumina Infinium MethylationEPIC BeadChip Arrays (850K CpG sites)

- Primary assay for genome-wide DNA methylation profiling.
- Estimated cost per sample (academic pricing): **$200–225**.
- For 120 samples: **$24,000–26,000**.

Total Costs (Materials Only):

- DNA extraction: $1,200
- Bisulfite conversion: $900
- Methylation arrays: $22,900

Grand Total: $25,000

Research Team

Dr. Gelareh Zadeh is a Senior Scientist at the University Health Network (UHN) in Toronto and PI of this project. She will determine the direction of this study and provide the required resources and expertise. Dr. Andrew Ajisebutu is a PhD student in Dr. Zadeh's lab whose PhD's focus is biomarker and predictive modelling for brain metastasis. Dr. Vikas Patil is a Post Doctorate computational scientist in the Zadeh Lab with expertise in specific on analysis of DNA methylation and genomic information.

Reference

1. Ostrom QT, Wright CH, Barnholtz-Sloan JS. Brain metastases: epidemiology. *Handb Clin Neurol*. 2018;149:27-42. doi:10.1016/B978-0-12-811161-1.00002-5
2. Suh JH, Kotecha R, Chao ST, Ahluwalia MS, Sahgal A, Chang EL. Current approaches to the management of brain metastases. *Nat Rev Clin Oncol*. 2020;17(5):279-299. doi:10.1038/s41571-019-0320-3
3. Sperduto PW, Mesko S, Li J, et al. Survival in Patients With Brain Metastases: Summary Report on the Updated Diagnosis-Specific Graded Prognostic Assessment and Definition of the Eligibility Quotient. *J Clin Oncol Off J Am Soc Clin Oncol*. 2020;38(32):3773-3784. doi:10.1200/JCO.20.01255
4. Lamba N, Muskens IS, DiRisio AC, et al. Stereotactic radiosurgery versus whole-brain radiotherapy after intracranial metastasis resection: a systematic review and meta-analysis. *Radiat Oncol*. 2017;12(1):106. doi:10.1186/s13014-017-0840-x
5. Loughan AR, Lanoye A, Aslanzadeh FJ, et al. Fear of Cancer Recurrence and Death Anxiety: Unaddressed Concerns for Adult Neuro-oncology Patients. *J Clin Psychol Med Settings*. 2021;28(1):16-30. doi:10.1007/s10880-019-09690-8
6. Byun J, Kim JH, Kim M, et al. Survival Outcomes and Predictors for Recurrence of Surgically Treated Brain Metastasis From Non-Small Cell Lung Cancer. *Brain tumor Res Treat*. 2022;10(3):172-182. doi:10.14791/btrt.2022.0016
7. Carminucci A, Zeller S, Danish S. Radiographic Trends for Infield Recurrence After Radiosurgery for Cerebral Metastases. *Cureus*. 2020;12(6):e8680. doi:10.7759/cureus.8680
8. Patel AJ, Suki D, Hatiboglu MA, et al. Factors influencing the risk of local recurrence after resection of a single brain metastasis. *J Neurosurg*. 2010;113(2):181-189. doi:10.3171/2009.11.JNS09659
9. Soffietti R, Ahluwalia M, Lin N, Rudà R. Management of brain metastases according to molecular subtypes. *Nat Rev Neurol*. 2020;16(10):557-574. doi:10.1038/s41582-020-0391-x
10. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LAJ, Kinzler KW. Cancer genome landscapes. *Science*. 2013;339(6127):1546-1558. doi:10.1126/science.1235122
11. Salomon MP, Orozco JIJ, Wilmott JS, et al. Brain metastasis DNA methylomes, a novel resource for the identification of biological and clinical features. *Sci Data*. 2018;5(1):180245. doi:10.1038/sdata.2018.245
12. Xu Y, Huang Z, Yu X, Chen K, Fan Y. Integrated genomic and DNA methylation analysis of patients with advanced non-small cell lung cancer with brain metastases. *Mol Brain*. 2021;14(1):176. doi:10.1186/s13041-021-00886-4
13. Capper D, Jones DTW, Sill M, et al. DNA methylation-based classification of central nervous system tumours. *Nature*. 2018;555(7697):469-474. doi:10.1038/nature26000
14. 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. doi:10.1038/s41591-020-0932-2